https://doi.org/10.52763/PJSIR.BIOL.SCI.69.1.2026.9.14

Pak. j. sci. ind. res. Ser. B: biol. sci. 2026 **69B**(1) 9-14

Antibacterial Activity of Artemisia

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(received October 2, 2023; revised May 1, 2024; accepted June 3, 2024)

Abstract. Currently, bacterial resistance to antibiotics has become a real problem in the world, which has pushed scientific research towards the search for alternatives represented by secondary metabolites synthesized by medicinal and aromatic plants, especially essential oils. The present study is a bibliographical synthesis characterizing the chemical composition of essential oils as well as the antibacterial activity of five species belonging to the '*Artemisia' genus: A. absinthium, A. mésatlantica, A. annua, A. herba alba et A.* campestris, against gram positive and gram-negative bacteria most resistant to antibiotics generally used in medicine. The study carried out gave the following results: the majority of gram-positive bacteria such as *Staphylococcus* and *Streptococcus* and gram-negative bacteria such as extended-spectrum beta-lactamase enterobacteriaceae: *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* have become resistant or even multi-resistant to greater or lesser percentages with conventional antibiotics, even with third generation antibiotics. The essentials oils of the plants studied had a very significant effect against all gram-positive bacteria: *Staphylococcus aureus, Listeria monocytogenes*, *Bacillus subtilis*, *Micrococcus luteus*, *Bacillus cereus* and *Streptococcus* as well as against gram negative bacteria*: Escherichia coli, Salmonella, Klebsiella, Pseudomonas aeruginosa* and *Proteus mirabilis*. These oils are generally characterized by their richness in powerful antibacterial substances, especially those belonging to oxygenated monoterpenes. Antibacterial activity is also related to stereochemistry, synergy effect and type of bacteria. Indeed, the essential oils of medicinal and aromatic plants are an excellent natural alternative to the antibiotics currently used in medicine.

Keywords: antibacterial activity, antibiotic, artemisia, essential oil, monoterpenes

Introduction

Public health suffers, especially in recent decades from several infectious diseases whose causative agents have become resistant or even multi-resistant to existing antibiotics WHO (1999). This has caused interested parties to start looking for alternatives to deliver antimicrobial molecules to overcome the problem of bacterial resistance to antibiotics. Medicinal plants, especially those used traditionally, are the main source of these antimicrobial molecules Bouharb *et al*. (2014). Morocco is characterized by its wealth of medicinal and aromatic plants, hence the economic importance of this sector in the country. Morocco exports the equivalent of 250 million dirhams in PAM to the United States and the European Union USAID (2005). Economically, essential oils bring in almost 165 million dirhams USAID (2005). Artemisia is a genus of medicinal and aromatic plants belonging to the Asteraceae family and which is characterized by a very significant chemical polymorphism. This chemo variety can be observed from one stand to another or even between two plants of the same species. This influences the yield and chemical quality of the essential oil from these plants Abad *et al*. (2012). Species belonging to the genus Artemisia are generally located in the north in temperate regions, while their distribution in southern regions remains limited Oberprieler *et al*. (2009). The genus Artemisia includes about 500 species distributed between herbaceous plants and shrubs. These species can be divided into 5 or 6 groups or subgenera given the great polymorphism that exists within this genus. The 6 representative species of these different subgenera are: *Artemisia annua* and *Artemisia vulgaris* of the subgenus Artemisia, *Artemisia absinthium* of the subgenus Absinthium, *Artemisia tridentata* of the subgenus Tridentata, *Artemisia dracunculus* of the subgenus Dracunculus and *Artemisia chinensis* of the subgenus Pacifica Vallès *et al*. (2011). In Morocco, Artemisia is represented by 19 species, the most important of which are: *Artemisia herba alba*, *Artemisia chitachensis*, *Artemisia negrei*, *Artemisia ifranensis*, *Artemisia arborescens* and *Artemisia absinthium* Ibn Tatou and Fennane (2008).

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Bacterial resistance to antibiotics. Antibiotic resistance has become a real problem in recent decades, especially with the overuse of antibiotics. These can no longer kill certain bacteria or slow down their growth. This resistance concerns both gram-positive and gram-negative bacteria.

***Gram positive bacteria*.** The bacteriology laboratory of the Mohammed V military Hospital of Rabat in Morocco carried out a study between April 2012 and March 2016 on the resistance of gram-positive bacteria to antibiotics. *Staphylococcus aureus* was resistant to methicillin in 8% of cases. Methicillin resistant *Staphylococcus aureus* isolates were resistant to gentamicin with a percentage of 48%, Ciprofloxacin (37.5%), Tetracycline (50.6%) and Tobramycin (52.6%). No isolate was resistant to Glycopeptides. Resistance to Fusidic acid, Rifampicin, Fosfomycin was respectively 48%, 15%, 17%. For *Streptococcus*, all species remain highly sensitive to antibiotics, except *Streptococcus B*, which are 90% resistant to Tetracycline.

***Gram negative bacteria*.** The majority of *Enterococcus* isolates were represented by *Escherichia* species. *Faecalis* at 90.5%, with resistance to tetracycline at 78.5%, ampicillin at 1.6% and rifampicin at 12.5%. *Escherichia faecium* was resistant to 80% erythromycin and 68% lincomycin. No isolate was resistant to Gycopeptides Lahlou *et al*. (2020). ESBL enterobacteriaceae represented mainly by *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae, Enterobacter aerogenes, Klebsiella oxytoca, Citrobacter koseri, Citrobacter braakii* and *Serratia marcescens* showed an antibiotic resistance rate of 60.14%: 89.13% to Norfloxacin, 87% to Ciprofloxacin, 50% to Gentamicin, 11% to Amikacin, 80.43% to trimethoprim-sulfamethoxazole combination and 2.17% to Imipenem. The strains studied were all sensitive to colistin. *ESBL Escherichia coli* had a resistance rate of 42% to gentamicin, 74.19% to the trimethoprim-sulfamethoxazole combination, 16.12% to Amikacin and 87.09% to ciprofloxacin and norfloxacin. The strains studied were sensitive to imipenem and colistin. The *ESBL Klebsiella pneumonia* resistance rate was 100% to norfloxacin, 93% to ciprofloxacin as well as to the trimethoprim-sulfamethoxazole combination, 64.28% to gentamicin, 7.14% to imipenem and all strains were sensitive to colistin and amikacin. The resistance rate of *Pseudomonas aeruginosa* is 100% to aztreonam, to ticarcillin-clavulanic acid combination, as well as to ticarcillin. The strains studied were all sensitive to colistin. Their resistance to ceftazidime was 10% with a resistance rate of 66% to amikacin, gentamicin, ciprofloxacin, Imipenem, as well as to the combination Piperacillin-tazobactam Amhal (2017).

The antibacterial activity of Artemisia. *Artemisia absinthium*. Artemisia absinthium or wormwood is a perennial shrub plant that has great medical interests, it is native to Asia, middle east, Europe and north Africa Sharopov *et al*. (2012). It contains many bioactive phytochemicals such as: terpenoids (trans-thujones, γ-terpinene, 1.4 terpineol, myrcene, bornyl acetate, cadinene, camphene, trans-sabinylacetate, guaiazulene, chamazulene, camphor and linalool), lactones, organic acids, tannins, resins and phenols Omer *et al*. (2007). It also contains flavonoids, glycosides and phenolic acids Kordali *et al*. (2005). The essential oil of *Artemisia absinthium* is rich in myrcene, trans-thujones, cis-epoxyocimene, cis chrysanthenyl acetate and trans-sabinyl acetate which are the compounds generally most found in this species Dhen *et al*. (2014); Rezaeinodehi and Khangholi (2008). Absinthe is also known by absinthin, substances that characterize this species. The fresh plant is an important source of azulene El Saber Batiha *et al*. (2020); Bhat *et al*. (2019).

Ethanolic extract of *Artemisia absinthium* inhibits *Staphylococcus aureus ATCC29213* with a zone of inhibition between 10 and 15 mm in diameter. They have no remarkable activity against *Escherichia coli, Staphylococcus faecolis* and *Bacillus subtilis var niger ATCC10* Dulger *et al*. (1999). *Artemisia absinthium* had a significant effect against gram-positive pathogenic bacteria Fiamegos *et al*. (2011). The methanolic extracts of *Artemisia absinthium* inhibit *Bacillus subtilis ATCC6633*, *Salmonella typhimurium RSSK95091, Bacillus cereus 6230, thermophilic Salmonella 6453, Providencia alcalifaciens 3215* and *Pseudomonas putida 1617*. Moslemi *et al*. (2012) that the hydroalcoholic extract has an antibacterial effect against *Staphylococcus aureus.* The antibacterial activity noted may be due to the synergistic effect between the majority compounds (camphor, cymene…etc) and the minority compounds (∝-pinene, β- pinene etc.) contained in the essential oil. This activity is strongly linked to the bioactive compounds which are the hydrocarbon monoterpenes, which they are (∝-pinene, β- pinene, camphor, caryophyllene and p-cymene. These molecules make the biological membrane of bacteria permeable Moslemi *et al.* (2012). The antibacterial action of essential oil extracted from *Artemisia absinthium* was more accentuated than

gentamicin against *Staphylococcus aureus* (the resistant and sensitive strains), *Salmonella typhimurium, Escherichia coli ATCC8739, Proteus vulgaris, Klebsiella pneumoniae 10031* and *Pseudomonas aeruginosa 9027.* This activity is related to the presence of ∝-phellandrene and chamazulene which are the important components of essential oil Assili *et al*. (2015); Rezaeinodehi and Khangholi (2008). Mihajiliv and Krstevetal recorded that the minimum inhibitory concentration of *Artemisia absinthium* essential oil was less than 0.08 mg/mL for *Staphylococcus aureus 25923* and *Pseudomonas aeruginosa 9027* extracted from wounds and for *Pseudomonas mirabilis* extracted from human stool and 2.43 mg/mL for *Klebsiella oxytoca* isolated from stool and 0.14 µL/mL for *Listeria monocytogenes*. These actions are related to the suppression of protein, polysaccharide, DNA and RNA biosynthesis in bacterial cells. (Table 1) Mihajilov-Krstev *et al*. (2014); Belay *et al*. (2011).

***Artemisia mesatlantica*.** The study of the essential oil from *Artemisia mesatlantica* collected in the region of Ifrane in Morocco revealed the presence of terpene esters with terpinyl acetate, terpene alcohols (∝-terpineol), monoterpenes (camphene, limonene, myrcene, tricyclene), oxides with 1.8 cineole, sesquiterpene ketones (piperidone) and sesquiterpenes. The essential oil of *Artemisia mesatlantica* is characterized by the dominance of β-thujone followed by camphene and camphor. In addition to the majority compounds, there are other minority compounds such as 1.8 cineole, D-germacrene, 1-epicubenol, ∝-thujone and davanone. The classification of these molecules according to their functional chemical groups shows the predominance of oxygenated monoterpenes Bencheqroun *et al.* (2012).

For antibacterial activity, the essential oil of this plant has an antibacterial effect against *Escherichia coli* and *Staphylococcus aureus* with a dilution of 1/2000 v/v, *Bacillus subtilis* with a dilution of 1/3000 v/v and *Micrococcus luteus* with a dilution of 1/1000 v/v (Table 2) Bencheqroun *et al*. (2012).

***Artemisia annua*.** The essential oil of *Artemisia annua*, collected in the southern slopes of the middle Atlas in Morocco, is rich in bioactive molecules such as: oxygenated monoterpenes (1.8 cineole, artemisia ketone, artemisia camphor alcohol, borneol), hydrocarbon sesquiterpenes (copaene, γ-cadinene, caryophyllene, β-farnesene, germacrene D and β-selinene), oxygenated sesquiterpenes (caryophyllene oxide).

Table 2. Antibacterial activity of the essential oil of *Artemisia mesatlantica*

Bacteria	*E. coli*	*B. subtilis*	*M. luteus*	*S. aureus*
Dilution(v/v)	1/2000	1/3000	1/1000	1/2000

Chebbak *et al*. (2023) showed that *Artemisia annua* studied has a significant antibacterial effect against gram-positive bacteria but especially against gram negative bacteria especially *Escherichia coli* with a zone of inhibition close to 75.67 mm and a minimum inhibitory concentration of 5.34 µL/mL plus or minus 0.50 µL/mL followed by *Bacillus subtilis* with an inhibition zone of 37 mm and minimum inhibitory concentration of 5.64 µL/mL plus or minus 0.97 µL/mL then *Staphylococcus aureus* with a zone of inhibition close to 23.33 mm and a minimum inhibitory concentration equal to 4.98 µL/mL plus or minus 1.82 µL/mL, then *Klebsiella pneumoniae* with a zone of inhibition close to 22.33 mm and a minimum inhibitory concentration 5.44 µL/mL followed by *Salmonella sp* with a zone of inhibition close to 16 mm and a minimum inhibitory concentration of 1.09 mg/mL and finally *Bacillus cereus* which is the least sensitive bacterium with an inhibition zone of 11 mm and a minimum inhibitory concentration of 5.44 mg/mL. (Table 3) Chebbak *et al*. (2023).

***Artemisia herba alba*.** The essential oil of *Artemisia herba-alba* of Moroccan origin has been studied from a chemical point of view and antibacterial power. This essential oil is composed of: oxygenated monoterpenes (56.4%) followed by oxygenated sesquiterpenes (2.5%). The other constituents represent 38.7%. the components found are: cis-thujones, trans-thujones, vanillyl alcohol, davanone, cis-threo-davanafuran, camphor, isobornyl *n*-butyrate, cis-chrysanthenyl acetate, trans-arbusculone, jasmolactone, n-dodecanol, artedouglasia oxide C and cyclosativene. *Artemisia herba-alba* essential oil had

Table 1. Antibacterial activity of the essential oil of *Artemisia absinthium* Mihajiliv -Krstev *et al*. (2014)

Bacteria	*S. aureus 25923*	*P. aeruginosa 9027*	*P. mirabilis*	*K. oxytoca*	*L. monocytogenes*
MIC (mg/mL)	0.08	0.08	0.08	2.43	0.14

Table 3. Antibacterial activity of the essential oil of *Artemisia annua*

Bacteria	Zone of inhibition (mm)	MIC(µl/mL)
E. coli	75.67	5.34
B. subtilis	37	5.64
S. aureus	23.33	4.98
K. pneumoniae	22.33	5.44
Salmonella sp.	16	1.09 mg/mL
B. cereus	11	5.44 g/mL

antibacterial activity against 12 bacterial species from the most sensitive to the least sensitive: *Bacillus clausii 2226, Brochotrix thermosphacta D274, Staphylococcus sp GB1, Streptococcus salivarius, Salmonella typhimurium, Carnobacterium maltaromaticum 9P, Pseudomonas fragi 6P2, Staphylococcus sp ES1, Serratia proteamaculans 20P, Brochotrix thermosphacta 7R1*. A very weak effect on *Escherichia coli 32* and on *Staphylococcus aureus* Amor *et al*. (2019).

Artemisia herba-alba from other countries and whose main constituents are: cis and trans-thujones have shown the same results Majdouli and Elazzouzi (2015); Sbayou and Ababou (2014).

Artemisia herba-alba is characterized by significant chemical polymorphism. Thus, several chemotypes have been studied such as the eucalyptol chemotype and the caryophyllene acetate chemotype found in Jordan Aldosary *et al*. (2018); Qnais *et al*. (2016). Camphor has been noted as the main component of artemisia herba alba from Algeria and Tunisia Bertella and Benlahcen (2018); Younsi *et al*. (2017).

***Artemisia campestris*.** The volatile compounds contained in the essential oil of *Artemisia campestris* belong to four groups: oxygenated monoterpenes (49.5%), hydrocarbon monoterpenes (42.2%), oxygenated sesquiterpenes (2.9%) and hydrocarbon sesquiterpenes (2.8 %). The other constituents represent only 0.2%. the isolated components are diverse. Among them are: ∝-pinene (18.4%), camphene (7.7%), limonene (5.2%), camphor (9.2%), borneol (5.2%), ∝-terpineol (3.6%), ∝-terpinyl acetate (18.8%), bornyl acetate (2.7%), ∝-thujene (0,3%), trans- β-ocimene (2%) Belhattab *et al*. (2011). The constituents of essential oil of *artemisia campestris* differ according to the chemotype or variety. However, generally, the constituents in common and which characterize this species are: β-pinene (24,2_27,9%), p-cimene (17,4_22,3%) et ∝-pinene (4,1_11%) Gucker and Coreyl (2007).

The antibacterial activity of *artemisia campestris* essential oil is tested against *Escherichia coli ATCC 25922, Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa 27853*, *Salmonella typhimurium*, *Staphylococcus aureus ATCC 43300* and *Staphylococcus aureus*. The most potent antibacterial effect was observed against *Pseudomonas aeruginosa ATCC 27853* with a zone of inhibition of 23 mm and against *Escherichia coli* with a zone of inhibition of 20 mm Djidel and Khennouf (2014).

Factors influencing antibacterial activity. In general, the presence of a significant concentration of oxygenated monoterpenes like thujones, camphor, camphene and 1.8-cineole in the essential oil increases its antibacterial activity. This may explain the pronounced effect of essential oil like that of *artemisia mesatlantica* against *Staphylococcus aureus* and *Bacillus subtilis* because *Staphylococcus aureus* has been shown to be most affected by ketone monoterpenes like thujones Oussalah, *et al*. (2007); Dorman and Deans (2000).

Other studies have shown that the majority of essential oil tested for their antibacterial effects had more pronounced activity against gram positive bacteria compared to gram negative bacteria, this is due to the structure of the outer membrane of gram-negative bacteria which is hydrophilic which prevents the entry of volatile water insoluble compounds Wan (1998).

Antibacterial activity is also influenced by stereo-chemistry. Studies by Dorman and Deans in 2000 that β-isomer compounds are more active against bacteria than ∝-isomers Dorman and Deans (2000). Synergy is a factor that also influences antibacterial activity. Indeed, the molecular interaction of the functional groups of different compounds of the essential oil with the wall of the bacteria leads to strong lesions. The cooperation therefore between the different majority and minority compounds of essential oils reinforces their antibacterial power Felice *et al*. (2004).

Conclusion

The present work has made it possible to study some species belonging to the genus artemisia with the aim of drawing conclusions that may be useful in the medical field. Indeed, artemisia is characterized, in general, by a powerful antibacterial power, whether against gram positive or gram-negative bacteria. This allows it to become a good alternative to current antibiotics in the medical and pharmaceutical field and specific formulas

that bring together specific compounds that can have a significant effect depending on the type of bacteria.

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