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Antibacterial activity of Eudesmanolide compounds isolated from medicinal plant *Artemisia herba-alba*

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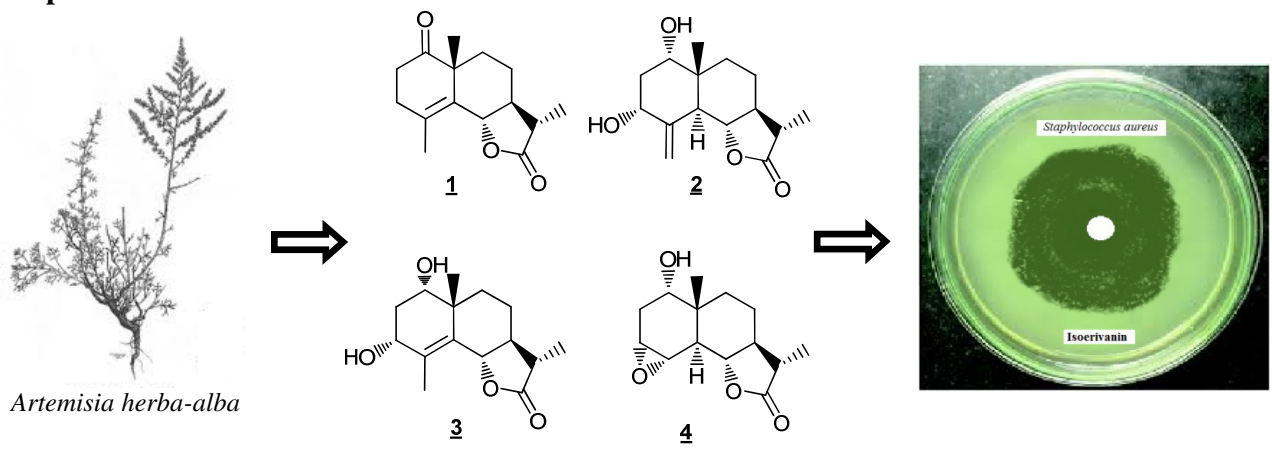
Abstract
The sesquiterpene lactones from chloroform extract of the *Artemisia herba-alba* (Taurin **1**, Erivanin **2**, Isoerivanin **3** and Herbalbin **4**) were tested for their antibacterial activities against five bacterial species: *Escherichia coli* (ATCC 10536), *Staphylococcus aureus* (ATCC 9144), *Streptococcus faecalis* (ATCC 10541), *Pseudomonas aeruginosa* (IPP 10536) and *Mycobacterium smegmatis* (IPP 7316). All tested compounds possess an antibacterial activity except *Mycobacterium smegmatis* that shows resistance towards all theirs compounds.

Keywords: *Artemisia herba-alba*, Sesquiterpene lactones, Eudesmanolide, Antibacterial activity.

Graphical Abstract



Artemisia herba-alba

Introduction
The genus *Artemisia L.* (family *Asteraceae*, tribe *Anthemideae*), comprises a several number of species (from 200 to over 400 species) found throughout the northern half of the world. [1] The genus may be divided into sections *Artemisia* and *Dracunculus*. [2] *Artemisia herba-alba* is a greenish-silver perennial herb, grows 20 to 40 cm in height, the vegetative growth of this plant takes place in the autumn, the flowering starts from September to December and basically develops at the end of the summer with many basal, erect and leafy stems covered by woolly hairs. [3-4] Which is a medicinal and strongly aromatic dwarf shrub that grows wild in arid areas of the Mediterranean basin extending into north western Himalayas. [5] In Morocco, this plant commonly known as the white wormwood in Arabic as "CHIH". *Artemisia herba-alba* is one of five spontaneous *Artemisia* species that were identified and was used as aromatising for tea. [6] In folk medicine, it was known for its therapeutic and medicinal properties, was used for treatment of colds, coughing, intestinal disturbances, as antidiabetic agent, for

bronchitis, diarrhea, neuralgias and hypertension. [7-10] Many researchers have reported various biological and/or pharmacological activities of *Artemisia herba-alba* as an antimicrobial (bacteria and fungi), [11-13] antileshmanial [14], anthelmintic [15] and antispasmodic agent [16] On the other hand, previously isolated classes of constituents: Coumarins [17], monoterpenes [18], davanones [19], flavonoides [20], sesquiterpene lactones. [21]
The present study is a continuation of the work of Boriky et al., [22] from which four molecules isolated from *Artemisia herba-alba* were tested by the antibacterial activity against five strains: *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus faecali*, *Pseudomonas aeruginosa* and *Mycobacterium smegmatis*.

2. Materials and methods

2.1. Plant material

Artemisia herba-alba was obtained from plants growing in a forest in Taza (Morocco), the plant was confirmed by Professor Fougrache Hassan (Department of biology, Hassan II University, Casablanca)

2.2. Tested material

Taurin **1**, Erivanin **2**, Isoerivanin **3** and Herbalbin **4** compounds (yields: 0.33, 0.60, 0.41 and 0.50 %, respectively) isolated from the chloroform extract of *Artemisia herba-alba*, were tested as antibacterial agent towards some bacterial species. Extraction, purification and identification procedure as described in a previous work. [22] Briefly, Air-dried plant material (1 kg) was crushed and soaked overnight in Petroleum ether. This extract was discarded and the residue was allowed to stand for several days covered with $CHCl_3$ and then filtered. After removal of the solvent, the new residue (80g) was extracted (×3) with Hexane/EtOH/H_2O. The combined aqueous phases were extracted with CH_2Cl_2. The CH_2Cl_2 extract, upon evaporation, yielded 25 g of a brown residue. One portion of this residue (6 g) was fractionated by column chromatography on silica gel under atmospheric pressure by a gradient of eluent A: Hexane/EtOAc (9:1), B: Hexane/EtOAc (7:3), C: Hexane/EtOAc (4:6) and D: hexane/EtOAc (3:7). The fraction A (0.16g) was fractionated in the second time, by a column chromatography on silica gel by CCl_4/CH_2Cl_2 (2:3) to give Taurin (**1**) (0.02 g). The fraction C (0.24g) was separated on silica gel with EtOAC (5-8%) in CCl_4/CH_2Cl_2 (1:2) and yielded three intermediate fractions. The first lactone fraction (0.08 g) was purified by preparative TLC in Hexane/EtOAc (2:3) to give Erivanin (**2**) (0.036g). The second crystallization of lactone fraction (0.066 g) from EtOH afforded 0.025 g of Isoerivanin (**3**). The third lactone fraction (0.074 g) was crystallized from EtOH to give Herbalbin (**4**) 0.03 g. All compounds were identified by nuclear magnetic resonance

2.3. Microorganisms

Two bacteria gram-positive *Staphylococcus aureus* (ATCC 9144) and *Streptococcus faecalis* (ATCC 10541), two bacteria gram-negative *Escherichia coli* (ATCC 10536), *Pseudomonas aeruginosa* (IPP 10536) and one bacteria alcoolo-acido resistant *Mycobacterium smegmatis* (IPP 7316) were used. The stocks are maintained at -32 ° C in Nutrient broth at 10 % of glycerol.
ATCC: Standard American Cultur Collection, IPP: Pasteur Institute of Paris.

2.3. Antibacterial activity

Antibacterial assays were conducted using the standard disc-diffusion method. [23] Chloroform extract and products were applied to sterile discs (Whatman, 6 mm) in aliquots of 5 µl of solvent, allowed to dry at room temperature, and placed on agar plates inoculated with microbes. Each product was tested in triplicate. Control discs contained 10 µl pure DMSO (100%). Standard antibiotic, Ampicillin (5 µg/disc) was used to eliminate variation between plates. The bacteria were maintained on nutrient agar plates and incubated at 37 °C for 24 h. Zones of growth inhibition, if any, were measured following incubation. All products were tested at a concentration of 5 µg/disc.

3. Results and discussion

Such as we previously described, we completed the work of separations and purifications bio-guided, hence we have made antibacterial tests of the isolated compounds in the plant *Artemisia herba-alba*. The antimicrobial activities of *Artemisia herba-alba* compounds originating from Morocco were evaluated by a paper disc diffusion method against tested bacteria. The results showed that compounds were active against the microorganisms

assayed. Related to the inhibition of growth, significant differences were detected among these cited works types, since all of them showed an interesting activity for all tested strains (Table 1).

Table 1: Antibacterial activity of the chloroform extract and eudesmanolides compounds from *Artemisia herba-alba*.

Extract/Compound	Inhibition zone (mm)				
	E. coli	*S. aureus*	*S. faecalis*	*P. aeruginosa*	*M. smegmatis*
Chloroform extract	10	14	10	5	5
Taurin **1**	-	-	-	10	-
Erivanin **2**	-	7	6	8	-
Isoerivanin **3**	10	12	11	11	-
Herbalbin **4**	-	7	6	7	-
Ampicillin	16	15	20	12	-

At first, we targeted the chloroform extract (extract contains all the isolated compounds **1**, **2**, **3** and **4**), which showed interesting activity against the five strains, so this extract presented Inhibition zone diameters: 10 mm, 14 mm, 10 mm, 5 mm and 5 mm respectively for *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Pseudomonas aeruginosa* and *Mycobacterium smegmatis*. On the other hand, the four isolated compounds **1**, **2**, **3** and **4** showed important antibacterial activity, while the Taurin **1**, which has an inhibition only for *Pseudomonas aeruginosa* strain with a zone inhibition zone diameter 10 mm, other bacteria have a resistance to this compound. Erivanin **2** demonstrated a medium antibacterial activity against three strains of *Staphylococcus aureus*, *Streptococcus faecalis*, *Pseudomonas aeruginosa* hence the inhibition zone diameters are respectively 7 mm, 6 mm and 8 mm. Isoerivanin **3** provides an interesting activity compared other isolated compounds, it is active against four bacteria, and it has inhibition zone diameters 10 mm, 12 mm, 11 mm and 11 mm respectively *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus faecalis* and *Pseudomonas aeruginosa*. The fourth compound isolated Herbalbin **4** showed an antibacterial activity similar to Erivanin **2**, then it has an activity only against three strains, *Staphylococcus aureus*, *Streptococcus faecalis*, *Pseudomonas aeruginosa*, the inhibition zone diameters are respectively 7 mm 6 mm and 7 mm.

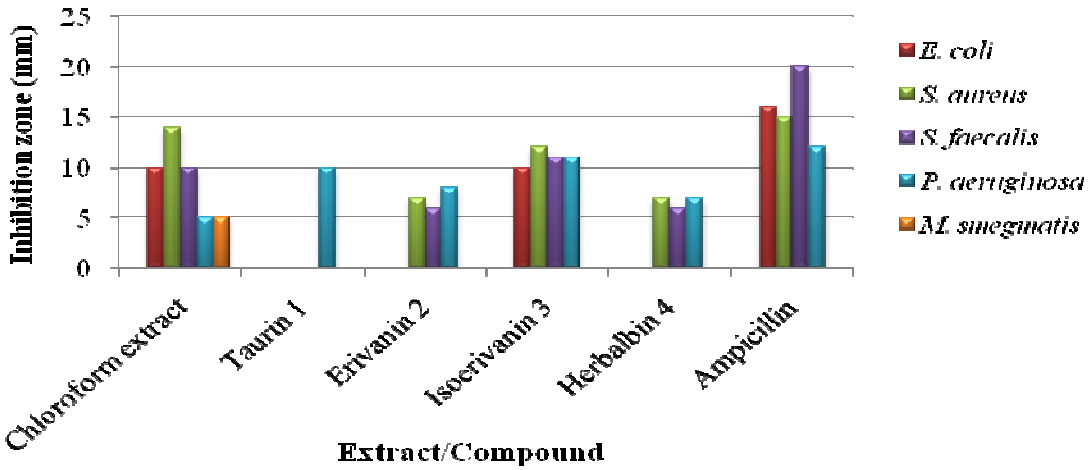


Figure 1: results of antibacterial activity

Finally, the compounds isolated from the chloroform extract did not show any activity against *Mycobacterium smegmatis*, these results are contradictory to the activity of the extract of the mother (chloroform) which gave 5 mm of Inhibition diameter. Two scientific hypotheses to explain these results, the first hypothesis is that compounds isolated were synergistic activity against *Mycobacterium smegmatis*, and second hypothesis that there are other compounds that have not been isolated and are activity antibacterial against this strain. Figure 1 is a histogram that shows all the results obtained in this study and its comparison with positive control Ampicillin (standard).

Conclusion

The isolated compounds from chloroform extract of the *Artemisia herba-alba* possess antibacterial activity, except *M. smegmatis* such it's showed resistance. The chloroform extract showed an antibacterial activity against *E. coli*, *S. aureus*, *S. faecalis* and *P. aeruginosa*. The sesquiterpene lactones showed a variable antibacterial activity and the most antibacterial activity was obtained with Isoerivanin **2**. Taurin **1** presented a limited activity with only action toward *P. aeruginosa* , the Erivanin **2** and the Herbalbin **4** presented a comparative antibacterial activity. At light of these results it appears clearly that the antibacterial activities is dependent as well as of the structure of compounds that the nature of bacteria used.

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