Abietane diterpenes from the cones of Abies numidica de Lannoy ex Carrière (Pinaceae) and in vitro evaluation of their antimicrobial properties

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Maya Belhadj Mostefa [a], Amin Abedini [b], Laurence Voutquenne-Nazabadioko [b], Sophie C. Gangloff [c], Ahmed Kabouche [a] and Zahia Kabouche [a]*

[a]*Université des frères Mentouri-Constantine, Département de chimie, Laboratoire d'Obtention de Substances Thérapeutiques (L.O.S.T), 25000 Constantine, Algeria.*

[b]*Groupe Isolement et Structure, Institut de Chimie Moléculaire de Reims (ICMR), CNRS UMR 7312, Moulin de la Housse, BP 1039, 51687 Reims, France.*

[c]*Laboratoire de Microbiologie, EA 4691, UFR de Pharmacie, 1 Rue du Maréchal Juin, 51096 Reims Cedex, France.*

*Corresponding Authors

Pr zahia Kabouche, Université Mentouri-Constantine

E-mail : zahiakabouche@gmail.com Phone :

Abstract

Eight known abietane diterpenes (**1-8**) were isolated for the first time from *Abies numidica* cones (Pinaceae). The structures of all compounds were established by means of 1D and 2D NMR, and UV spectral analyses. The hydromethanolic extract of *A. numidica cones* was tested for its antimicrobial activity against 17 Gram-positive and Gram-negative bacteria and against 5 yeasts by the use of liquid and solid medium and bioautography methods. The best antimicrobial activity was found against Gram-positive bacteria (MIC ≤0.3mg/mL) *Bacillus subtilis, Enterococcus faecalis* ATCC 1034, *Staphylococcus aureus* 8325.4, *Staphylococcus aureus* CIP 53.154, *Micrococcus luteus* and *Listeria innocua* and against *Candida* yeasts. The determination of MIC's of isolated products showed a high activity of compounds **4** and **6** against *S. aureus, L. innocua* (MIC=62.5µg/mL) and *E. faecalis* (MIC=125µg/mL).

Keywords: *Abies numidica*; Pinaceae; abietane diterpenes; antimicrobial activity; solid medium; liquid medium; bioautography

1. Introduction

Abies is an important genus of the Pinaceae family. About 50 species are distributed in North Africa, Europe, North and Middle America and highlands of Asia (Zheng et al. 1978). Triterpenoids, steroids, flavonoids, phenols, fatty acids, and lignans have been previously reported (Yang et al. 2008). *Abies* species have exhibited several activities, including antitumour (Kim et al. 2004), anti-inflammatory, antihypertensive, anti-ulcerogenic (Singh et al. 2000), antibacterial (Vishnoi et al. 2007), antifungal (Aoyama et al. 1992), antitussive (Nayak et al. 2003), and central nervous system activities (Nayak et al. 2004). The cones of *Abies numidica* de Lannoy ex Carrière were used in folk medicine against cold, indigestion, stomachache, pulmonary, vascular and venereal diseases (Fujita et al. 1995). We report here, the phytochemical composition and the *in vivo* antimicrobial activity of the cones of *A. numidica*.

2. Result and discussion

Chromatography of the hydromethanolic extract of the cones of *A. numidica* afforded eight known compounds (**1-8**). They were unambiguously identified as: 7α,15-dihydroxydehydroabietic acid (**1**) (Prinz et al. 2002),13β-hydroxy-7oxo-8(14)-abieten-18-oic acid (**2**) (Ohtsu et al. 2000), 15-hydroxy-7-oxo-dehydroabietic acid (**3**) (Matsumoto et al. 1988), 7α-hydroxydehydroabietic acid (**4**) (Miguel Del Corral et al. 1994), 7-oxodehydroabietinol (**5**) (Tanaka et al. 1997), 7α-hydroxy-15-methoxy-dehydroabietic acid (**6**) (Xian-Wen et al. 2010), 4-hydroxy-18-nor-8,11,13-abietatrien-7-one (**7**) (Lee et al. 1995) and 15-hydroxydehydroabietic acid (**8**) (Cheung et al. 1993) (figure 1) by comparison of their spectroscopic data with literature values.

The antimicrobial potential of the hydromethanolic extract of *A. numidica* cones was evaluated against 22 microorganisms including Gram-positive and Gram-negative bacteria as well as fungi by the MIC's evaluation in solid and liquid medium (Abedini et al. 2014; Acebey-Castellon et al. 2011). In solid media, the hydromethanolic extract was active at different degrees against 12 bacterial strains, particularly Gram-positive bacteria (Table 1). No antimicrobial activity was found against the Gram-negative bacteria strains, *Escherichia coli* CIP 54.127, *Enterobacter cloacae*, *Salmonella enterica*, *Klebsiella pneumoniae* and *Providencia stuartii*. The best antibacterial activity was obtained against six microorganisms namely *Bacillus subtilis*, *Enterococcus faecalis* ATCC 1034, *Staphylococcus aureus* 8325-4, *Staphylococcus aureus* CIP 53.154, *Micrococcus luteus* and *Listeria innocua* with MIC's values on agar below ≤0.3 mg/mL. The exact MIC values, in the liquid media, against these

six bacterial strains, reveal that the hydromethanolic extract have a strong activity against *Bacillus subtilis* (MIC=62.5 µg/mL) and a good antimicrobial activity against *E. faecalis*, *S. aureus* 8325-4, *S. aureus* CIP 53.154, *M. luteus* and *L. innocua* (MIC ≤250 µg/mL) .This activity is very interesting for a crude extract (Toyang et al. 2012). To identify the compounds having an antibacterial activity in this extract, a bioautography assay (Abedini et al. 2013; Acebey-Castellon et al. 2011) was performed with the pure compounds (**1**-**8**) against *Staphylococcus aureus* CIP 53.154, one of the most sensitive microorganisms. Bioautography showed good to moderate activity for the five dehydroabietic acid derivatives (**1**, **3**, **4**, **6**, **8**) and low activity for compound **7** (Table 2). Therefore, these compounds were tested separately in liquid medium against *S. aureus* CIP53-154, *L. innocua* and *E. faecalis* ATCC 1034 (Table 2). Like bioautography, **4** and **6** showed the greatest activity against *S. aureus* and *L. innocua* (MIC=62.5 µg/ml) which is very close to MICs of antibiotics. They were less active against *E. faecalis* (MIC= 125µg/mL). Compound **1** showed a good activity against *L. innocua* (MIC=62.5µg/mL) and a moderate activity against *S. aureus* and *E. faecalis* (MIC= 125µg/mL). Compounds **3**, **7** and **8** exhibited a moderate activity with MICs ranging between 125 and 500µg/mL. These results suggest that the presence of hydroxyl group in 7α position (**1**, **4**, **6**) may increase the antimicrobial activity of dehydroabietic acid derivatives compare to a ketone group (**2**, **3**, **5**, **7**) as observed by Gouiric et al (2004). On the other hand, a carboxyl group in C-18 position in dehydroabietic acid derivatives seems to be more selective to the antibacterial activity.

The *in vitro* antifungal activity of the hydromethanolic extract was also tested against five yeasts and was active at 5mg/mL MIC's value against *Candida glabrata* and at 10mg/mL MIC's value against *Candida tropicalis*, *C. kefyr* and *C. albicans* (Table 1).

3. Conclusions

Eight abietane diterpenes (**1**-**8**) were isolated for the first time from the cones of *Abies numidica*. The hydromethanolic extract was screened for their antibacterial and antifungal activities and demonstrated antibacterial activity against *Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus* 8325-4, *Staphylococcus aureus* CIP 53.154, *Micrococcus luteus* and *Listeria innocua* with MIC's ranging from 62.5 to 250 µg/mL.

Compounds **4** and **6** showed strong activity against *S. aureus* 8325-4 and *L. innocua* (MIC=62.5µg/mL), and compounds **1**, **3**, **7** and **8** a good antimicrobial activity. This work reveals that this plant can be used as potent sources of new antimicrobial agents against bacteria which are increasingly becoming resistant to traditional antibiotics.

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Supplementary material

The experimental part of this article can be accessed on line at ….

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