

The Therapeutic Importance of Products Extracted from the Fir Tree of Numidia (*Abies numidica*) and Research on its Antibacterial Activity

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Summary

Conifers, "Trees of the records", contain an extraordinary collection of species, represent one third of the world's forests, and their origins go back up in the mists of time. A pyramidal port, leaves in needles, fruits in cones and especially the resin make the main characteristics of these species. The ecological plasticity and high wood quality as well as their non-ligneous forest products (resin and rosin) confer remarkable ecological and economic interests to these species. Although the traditional medicines originating from conifers have always showed their efficiency through the history, they still remain badly known.

The essential oil of the Fir tree of Numidia (*Abies numidica*) from the region of Seraidi (Annaba) was the topic of our work. The chemical identification of essential oil revealed their wealth in terpene compounds of which the monoterpenes had shown the highest content.

The research for the antibacterial activity of various studied samples showed variable results regarding the drug and the tested origin. A good antibacterial activity was noticed for essential oil.

Introduction

The fir tree of Numidia (*Abies numidica*) is a tree 25 m tall, evergreen, its pyramidal feature becoming conical with years. It is a dense conifer thus very branchy. The trunk is straight, covered with a grey pale pink to orange bark cracking at older ages. Twigs are many hairless, of green-brown color when young, then becoming grey-brown.



Figure 1. The fir of Numidia in Seraidi (eastern Algeria)

The needles are arranged around twigs and quite upward. They are blue-green sometimes with a triangular green-grey spot towards the tip. On the lower side of needles, two stomata whitish stripes are found. Needles are dense, short, from 1 to 2 cm long and rounded off to the apex.



Figure 2. Twigs of the fir tree of Numidia (*Abies numidica*)

The bloom takes place in April. The male flowers have their floral buttons of red color and become yellow when blooming. The female flowers are green and give rise to cones 15 cm long and 3-4 cm in diameter, cylindrical, of grey brown colour with mature. They contain winged seeds 2-3 cm long. The fir tree of Numidia is also named *Abies numidica* of Lannoy, Abete d'Algeria, and Numidian fir.

Our choice of the fir tree of Numidia is understandable because of the low abundance and the misunderstanding of this species although it is endemic in Algeria.

Material and methods:

Plant materiel: the needles of the fir tree of Numidia were harvested in the region of Seraidi

(experimental station, east of Algeria) in October 2010.

Bacterial strains: five bacterial strains (*Staphylococcus aureus* (MRSA), sensitive *Escherichia coli*; *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Acinetobacter* sp. and *Staphylococcus epidermidis*) were selected.

Methods: several methods were used in our study: (i) the histological study based on the double coloring, (ii) the extraction of essential oil by the method of vapor training; (iii) the chromatography on thin layer; (iv) the search for an antibacterial activity.

Results and discussion

1-Histology: The histological study was carried out on the needles of the fir tree of Numidia in order to determine the glandular structure of this species. It is well-known that most of the essential oils are found in glands. We found lysigen glandular canals in all the conifer wood (Figure no. 3) what is particular to Abietaceae of which the fir tree of Numidia is an example.



Figure 3. Cross section of a needle of fir tree of Numidia (*Abies numidica*) observed in the microscope in Gr 10

2-Extraction of the essential oil: the essential oil of needles of fir tree of Numidia was extracted by the method of vapor training using the Likens Nickerson device (method standardized by the pharmacopoeia).

Table 1. Yield and characteristics of extracted essential oil

Sample	Extraction	quantity of the sample (g)	Characteristic of the essential oil	Yield (efficiency) (ml)
Needles of the fir tree of Numidia	1st extraction	100	Crystal clear and light	0.32
	2nd extraction	100	yellow	0.26
	3rd extraction	100		0.37

Based on this study, one may say that the yield of essential oil is low compared to the literature. The best content of this essential oil is 0.37 ml/10 g of the dried drug. We can explain this difference by the characteristic bioclimatic conditions of the region of Séraïdi (Annaba) as well as the period of harvest.

3-Chromatography on thin layer: these analyses have allowed us to obtain various results; each of them corresponds to a component of the chemical composition of our sample (figure n 4). The distance crossed by the mobile phase is 11.7 cm.

Table 2. The crossed distances and the HRF of the studied samples

Tasks	The crossed distances (cm)	HRF
Spot 1 (S6)	1	52
	2	100
Spot 2 (S7)	11.1	95
Spot 3 (S8)	1	19
	2	54
	3	70
	4	83

- Spot 6: essential oil in the needles of fir tree of Numidia (Seraidi)
- Spot 7: essential oil in the needles of fir tree of Siberia (control)
- Spot 8: essential oil in the needles of fir tree of Numidia (Seraidi)

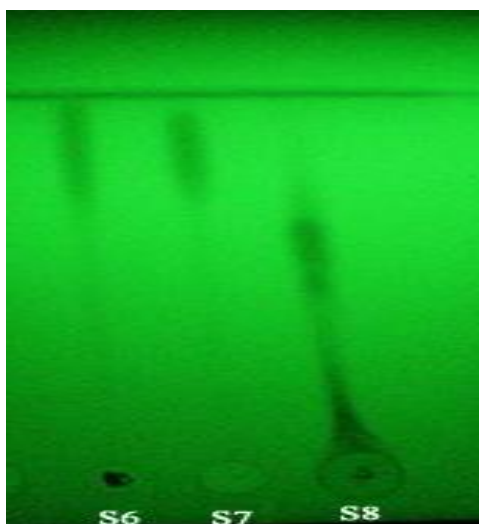


Figure 4. Chromatograph image under wood lamp

For essential oils: various spots were revealed by the chromatogram of control sample. It involves the presence of composites outbuildings between tested essential oil and essential oil of the fir tree of Siberia. This latter oil is rich in bornyle acetate (40-50 %), has it-pinene, camphene and Delta 3 hulls in-line with the literature; we can assume the presence of α -pinene, camphene, Delta 3 carene, and bornyle acetate in our essential oil. This work presents only preliminary results in absence of works in the literature. It will need more thorough research which will probably allow a better identification of chemical composition of fir tree. Our work will be pursued by a chromatography in gas phase.

4-Research for an antimicrobial activity: the purpose was to evaluate the essence produced by the fir tree of Numidia. The work consisted of the search for a possible antibacterial activity of the essential oil of needles on the 5 bacterial strains.

Evaluation of the antimicrobiologic activity: It was based on the studies

demonstrating the antibacterial activity of the essential oil of the fir of Numidia; we have also tried to widen the range of tested bacteria: positive and negative Gram. Besides, these species are the most implied in the healing of diseases for which fir is traditionally used.

- **The method of the aromatogram:** the antibacterial activity of essential oil was estimated using the method of distribution in solid environment (Bauer and al., 1966).

- **The research for an antimicrobial activity of the essential oil of fir:** bacterial strains are maintained by transplanting on nourishing gels favorable to their growth (to obtain young origins from pure and ancient cultures, brooded for 24 hours at 37°C before being used during the tests of antimicrobial activity).

- **Preparation of the dilutions of essential oil:** the dilutions of the oil in 1/10, 1/100, 1/1000 by means of DMSO. Beforehand washed and sterilized disks of blotting paper were soaked in essential oil.

Table 3. Diameters of inhibition from bacterial strains

Bacteria	Diameter (mm)			
	Raw Extract	Dilution 1/10	Dilution 1/100	Dilution 1/1000
<i>Escherichia coli</i>	Resistant	9.6	9	10.7
<i>Klebsiella pneumoniae.</i>	Resistant	10	12.5	8.2
<i>Pseudomonas aeruginosa</i>	Resistant	8.7	7.2	7.2
<i>Acinetobacter sp.</i>	Resistant	8.2	Resistant	7.4
<i>Staphylococcus aureus(MRSA)</i>	Resistant	9.2	8.7	7.5

The raw essential oil was inactive on all tested germs.

The dilutions of the various studied samples had allowed to reveal an antibacterial activity (d) ranging from 7.2 mm to 12.5 mm.

The comparison between the antibiogram and the aromatogram is indispensable for every bacterial strain as follows:

Pseudomonas aeruginosa and Escherichia coli: although the raw extracts of oil were inactive, the dilutions were moderately active but unimportant compared with some antibiotics.

Acinetobactersp: the raw extract had no antibacterial activity as for the dilution of 1/100. The dilutions had shown a remarkable activity especially for the dilutions 1/10 and 1/1000 of HEY of the fir tree of Numidia which were more active.

Klebsiella pneumoniae: the raw extract had no antibacterial activity. 3 dilutions had a good antibacterial activity. A specific activity marked for the dilution 1/100 and a little less for the dilution 1/10.

***Staphylococcus aureus* (MRSA)**: the raw extract was inactive. 3 dilutions had an antibacterial activity especially for the dilutions 1/10 and 1/100.

The antibacterial inactivity of the raw extract of our samples forced us to work with the dilutions which gave a very good activity. This fact can be understandable based on the phenomenon of "saturation": the strong concentration of the sample prevented the distribution of the active molecules in the nourishing environment.

Conclusion

During this work, we have tried to evaluate the fir tree of Numidia by demonstrating the importance, yield, chemical composition and antibacterial activity of its essential oil.

The chemical analyses showed the wealth of our essential oil. Selected drugs proved their efficiency against the resistant and sensitive bacteria, what gives rise to possible uses as preventing agent against germs. It seems that the traditional use as antiseptics of this species aiming at curing certain infections is validated.

Perspectives

We think that it could be interesting to complete this study by treating the bark powder of the fir tree of Numidia. Would be also interesting to evaluate the other conifer species of the Algerian forests (i.e. the Atlas cedar). In order to widen the microbiological study, a

research on the possible antimicrobial activity of micro-organisms such as bacteria (*Bacillus subtilis*, *Micrococcus luteus*) or mushrooms (*Penicillium* and *Aspergillus*) contaminating the foodstuffs can be started, without forgetting the evaluation of a synergy between the studied drugs and antibiotics.

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