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Phytochemistry, antimicrobial activities of the essential oils of the branches of *Juniperus phoenicea* in Bechar (Algeria)

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Abstract – The branches of *Juniperus phoenicea* harvested in the Bechar region, contain (2.004%) of essential oils extracted by steam distillation, they show a density of (0.91) and an optical rotation of (+19.74°). Phytochemical tests reveal the presence of chemical compounds, including coumarins, reducing compounds, sterols and steroids, saponins, starch, tannins, flavonoids, anthocyanins and anthracénosides. The activity of the essential oils of *Juniperus phoenicea* a is tested on seven bacterial strains: *Staphylococcus aureus, Enterococcus faecalis, Listeria monocytogenes, Pseudomonas aeruginosa (*yellow*), Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa (*green*) and two fungal strains: Aspergillus flavus* and *Aspergillus ochraceus*. Allowed to claim that it has an inhibitory power vis-à-vis all microorganisms tested despite their Gram, mold proved to be more vulnerable than bacteria. Moreover, the minimum inhibitory concentration shows a value of (8µl / ml) for mold and (22.2µl / ml) for the bacteria.

Keywords: Antimicrobial activity, essential oil, *Juniperus phoenicea*, Arid Zone.

1. Introduction

The essential oils of plants have found their place in aromatherapy, pharmacy, perfumery, cosmetics and in the conservation of food. Their use is linked to their broad spectra of recognized biological activities (Lamiri *et al.*, 2001; Cimanga *et al.*, 2002).

The region of Bechar is located in the south-west of Algeria. It is located at an altitude of 769 m and belongs to the pre-Saharan climatic zone with two main seasons (summer and winter).

Figure 1. Plant *Juniperus phoenicea* L. of the region of Bechar. A: aerial part; B: branches (berries and leaves)

With a strong sunshine, exceeding 3500h / year, and an intense direct solar radiation which can reach 800 W / m² on a horizontal plane. In summer the temperature easily exceeds 50 ° C in the shade, and the relative humidity remains

low around 27%. Moreover, in winter the outside temperature can drop to -5 ° C at night with rare and irregular rainfall of the order of 60 mm / year. In addition to this latter, during the half-seasons, there are violent sand winds that can reach 100 km / h (Mokhtari *et al.*, 2008; Ozenda, 2004).

Dioecious plant is rarely monoecious Shrub 2 to 5 m, with divided and cylindrical branches. Persistent leaves, numerous, in the form of very small scales, ovals losangic, tight, imbricated and applied one on top of another, regularly dispersed and form a body with the twig, rounded at the base, pointed at the top, bulging and marked with a rib, dry and rough, supported green, sometimes bluish. Flowers, Male and female cone usually on the same foot but also on separate feet, March to April (Varlet, 2008).

This species is widely used in traditional medicine: leaves are used as a decoction to treat diabetes, diarrhea and rheumatism, while dried and powdered fruit can heal skin ulcers and abscesses (Akrout, 2004).

The methanoic extract of the leaf of *Juniperus phoenicea* L. shows a remarkable effect in improving the functions of the liver and queens and can therefore be a therapeutic potential in hepatotoxicity treatment and nephrotoxicity (Ahmed *et al*., 2010).

In order to contribute to a better valorization of our plant resource *Juniperus phoenicea*, harvested at the Wilaya of Béchar, this work addresses the antibacterial and antifungal efficacy of their essential oil.

2. Materials and methods

2.1 Plant material

Based on certain connoisseurs of medicinal plants in the Bechar region and the Sahara flora of Ozenda (2004) the species studied have been identified. The plant was harvested in full bloom in June 2010 at djebel Bou-Grone located about (60km) north of the town of Béchar. The parts used are recovered in clean bags. The plants, freshly harvested, are washed and allowed to dry in the shade in a dry and ventilated place. The parts used are recovered in clean bags. Noting that prior to washing, a certain amount of plants is recovered to measure their moisture levels. The process of extracting the essential oils used in our work is hydrodistillation, for which it has been introduced 100g of dry plant in a balloon, filled with distilled water: the whole is brought to boil for 3h to 3h: 30. The recovered oil is stored at 4 ° C. in the dark in the presence of anhydrous sodium sulphate. The yield of essential oil is the ratio between the weight of the extracted oil and the weight of the plant to be treated (Caree ,1953).

3. Phytochemical tests

phytochemical screening of the *J. phoenicea* was performed using the methods described by Lazouni (2007) and Raaman (2006), detection of steroids, alkaloids, flavonoids, saponins and tannis.

4. Physicochemical analysis of the essential oil of *Juniperus phoenicea* L.:

These analyzes are made according to the AFNOR standards (1982). the physical and chemical characteristics have been determined including density, refractive index, rotatory power, acid number, ester index, iodine value and saponification index.

5. Antimicrobial activity

The essential oil was tested for antibacterial and antifungal activities on seven bacterial strains: *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Listeria monocytogenes* (ATCC 19115), *Bacillus*

cereus (ATCC 11778), *Escherichia coli*, *Pseudomonas aeruginosa* (yellow, green) (ATCC 27853) and two molds isolated from wheat: *Aspergillus flavus* and *Aspergillus ochraceus*, have been implicated in various pathologies and often responsible for infections in hospitals (nosocomial infections) (Skurnik, 2008).

6. Preparation of inoculas

For bacteria, the method of preparation of inoculas is that recommended by SFM (Rahal, 2005), which consists in preparing, from a culture of 18 to 24 h of the bacterium studied on agar medium, a suspension equivalent to the McFarland standard 0.5 (≈ 10^8 CFU / ml) (Rahal, 2005; Amhis & *al.*, 2000).

For fungi, a 1 cm^2 mycelial disc from a 7-day culture poured into a 0.2% agar solution, this sporular suspension is about 10^5 conidia / ml measured with a cell of Malassez, is inoculated according to the single spore technique (Choi *et al.*, 1999) on acidified PDA agar media.

7. Antibacterial activity by direct contact method

Suitable amounts of the essential oil were added to the still liquid Mueller-Hinton culture medium to obtain different concentrations (v / v) (Fandohan & *al.*, 2004) : 1/270, 1/135, 1/27, 1/45, 1/10. Boxes without essential oils as a control are also prepared, the incubation is done at 37 ° C. for 24 h.

8. Antibacterial activity by diffusion from disk

The agar is seeded with an inoculum 10^8 CFU / ml. Filter paper discs (6 mm in diameter) were impregnated with the two essential oils and distributed over the agar. After incubation at 37 ± 1 ° C for 24 h, the diameters of the inhibition zones were measured in mm. All experiments were carried out in three replicates (Duraffourd, 2002).

9. Antifungal activity by the radial growth method

The antifungal activity of essential oil was tested by the method of radial growth on a solid medium consisting in mixing different volumes of essential oil to be tested with a volume of acidified PDA medium in order to obtain the following concentrations (v/v): 1/5000, 1/1000, 1/125, 1/100, 1/30, 1/20 (Hibar & *al.*, 2006), a drop of the prepared slurry is deposited in the center of each box. Incubation is at a temperature of 25 ± 2 ° C for 7 days. Measurements of the diameters of the radial growth of the mycelial carpet are taken from the third day of incubation to the seventh day; before the mycelial filaments reach the peripheral of the control boxes (Rahal, 2005). According to the formula below, a percentage of growth inhibition (T) is evaluated with respect to mycelial growth in the control boxes (Singh & *al.*, 2005):

$$T = \frac{Db - Da}{Db} \times 100$$

Db: diameter of control mycelial colony, in centimeter

Da: diameter of the mycelial colony in the experiment

T: percentage inhibition of mycelial growth.

10. Determination of the minimum inhibitory concentration (MIC)

The minimum inhibitory concentration was expressed in $\mu l.ml^{-1}$. It corresponds to zero growth for the duration of incubation adapted to each species (Koba, 2004) .

11. Results and discussion

The extraction of the essential oil of *Juniperus phoenicea* by the technique of hydrodistillation, allowed us to have a higher yield (2.004%) compared to that found in the work of Bouzuita & *al.* (2008) in Tunisia and those of MAZARI & *al.* (2010) (0.50%) and (0. 52%). Several factors influence the variation in yield, in particular, the difference in the mode of extraction with respect to the device used, the temperature and the duration as well as the origin of the plants studied, and the harvest period (Rohloff, 2003).

The physicochemical aspects of the essential oil of *Juniperus phoenicea* show a density of 0.91, a rotatory power (1/100) + 19.74 °, a refractive index R of 1.44, an acidity index A 1.44 mg KOH / ml, ester E of 15.75 and SI of 22.81. The essential oil of the twigs of *Juniperus phoenicea* has a lower density than water and their rotatory powers are dextrorotatory.

The phytochimique test revealed the presence of the chemicals compounds in particular the reducing coumarins compounds, sterols and steroids, the saponosides, the starch, the tannins, the flavonoïdes, the anthracénosides and the anthocyanosides (**Table 1**).

12. Antibacterial activity by direct contact method and determination of the minimum inhibitory concentration (MIC)

The results shown in **Table 2** indicates an antibacterial effect on all the strains from the minimum inhibitory concentration of 22.2 µl / ml, with the exception of *Escherichia coli* and *Pseudomonas aeruginosa* (green).

BOUZUITA (2008) confirmed the inhibitory potency of *J. phoenicea* essential oil on other bactéria namely: *Klebsiella oxytoca, Lactobacillus plantarum, Saccharomyces cereviseae* and *Geotrichum candidum.*

Table 1. Phytochemical tests results of twigs of *Juniperus phoenicea*

Phytochemical tests	Results
Alkaloid salts	+
Coumarins	++
Antracenosides	++
Anthocyanosides	-
Steroids	+++
Flavonoids	+++
Starch	+
Saponosides	++
Tannins	-

12. Antibacterial activity by diffusion from disk

Antibacterial activity represented by the diameter of the zone of inhibition reveals differences in sensitivity between strains (**Figure 5**). It is superior with the highest areas of inhibition (14.1), (13.6), (13.5) and (11.1 mm), of which they are observed with P. *aeruginosa* (yellow), *P. aeruginosa* (green) *L. monocytogenes* and *B. cereus*, compared with those (8.8) and (7.8) observed at *E. faecalis* and *S. aureus* successively. On the other hand, no zone of inhibition was observed around disk for *E. coli.*

13. Antifungal activity by the radial growth method

The results obtained during a follow-up of the mycelial radial growth during 7 to 10 days of incubation are presented in figure 3 and 4, note that (T) represents the inhibition rate calculated from the mean diameter of the radial growth .

Table 2. Results of antimicrobial activity according to the direct contact method

Concentration EO µl/ml / Bacteria testeds	3,7	7,4	22,2	37	90	100
Staphylococcus aureus	+	+	-	-	-	-
Entérococcus faecalis	+	+	-	-	-	-
Listeria monocytogenes	+	+	-	-	-	-
*Pseudomonas aeriginosa (*yellow*)*	+	+	-	-	-	-
Bacillus cereus	+	+	-	-	-	-
Escherichia coli	+	+	+	+	+	+
Pseudomonas aeriginosa (green)	+	+	+	+	+	+

(+) presence of growth; (-) absence of growth.

Figure (3) and **(4)** show a significant slowdown which appears at concentrations (1 µl / ml) and (8 µl / ml), which correspond to a T (inhibition rate) of (18.78%) and (43.51% to (90.92%) and (63.09%) at the highest concentration (50 µL / ml) respectively as against *Aspergillus flavus* and *Aspergillus ochraceus* **(Figure 6, Figure 7).**

Bouzuita (2008) also revealed that the essential oil of *J. phoenicea* possesses an inhibitory effect on molds notably *Saccharomyces cereviseae and Geotrichum candidum.*

14. Conclusion

The evaluation of the antimicrobial effect of the essential oils of the J*uniperus phoenicea* branches made it possible to claim that it has an inhibitory power against all the microorganisms tested in spite of their Gram. Molds have proven to be more vulnerable than bacteria. Our study will have a flavor of incomplete ? if we do not carry out a chromatographic analysis. The valorisation of these plants, which is the subject of our study, will be a source of value creation. It will have a visible impact on the generation of employment and local development. It is a long-term task, but it is within our grasp, as long as we diversify the panel of scientific disciplines that have to separate their efforts to enhance the properties of these plants.

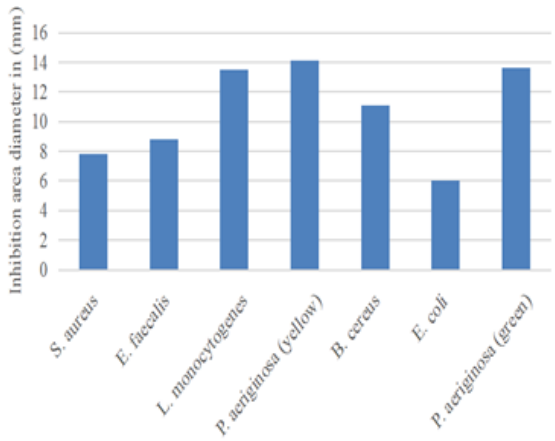

Figure 2. Antibacterial activity by diffusion from disk of Essential oil Juniperus phoenicea L.

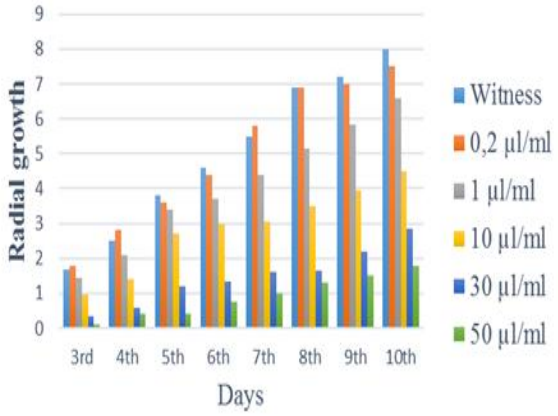

Figure 3. Rate of inhibition of the growth of Aspergillus ochraceus as a function of different concentrations of essential oil of J. phoenicea.

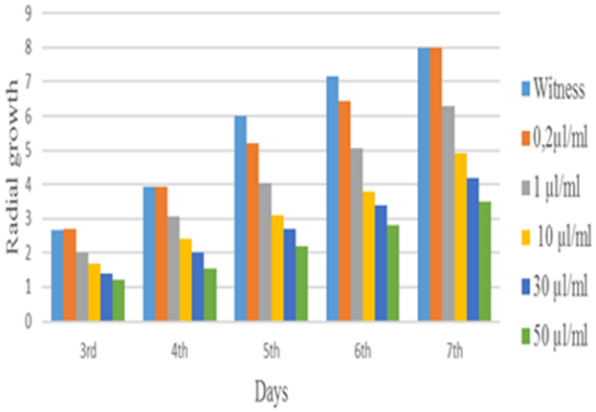

Figure 4. Rate of inhibition of the growth of Aspergillus flavus as a function of different concentrations of essential oil of J. phoenicea

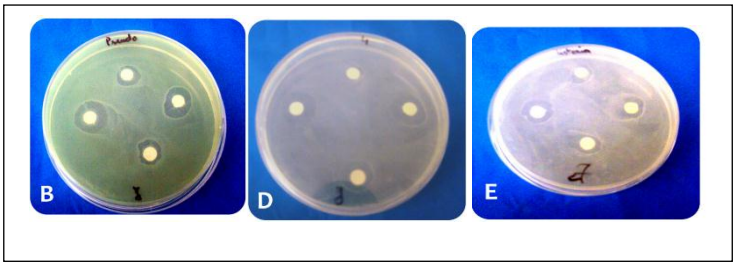

Figure 5. Results of the antibacterial activity of the *juniperus phoenicea* L. urea oil opposite; B: *P. aeruginosa* (green); D: *P. aeruginosa* (yellow) and E: *L. monocytogenes.*

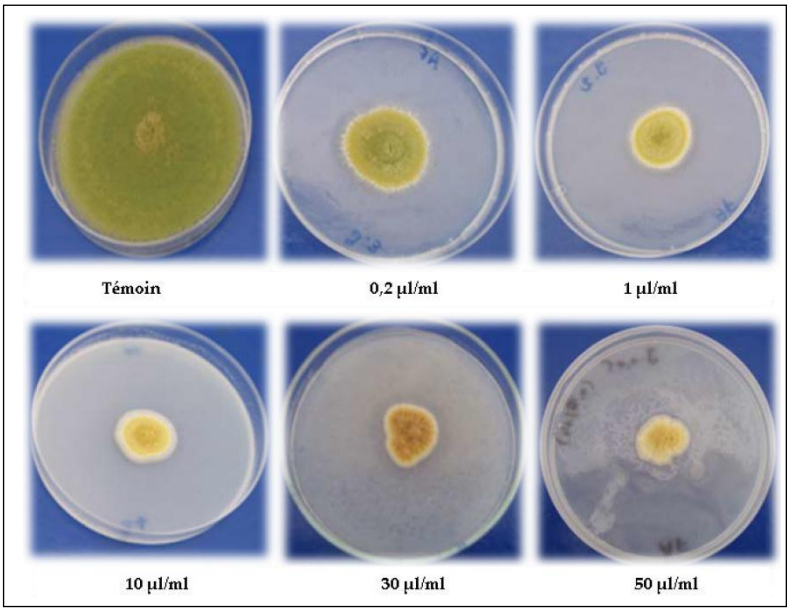

Figure 6. Specimens of the radial growth of the *Aspergillus ochraceus* strain due to the different concentrations of *J. phoenicea* oil.

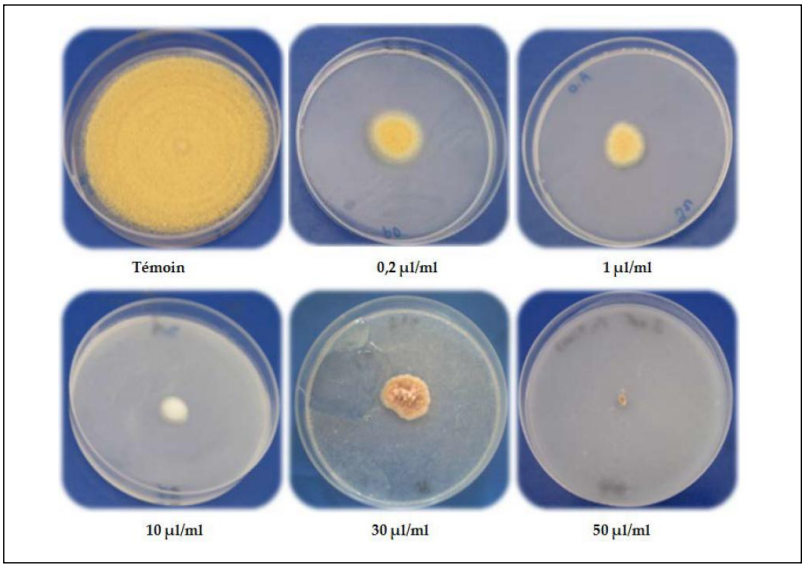

Figure 7. Specimens of the radial growth of the *Aspergillus flavus* strain due to the different concentrations of *J. phoenicea* oil.

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