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## **Evaluation *in vitro* antimicrobial and antioxidant abilities of aerial part extracts of *Rhanterium adpressum* Cossion & Curieu (Algerian and Moroccan endemic plant)**

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### **Abstract**

Evaluation of *in vitro* antibacterial and antioxidant activities of essential oil and organic extracts (methanolic, chloroformic and hexanic) of the aerial part of an aromatic and medicinal plant harvested in southern Algeria, belonging to the Asteraceae (*Rhanterium adpressum* Cossion & Durieu). The agar diffusion method was used to assess the antimicrobial activity of different extracts. These extracts were tested against eleven (11) microbial strains, ten (10) of them are bacterial species (gram positive and gram negative) and one (01) is yeast species. To evaluate the antioxidant activity, the DPPH free radical catching method was used.

For the antimicrobial activity, all extracts showed inhibitory ability against at least one of the tested microbial strains, the essential oil showed the best antibacterial and antifungal activity followed by the hexanic (In-Hex), the chloroformic (EDCM) and the methanolic (EMeOH) extracts respectively. In fact, nonpolar extracts are the most active. The results of the antioxidant activity showed that the methanolic extract (EMeOH) presented the best antiradical capacity, followed by the chloroformic (EDCM) and the hexanic (In-Hex) extracts and ultimately the essential oil. Indeed, methanol is the best solvent for the concentration of the plant active substances. Extracts of *Rhanterium adpressum* have antioxidant and antimicrobial activities.

**Keywords:** Antibacterial, antioxidant, *Rhanterium adpressum*, Algeria

### **1. Introduction**

The Asteraceae family is the largest of the vegetable world, with about 25000 species distributed in 1300 genus, spread all over the earth [1]. In this family, the genus *Rhanterium* covers seven species distributed worldwide: northwest Africa, Peninsula, Iraq and Iran, among these species *Rhanterium adpressum* Cossion & Durieu, it is endemic in Algeria and Morocco [2]. In Algeria, this species is generally propagated in the Sahara (Ain-Sefra, Mzab, Laghouat, Bousaada and Biskra) [3-5].

*Rhanterium adpressum* Cossion & Durieu known (El Arfedj), it is a multi-stemmed shrub, with small alternate leaves, entire and toothed with monocephalic branches, stands, countless, tight in tufts, heterogamy heads, disbarred, small, yellow flowers; tri-toothed femal ligules; hermaphrodite florets with 5 teeth; overlapping scales and cylindrical channels, narrow, with 4-5 spines [6]. *R. adpressum* is traditionally used with another plant called *Haplophyllum tuberculatum* to treat stomach pain, it used also by the local population in traditional cheese production and as an anti-diuretic [7].

According to our investigations, three studies have been carried out on this species in Algeria. Bouheroum *et al.* (2007) have isolated terpenic compounds from MeOH / H<sub>2</sub>O extract. In 2009, Kala *et al.* have performed a study of the chemical composition of the aerial part essential oil. Hamia *et al.* (2013) have studied the antioxidant effect of the essential oil and fatty acids extracted from the leaves of the plant. In 2014, Boussoussa *et al.* have studies the effect of different solvent polarity (methanol-water) and (acetone-water) on extraction of phenolic compounds from the flowers of the plant and their antimicrobial and antioxidant activities.

The aim of our study is to assess the antimicrobial and antioxidant effects of different extracts (essential oil, methanolic, chloroformic and hexanic extracts) of the aerial part of *Rhanterium adpressum*.

## 2. Materials and methods

### 2.1. Preparation of crude extracts

The aerial parts of the plant have been collected from the Biskra province, during the flowering period (May, 2013), they were then dried in open air, at room temperature for 15 days and then crushed into a fine powder which used for the preparation of various extracts.

The following solvents were used for the extraction: methanol, chloroform and n-hexane. 20g of this dry powder were first putted in 200 ml of each solvent in a recipient, which is covered later by aluminum foil, to preserve the secondary metabolites present in the extracts against photo-oxidation. The maceration was carried out under mechanic agitation for 72 hours, at room temperature, the macerates were then vacuum filtered, the obtained filtrates were then evaporated by a rotary evaporator at temperatures 35, 40 and 50 °C (following the solvent). The resulting dry extracts were dissolved in the DMSO for antimicrobial activity and ethanol for antioxidant activity.

The essential oil extraction by steam distillation was carried out using a Clevenger apparatus, 100 g of the vegetal material powder are completely immersed with distilled water in a 2L flask, then heated to boiling for 3 hours, the recovered essential oil was dried using  $K_2CO_3$  and stored in the refrigerator at 4 °C, until the establishing antimicrobial and antioxidants tests.

### 2.2. Tests of the biological activities of the extracts

#### 2.2.1. Antimicrobial Activity

##### Microbial strains

The germs that used in the experiences are ATCC strains and clinical isolates: *Staphylococcus aureus* (ATCC 43300), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus aureus*, *Streptococcus* group D, *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae*, *Salmonella typhi*, *Citrobacter freundii*, *Enterobacter* sp, *Candida albicans*.

##### Test of antimicrobial activity

The agar diffusion method was used to evaluate the antimicrobial capacity of the extracts.

The essential oil and the organic extracts were first diluted in DMSO to obtain the following concentrations: 8mg / ml, 4 mg / ml, 2mg / ml, 1mg / ml, 0.5mg / ml and 0.25mg / ml. The petri dishes containing Muller Hinton agar were inoculated using swabbing technique by the different microorganism suspensions with a density of 106 CFU and dried for 15min. Wattman paper discs of 6 mm diameter were deposited on the inoculated MH agar, using sterile pincers, then impregnated with 10 $\mu$ l of the tested extracts in different concentrations. The dishes were then leaved to diffuse the extracts for 30 minutes, at room temperature and finally incubated at 37 °C for 24 hours for bacteria and 30°C or 37°C for 48 hours for yeast. Antimicrobial activity was determined by measuring the diameters of inhibition zones.

#### 2.2.2. Antioxidant Activity

##### DPPH test

The antioxidant activity was carried out using the DPPH catching method with some modifications [8]. This free radical (2, 2'-Diphenylpicrylhydrazyl) has a dark purple color, in reduced state, the color becomes pale yellow.

The extracts samples were dissolved in ethanol (EtOH) to obtain the following concentrations ( $1M$ ,  $10^{-1}M$ ,  $10^{-2}M$ ,  $10^{-3}M$ ,  $10^{-4}M$  and  $10^{-5}M$ ). The test consists to add 1 $\mu$ l of the tested extract at different concentrations on 1 ml of the DPPH

solution obtained by dissolving 4 mg of the DPPH powder in 100 ml of ethanol. After stirring using a vortex, the tubes were placed in the darkness at room temperature for 5 min. The results reading was performed by measuring the absorbance at 517 nm using a UV / VIS spectrophotometer, against a whit containing pure ethanol. The positive control was represented by the vitamin C, the absorbance was measured in the same conditions as the samples.

The results were expressed using the following formula:  
 $I\% = (\text{Abs test} - \text{Abs White}) / 100$

## 3. Results and discussion

### 3.1. Antioxidant Activity

The results, expressed as a percentage of the anti-radical activity (Fig. 1), showed that the methanolic extract (EMeOH) of *R. adpressum* submitted the best radical scavenging capacity of about (80.91%); followed by the chloroformic extract (EDCM) in the range of (45.93%) and the hexanic extract (In-Hex) with (40.54%) and finally the essential oil with (3.97%). This allowed us to classify our extracts following their antiradical activity as follows: EMeOH> EDCM> In-Hex> EO. This difference in antiradical activity may be caused by the difference on chemical composition of these extracts.

The high antioxidant power of EMeOH of *R. adpressum* can be explained by the presence of different constituents, in particular flavonoids and other polyphenolic substances known for their antioxidant activity. According to Fellah *et al.* (2008) for a higher recovery of polyphenols, methanol is the suitable solvent [9]. Many studies have highlighted the great antioxidant power of methanolic extracts [10, 11].

Some authors have worked on the leaves of *R. adpressum*, they found that the lipidic (hexanic) extract has activity against DPPH free radicals three times over than that of the essential oil. This is related to the phenolic content which is recorded for the lipidic extract with a value ten times higher than that of the essential oil [12].

The extracts carried out using organic solvents contain several families of compounds (flavonoids, tannins, saponins, pigments, terpenes, etc.), while the essential oil consists only of terpene molecules, which have a weak antioxidant activity [13].

We also noted that the solvent polarity has an effect on the antioxidant capacity of the extract, polar extracts showed a high antioxidant activity, compared with no polar extracts.

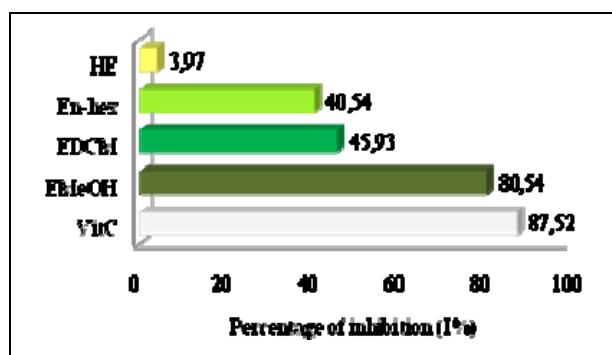


Fig 1: Relative antioxidant activity of *R. adpressum* and Vit C extracts.

### 3.2. Antimicrobial Activity

The various extracts showed a significant *in vitro* antibacterial activity against a series of pathogenic human strains. The results are summarized in Table 1.

**Table 1:** Effect of different *R. adpressum* extracts on the growth of the tested strains.

|        | <i>Strains</i> | <i>E.c</i><br>ATCC 25922 | <i>P.a</i><br>ATCC 27853 | <i>K.p</i> | <i>S.t</i> | <i>C.f</i> | <i>E.sp</i> | <i>S.a</i><br>ATCC 43300 | <i>S.a</i><br>ATCC 25923 | <i>S.a</i> | <i>S.g D</i> | <i>C.a</i> |
|--------|----------------|--------------------------|--------------------------|------------|------------|------------|-------------|--------------------------|--------------------------|------------|--------------|------------|
| HE     | 8              | 12,00                    | -                        | -          | 9,33       | 16,66      | -           | 14,00                    | 12,66                    | 13,33      | 12,00        | 19,00      |
|        | 4              | 10,66                    | -                        | -          | 8,33       | -          | -           | 13,33                    | 12,33                    | 12,00      | 10,00        | 18,00      |
|        | 2              | 10,50                    | -                        | -          | -          | -          | -           | 12,33                    | -                        | 10,33      | -            | 13,00      |
|        | 1              | 10,33                    | -                        | -          | -          | -          | -           | 7,66                     | -                        | -          | -            | 12,66      |
|        | 0,50           | 10,33                    | -                        | -          | -          | -          | -           | 7,00                     | -                        | -          | -            | 10,00      |
|        | 0,25           | 10,33                    | -                        | -          | -          | -          | -           | 7,00                     | -                        | -          | -            | 10,00      |
|        | 8              | 10,00                    | -                        | 12,66      | -          | -          | -           | -                        | -                        | -          | -            | 11,66      |
|        | 4              | 9,33                     | -                        | 9,66       | -          | -          | -           | -                        | -                        | -          | -            | 10,33      |
|        | 2              | 9,00                     | -                        | 9,00       | -          | -          | -           | -                        | -                        | -          | -            | 9,66       |
|        | 1              | -                        | -                        | -          | -          | -          | -           | -                        | -                        | -          | -            | 9,33       |
|        | 0,50           | -                        | -                        | -          | -          | -          | -           | -                        | -                        | -          | -            | 9,00       |
|        | 0,25           | -                        | -                        | -          | -          | -          | -           | -                        | -                        | -          | -            | 9,00       |
| EMeOH  | 8              | -                        | -                        | 11,66      | -          | -          | -           | -                        | -                        | -          | -            | 13,66      |
|        | 4              | -                        | -                        | 10,00      | -          | -          | -           | -                        | -                        | -          | -            | 10,66      |
|        | 2              | -                        | -                        | 9,66       | -          | -          | -           | -                        | -                        | -          | -            | 10,66      |
|        | 1              | -                        | -                        | 8,66       | -          | -          | -           | -                        | -                        | -          | -            | 10,66      |
|        | 0,50           | -                        | -                        | 8,33       | -          | -          | -           | -                        | -                        | -          | -            | 10,33      |
|        | 0,25           | -                        | -                        | 8,00       | -          | -          | -           | -                        | -                        | -          | -            | 9,66       |
|        | 8              | 10,00                    | -                        | 11,66      | -          | 12,33      | -           | -                        | -                        | -          | -            | 13,66      |
|        | 4              | 9,00                     | -                        | 10,66      | -          | 11,00      | -           | -                        | -                        | -          | -            | 12,33      |
|        | 2              | 8,83                     | -                        | 10,00      | -          | 9,00       | -           | -                        | -                        | -          | -            | 10,66      |
|        | 1              | 8,33                     | -                        | 9,66       | -          | 8,33       | -           | -                        | -                        | -          | -            | 10,66      |
|        | 0,50           | 8,16                     | -                        | 8,33       | -          | 7,33       | -           | -                        | -                        | -          | -            | 10,33      |
|        | 0,25           | 7,66                     | -                        | 7,33       | -          | 7,00       | -           | -                        | -                        | -          | -            | 9,00       |
| EDCM   | 8              | -                        | -                        | 11,66      | -          | -          | -           | -                        | -                        | -          | -            | 13,66      |
|        | 4              | -                        | -                        | 10,00      | -          | -          | -           | -                        | -                        | -          | -            | 10,66      |
|        | 2              | -                        | -                        | 9,66       | -          | -          | -           | -                        | -                        | -          | -            | 10,66      |
|        | 1              | -                        | -                        | 8,66       | -          | -          | -           | -                        | -                        | -          | -            | 10,66      |
|        | 0,50           | -                        | -                        | 8,33       | -          | -          | -           | -                        | -                        | -          | -            | 10,33      |
|        | 0,25           | -                        | -                        | 8,00       | -          | -          | -           | -                        | -                        | -          | -            | 9,66       |
|        | 8              | 10,00                    | -                        | 11,66      | -          | 12,33      | -           | -                        | -                        | -          | -            | 13,66      |
|        | 4              | 9,00                     | -                        | 10,66      | -          | 11,00      | -           | -                        | -                        | -          | -            | 12,33      |
|        | 2              | 8,83                     | -                        | 10,00      | -          | 9,00       | -           | -                        | -                        | -          | -            | 10,66      |
|        | 1              | 8,33                     | -                        | 9,66       | -          | 8,33       | -           | -                        | -                        | -          | -            | 10,66      |
|        | 0,50           | 8,16                     | -                        | 8,33       | -          | 7,33       | -           | -                        | -                        | -          | -            | 10,33      |
|        | 0,25           | 7,66                     | -                        | 7,33       | -          | 7,00       | -           | -                        | -                        | -          | -            | 9,00       |
| En-Hex | 8              | -                        | -                        | 11,66      | -          | -          | -           | -                        | -                        | -          | -            | 13,66      |
|        | 4              | -                        | -                        | 10,66      | -          | -          | -           | -                        | -                        | -          | -            | 10,66      |
|        | 2              | -                        | -                        | 10,00      | -          | -          | -           | -                        | -                        | -          | -            | 10,66      |
|        | 1              | -                        | -                        | 9,66       | -          | -          | -           | -                        | -                        | -          | -            | 10,66      |
|        | 0,50           | -                        | -                        | 8,33       | -          | -          | -           | -                        | -                        | -          | -            | 10,33      |
|        | 0,25           | -                        | -                        | 8,00       | -          | -          | -           | -                        | -                        | -          | -            | 9,00       |

Ec : *Escherichia coli* ; Pa : *Pseudomonas aeruginosa* ; Kp : *Klebsiella pneumoniae* ; St : *Salmonella typhi* ;Cf : *Citrobacter freundii* ; E.sp : *Enterobacter*.sp ; Sa : *Staphylococcus aureus*; Sg D : *Streptococcus* groupeD ; C.a : *Candida albicans* ; (-) : absence of inhibition.

The essential oil of *R. adpressum* showed an antibacterial activity against *E. coli*, *Salmonella*, *Citrobacter*, both ATCC and clinical *Staphylococcus* and *Streptococcus* D, however it had no effect on the growth of *Klebsiella*, *Pseudomonas* and *Enterobacter*. From these results we can note that the essential oil has an antibacterial effect on gram-positive bacteria more than gram negative bacteria. This selective effect of essential oil has been observed by several authors [14,15,16,17,18]. The organic extracts (EMeOH, DCM and n-Hex) showed an antibacterial action only on certain Gram-negative bacteria (*E. coli*, *Klebsiella pneumoniae* and *Citrobacter freundii*), the highest activity was recorded with EMeOH extract against *Klebsiella pneumoniae*, with an inhibition zone diameter of 12, 66 mm at a dose of 8mg / ml.

The results of the antifungal activity have revealed the efficiency of all samples against the *Candida albicans*, the largest inhibition zone was observed with the essential oil (19mm), followed by the In-Hex and EDCM (13,66mm) and at least EMeOH (11,66mm) with dose of 8mg / ml. The yeast was inhibited with the lowest concentration of 0.25 mg / ml of all samples, while the bacteria are not inhibited by the same concentration of the extracts. For the essential oil, *E. coli* and *S. aureus* ATCC 43300 were inhibited with 0.25 mg / ml, *S. aureus* was inhibited with 2mg / ml, *S. typhi*, *S. aureus* ATCC 25923 and *Streptococcus* D were inhibited with 4mg / mL, and *C. freundii* was inhibited with 8mg / ml.

In general, all the extracts had a dose dependent antimicrobial activity. The highest activity was still obtained with a concentration of 8 mg / ml, but this activity is linked to the qualitative and quantitative diversity of compounds, present in the extracts. The dominance of oxygenates may explain the antimicrobial potency of the essential oil of this plant.

#### 4. Conclusion

Extracts of *Rhanterium adpressum* have antioxidant and antimicrobial activities. It would be interesting to reveal the

nature of the active substances that contribute to these activities.

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