# EFFECTS OF EXTRACTION METHODS ON ANTIBACTERIAL ACTIVITY OF DIFFERENT EXTRACTS OF CALLIGONUM COMOSUM L’HER. GROWING IN SAHARA ALGERIAN

**Chouikh A,1,2*, Mekki M1 and Adjal, EH1**

1Department of Biology, Faculty of Natural Science and Life, University of El Oued, BP 789 El-Oued (39000) Algeria

2Laboratory Development and Control of Hospital Pharmaceutical Preparations. Medical Science Faculty, University of Badji Mokhtar- Annaba, LP 205 Ezzafrania, Annaba (23000) Algeria

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**ABSTRACT**

The aims of this study are evaluated the in-vitro antibacterial activity of the crude ethanolic (EOH) and methanolic (MOH) extracts of *Calligonum comosum* L’her. extracted by Maceration (MM) and Ultra-sound (UM) methods on three strains bacteria’s: Two Gram negative (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 ) and One Gram positive (*Staphylococcus aureus* ATCC 25923).

The activity of different extracts of *C. comosum* reveals a high sensitivity of the tested germs except for *E. coli* especially with: EOH UM and MOH MM, also showed very expressive inhibition diameters (44 - 28 mm) with three antibiotics tested (AMC30, AK30, AM10), and showed medium resistance with different concentrations of the EOH MM and MOH UM.

*P. aeruginosa* is very sensitive to high concentrations of the antibiotic AK30 (37mm) and MOH UM (9.5 mm with concentration 0.5mg/ml and 14 mm with concentration 2mg/ml). As against this strain is resisted the two antibiotics AM10, AMC30 and the different concentration of MOH MM this is probably due to the possibility of the absent chemicals compounds in this extract.

The most important antibacterial effect is observed with *S. aureus* to all concentrations of Four extracts and Three antibiotics tested.

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**INTRODUCTION**

*Calligonum comosum* L’her. (Polygonaceae), is a small leafless shrub, under the local names “ghardaq”, “rusah” or “arta” (Muschler, 1912) a shrub distributed throughout Arabia and growing in sandy deserts (Halis, 2007), is used by the local healers to treat stomach ailments (Liu et al., 2001); has reputation in folklore medicine as a stimulant and astringent “ (Muschler, 1912), the stems and leaves are chewed for curing toothache (Liu et al., 2001). Dehydrodicatechin A compound isolated from this plant showed the best cytotoxic and antioxidant activity. (Badria et al., 2007). Besides, anthraquinones of *Calligonum* showed high antimicrobial potential (Zaki et al., 1984); Chemical analysis showed that anthraquinones and flavonoids are the common chemical constituents in this plant (Ghazanfar, 1994; Kamil et al., 2000).

The ethanolic extract of *Calligonum comosum* treat infected animals with fasciolosis and improved their health condition (Degheidy et al., 2013). The aims of this study are evaluated the in-vitro antibacterial activity of the crude ethanolic and methanolic extracts of *Calligonum comosum* extracted by maceration and ultra-sound methods on two Gram negative bacteria (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 ) and one Gram positive bacteria (*Staphylococcus aureus* ATCC 25923).

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**MATERIALS AND METHODS**

**Plant material**

*Calligonum comosum* L’her. was assembled in October 2014 in Oued Souf region (East South Algerian). The plant is then dried, crushed and stored in glass flasks to protect from light and moisture.

**Preparation of extracts**

We prepared the extracts in Methanolic and Ethanolic in two extraction methods:

**Maceration**

50g of plant material with 500 ml of solvents for 24 h, after filtration, the macerated are evaporated to dryness in Rota-

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*Corresponding author: Chouikh A*

Development of Biology, Faculty of Natural Science and Life, University of El Oued, BP 789 El-Oued (39000) Algeria
Extraction with ultra-sound

According (Khosravi et al., 2013) with slight modification, 200 ml of solvents is added to 20 g of plant material then take mixtures to ultra-sound Type JP SELECTA (3.1A; 720 W) under the conditions: 30°C for 30 min the extracts are evaporated in Rota-vapor.

Source of pathogens and cultures medium

Three pathogenic bacteria: two Gram negative bacteria (Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 ) and one Gram positive bacteria (Staphylococcus aureus ATCC 25923). Were provided from the Pasteur Institute, Algiers. Nutrient agar Mueller Hinton was used as a growth medium for investigated microorganisms in this study.

Antibacterial activity

The antibacterial activity of extracts and antibiotics (AMC<sub>30</sub>; AMOXYCLAV 30µg/disk, AK<sub>30</sub>; AMIKACIN 30µg/disk and AM<sub>10</sub>; AMPICILLIN 10 µg/disk) was determined by agar diffusion method cited by (Treki et al., 2009). Sterile disks (6 mm diameter) impregnated with 10 µl of different concentrations of extract (C<sub>1</sub>; 0.5mg/ml; C<sub>2</sub>; 1mg/ml and C<sub>3</sub>; 2mg/ml this concentrations were easing by DMSO) are then deposited on the agar surface previously seeded with bacterial suspension (10<sup>6</sup> CFU/ml) in exponential growth phase. The Petri dishes were incubated at 37° C for 24 h. The inhibition of microbial growth and Minimum inhibitory concentration (MIC) is determined by measuring the inhibition diameter (mm) of each bacterium (Andrews, 2001).

RESULTS

The results of the anti bacterial activity of extract Calligonum comosum L’her. are summarized in Graph 1.

The comparison of the activities of different extracts of C. comosum reveals a high sensitivity of the tested germs except for E. coli especially with: EOH UM and MOH MM, also showed very expressive inhibition diameters (44 - 28 mm) with three antibiotics tested (AMC<sub>30</sub>, AK<sub>30</sub> and AM<sub>10</sub>); also which showed medium resistance with different concentrations of the extract EOH MM and MOH UM.

P. aeruginosa germ is very sensitive to high concentrations of the antibiotic AK<sub>10</sub> (37mm) and MOH UM (9.5 mm with concentration 0.5mg/ml, 14 mm with concentration 2mg/ml. As against this strain is resisted the two antibiotics tested AM<sub>30</sub>, AMC<sub>30</sub> and the different concentration of MOH MM. Clearly not noticed effect of this strain in MOH MM, this is probably due to the possibility of the absent chemical compounds in this extract.

The most important antibacterial effect is observed with S. aureus to all concentrations of Four extracts and Three antibiotics tested.

Our results are consistent with those obtained by (Alkhalilifah 2013) who were able to show that ethanolic extracted from the same plant in their countries (SAUDI ARABIA) have good antimicrobial activity against gram-positive bacteria (S. aureus with inhibition zone 13 mm), and two strains gram negative (E. coli and P. aeruginosa with inhibitions zones 17.5 and 20.5 mm respectively).

With regard to the minimal inhibitory concentration (MIC) (Table 1) of plant extracts against the bacterial strains it was fixed with all strains (≤ 0.5 mg/ml). Except for the extract methanol extracted by maceration ( 2mg/ml).

Ours values of (MIC) are best of the values obtained by (Alkhalilifah 2013) that the MIC of leaves extract was between 3.13 and 6.5 mg/ml, while that of stem extract was 3.13 mg/ml and that for the root extract was in the range between 6.5 and 12.5 mg/ml.

Table 1 Minimal inhibitory concentration (MIC) in mg /ml of the different extracts of Calligonum comosum L’her. . plant against Strains bacteria’s tested.

<table>
<thead>
<tr>
<th>Strains bacteria’s</th>
<th>Escherichia coli ATTC 25922</th>
<th>Pseudomonas aerogenosa ATCC 27853</th>
<th>Staphylococcus aureus ATTC 25923</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOH MM</td>
<td>≤0.5</td>
<td>≤0.5</td>
<td>≤0.5</td>
</tr>
<tr>
<td>EOH MM</td>
<td>≤0.5</td>
<td>≤0.5</td>
<td>≤0.5</td>
</tr>
<tr>
<td>MOH UM</td>
<td>≤0.5</td>
<td>≤0.5</td>
<td>≤0.5</td>
</tr>
<tr>
<td>EOH UM</td>
<td>≤0.5</td>
<td>≤0.5</td>
<td>≤0.5</td>
</tr>
</tbody>
</table>

DISCUSSION

This results of present investigation clearly indicated that the antibacterial activity vary with methods extraction and the solvents used.

The observed differences in sensitivity the different concentration of extracts between Gram-positive and Gram-negative bacteria can probably be attributed to the structural and compositional variations in the nature of the cell wall between the two groups (Lambert, 2002).
This inhibitory effect of *Calligonum comosum* extracts might between: Ghazanfar (1994) and Kamil *et al* (2000) be due to the action of special organic compounds such as anthroquinones and flavonoids as reported in previous studies for the chemical composition of *Calligonum comosum* L’her.

**References**


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