



RESEARCH ARTICLE

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

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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). dehydrodicatichin 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).

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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vapor Type Buchi R-200 at 55 ° C with methanol and 60°C with ethanol ([Rebaya et al., 2015](#))

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

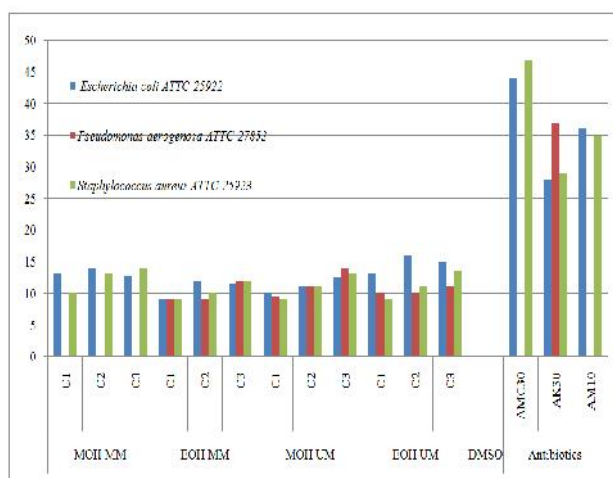
Three pathogenic bacteria: two Gram negative bacteria (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) and one Gram positive bacteria (*Staphylococcus aureus* ATCC 25923). Were provided from the Pasteur Institute, Algiers. Nutrient agar Mueller Hinton was used as a growth medium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

Antibacterial activity

The antibacterial activity of extracts and antibiotics (AMC₃₀: AMOXYCLAV 30µg/disk, AK₃₀: AMIKACIN 30µg/disk and AM₁₀: 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 (C₁: 0.5mg/ml; C₂: 1mg/ml and C₃: 2mg/ml this concentrations were easing by DMSO) are then deposited on the agar surface previously seeded with bacterial suspension (10⁶ 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.



Graph 1 Zone of inhibition in (mm) of different concentrations of extracts of *Calligonum comosum* L'her.

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 (AMC₃₀, AK₃₀ and AM₁₀); 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 AK₃₀ (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 AM₁₀, AMC₃₀ 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 ([Alkhalifah 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 ([Alkhalifah 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.

Strains bacteria's	<i>Escherichia coli</i> ATCC 25922	<i>Pseudomonas aeruginosa</i> ATCC 27853	<i>Staphylococcus aureus</i> ATCC 25923
MOH MM	0.5	2	0.5
EOH MM	0.5	0.5	0.5
MOH UM	0.5	0.5	0.5
EOH UM	0.5	0.5	0.5

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 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.

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