

Research Letter

Antimicrobial activity of an Algerian medicinal plant: *Carthamus caeruleus* L.

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ABSTRACT: Background: Medicinal plants constitute a source of bioactive substances to treat chronic as well as infectious diseases in many countries of the world. A wide range of natural antimicrobial agents can either inhibit the growth of microorganisms or kill them and represent a good alternative to chemical drugs for treatment of various infectious diseases to face the phenomena of germ resistance. **Methods:** In our study, we have used plants of the genus *Carthamus*, which are widely distributed in the Mediterranean region and have been traditionally used to treat skin diseases and burns. Our experiments were carried on the roots and leaves of a *Carthamus* species growing in Sétif area in the North-East of Algeria, in order to evaluate its antimicrobial activity. **Results:** Our results showed potent activity of root and leaf extracts *in vitro* against Gram-negative and Gram-positive bacteria strains as well as against pathogenic fungus. Variable antimicrobial effects of different extracts have been obtained against the tested microorganisms. For root extracts, high inhibition zone of 25 mm was exhibited on *Candida albicans*, 20 mm against *Staphylococcus aureus* and *Bacillus cereus*, and 15 mm on *Acinetobacter bowie*. Moderate activity of 12 mm and 11 mm was obtained for pathogenic plant fungi (*Aschochyta rabiei* and *Fusarium Var coeruleum*) and lowest activity of 8.3 mm was obtained for *Fusarium oxysporum albidinis*. Leaf extracts were only effective against *Acinetobacter bowie* and *Staphylococcus aureus* with inhibition zones of 18 and 20 mm respectively. The root extracts were more active than the leaf extracts against the tested microorganisms.

INTRODUCTION

Medicinal plants are intensely screened and tested for a wide range of applications including pharmacology, pharmaceutical botany, medical and clinical microbiology, phytopathology and food preservation. The use of herbal medicines has taken on a greater importance during the last years because of their potential antioxidant activity and antimicrobial effects against a wide range of pathogenic micro-organisms. Various parts of vegetables: roots, leaves and flowers, have been used in traditional medicine to treat chronic as well as infectious diseases in many countries of the world. Microbial resistance to currently used antibiotics is considered a public health problem, particularly in developing countries and

many efforts have been made to discover new bioactive compounds as natural antimicrobial agents. Their antimicrobial activity has been attributed to a wide diversity of secondary metabolites such as flavonoids,^[1,2] tannins^[3] and saponins.^[4] Phytochemical screening carried out on aerial parts of *Carthamus lanatus*^[5] and seeds of *Carthamus tinctorius*,^[6] have identified many of their bioactive agents such as fatty acids,^[7] serotonin derivatives,^[8] flavonoids^[9] and hydroxy safflor yellow A.^[10] These molecules possess pharmacological properties such as protection of bone,^[11] effect on lipid metabolism,^[12] anti-oxidation,^[13–16] anti-hypertension,^[17] treatment of heart ailments and rheumatism,^[18,19] and also curing male and female fertility problems.^[20–22] Other studies have reported on anti-inflammatory effect^[21] and anti-tumor activity of *Carthamus*.^[23–25] In dermatology, *Carthamus* spp. have many beneficial effects against skin problems such as erythematic, psoriasis and other dermatitis.^[26] The antimicrobial activity has previously been tested for flower and seed extracts and few reports were investigated on leaf extracts.^[6,26] However, all of these studies tested this extract on *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* only. Thus, our study was focused

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on the antimicrobial activity of root and leaf extracts of *Carthamus* species growing in Sétif area in the North-East of Algeria. Roots of this species are commonly used in traditional medicine by healers and the population of Sétif, because of their therapeutic benefits to treat skin problems and burns.

Carthamus is a biennial plant growing in the Mediterranean area and includes almost all plants of the family Asteraceae. This plant, which is also called false saffron, originates from Egypt and its flowers are used for colouring and flavouring foods. It is cultivated mainly in Algeria, in Corsica, in France, in the south of Italy and in Spain, for its oleaginous seeds. The most common species of this genus found in Algeria are: *Carthamus caeruleus*, *Carthamus daumasianus*, *Carthamus fauvei* and *Carthamus doumerguei*.^[27]

MATERIALS AND METHODS

Plant material

The vegetable material consists of leaves and roots collected in May 2010 from the area of Sétif especially Maouane vilage. The fresh plant parts were cleaned and shade dried at the room temperature during a two week period. Exposure to sunlight was avoided to prevent the loss of active components. The dried plant material was ground into powder using electric blender. Botanical identification of the plant was conducted by Pr. Laouer Hocine “Laboratoire de Valorization des Ressources Naturelles”, University Ferhat Abbas of Sétif Algeria. A Voucher specimen of the plant was deposited in the herbarium of this laboratory.

Preparation of the extracts

Extract of the roots

The extraction was carried out according to a previously established method.^[28] The same procedure was used with methanol and ethanol extractions: 50 g of vegetable powder are mixed with 500 ml of the relevant solvent 70% (7:3) and put under magnetic agitation at 80 °C for 30 minutes. This process prevents the enzymatic hydrolysis.^[29] The mixture was left for maceration during 24 hours and then filtered through muslin cloth and Whatman filter paper N°3. The marc was macerated three times. Finally, all the filtrates were collected and were evaporated to dryness under reduced pressure to 45 °C with a rotary evaporator (BUCHI R215). Each dry residue was weighed and treated with boiling distilled water to dissolve the flavonoids and left to settle in the refrigerator for 24 hours, then filtered through Whatman filter paper N°3. This filtrate (aqueous solution: Aq) was

firstly extracted with ethyl acetate (Fraction Ac), then with n-butanol (Fraction Bu). At the end of this treatment, various fractions were obtained:

- Two organic phases (Ac1 and Bu1) and an aqueous phase (Aq1) from methanol extract.
- Two organic phases (Ac2 and Bu2) and an aqueous phase (Aq2) from ethanol extract.

These fractions were evaporated to dryness under reduced pressure to 45 °C with a rotary evaporator (BUCHI R215) and each residue dissolved in 10 ml of methanol and stored in the refrigerator until used.

The percentage yield of extracts was calculated by following formula as the ratio of the mass of the dried extract to the mass of the ground plant sample.

$$\% \text{ yield} = \frac{\text{Weight of extract obtained}}{\text{Total weight of the sample}} \times 100$$

Extract of leaves

The same procedure of extraction was used for leaves. The extract was obtained by three successive macerations in a mixture methanol/water (8:2). With the leaf extract, we have used only ethyl acetate to remove the phenol compounds. Two fractions were obtained by this treatment: an organic phase (AcF) and an aqueous phase (AqF). Both fractions were evaporated to dryness under reduced pressure to 45 °C with a rotary evaporator (BUCHI R215) and each residue dissolved in 10 ml of methanol to test their antibacterial activity.

Antimicrobial susceptibility test

Bacteria and Fungi strains

The antibacterial tests were carried out using reference strains: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and clinical origins strains: *Acinetobacter bowie*, *Proteus sp*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Candida albicans* obtained from the Laboratory of Bacteriology at Sétif hospital. The other reference strains: *Bacillus cereus* (ATCC10876), *Enterobacter fecalis* (ATCC49452), *Salmonella thyphimurium* (ATCC13311), *Citrobacter freundii* (ATCC8090) and *Klebsiella pneumonia* (ATCC700603) obtained from Laboratory of Natural Substances at the University of Tlemcen, Algeria. Plant pathogenic fungus, *Aschochyta rabiei*, *Fusarium oxysporum albidinis* and *Fusarium Var coeruleum* were obtained from Laboratory of Applied Microbiology at the University Ferhat Abbas of Sétif, Algeria.

Antibacterial activity

Antimicrobial activity *in vitro* was screened by using disc diffusion and micro-dilution methods.^[30,31] For the

antibacterial assay, sterile Muller-Hinton agar plates were inoculated by the method of streak with inoculums of the tested bacterial suspension.

The bacterial suspension from young colonies of 18 to 24 hours was made in sterile distilled water for each strain. The turbidity of this suspension was adjusted to 0.5 Mc Farland (10^8 CFU/ml). Then, sterile paper discs of 6 mm diameter were impregnated with 20 μ l of plant extract. Negative controls were discs impregnated with 20 μ l of methanol. Discs impregnated with methanol plant extract solutions or absolute methanol were left in an oven till total evaporation of methanol. First, the discs were impregnated with 10 μ l and let to dry, then they were impregnated with another 10 μ l and then, the discs were delicately deposited on the surface of the inoculated mediums. Plates were incubated at 37 °C for 24 hours and at 30 °C for 72 hours for bacteria and fungi respectively. Each extract was tested in triplicate. The antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism and the average of the three measurements in all three replicates was calculated. An inhibition zone of 14 mm or more of plant extracts was considered as high antibacterial activity.^[6]

Antifungal susceptibility test

Antifungal activity *in vitro* was tested against pathogenic human fungi (*Candida albicans*) and plant pathogenic fungus (*Aschochyta rabiei*, *Fusarium oxysporum albidinis* and *Fusarium Var coeruleum*).

Sabouraud dextrose medium containing chloramphenicol or actidione was used for *Candida albicans*.^[32] The fungal suspension from two to three day old culture was made in sterile distilled water and its turbidity was adjusted to 5 Mc Farland (10^8 CFU/ml). An aliquot of 0.1 ml of this fungal suspension was spread over the surface of agar plate.

Plant pathogenic fungi, were grown on Malt agar during 5–7 days. Mycelia discs of 5 mm diameter were taken with a sterilized cork borer from each fungus culture and transferred into sterile distilled water. The suspension was diluted with sterile distilled water till obtain 10^7 spore/ml, then an inoculums of 0.1 ml was spread uniformly on Malt agar plate.

The disc technique was used for the antifungal activity; sterile paper discs of 6 mm diameter impregnated with 20 μ l of plant extract. The same procedure was used as described previously. Negative controls were discs impregnated with 20 μ l of methanol. Discs impregnated with methanol plant extract solutions

or absolute methanols were left in an oven till total evaporation of methanol. After that, they were placed aseptically on the surface of the inoculated agar plates. The tests were performed out in triplicate. After incubation for 3 to 6 days, the diameters of the inhibition zones against the tested fungus were measured in mm.

Determination of minimal inhibitor concentration (MIC)

The MIC was determined using the dilution method for extracts which showed activity on any of the test bacteria and fungus. The test was performed using three dilutions of each extract (1/2, 1/4 and 1/8) against the tested microorganisms. Each dilution was tested in triplicate with the presence of sterile disc as a negative control. The same procedure for preparing bacteria and fungi cultures was done as described previously. The MIC was the lowest concentration of plant extracts that exhibited no growth of microorganisms.

RESULTS AND DISCUSSION

The percentage yield of extracts from roots was 15.2% for methanol extract and 16.2% for ethanol extract. These yields were similar. The weights of different fractions of the methanol and ethanol extracts from roots were: 0.47 g, 2.72 g, 5.20 g, 0.40 g, 1.17 g and 4.70 g respectively for Ac1, Bu1, Aq1, Ac2, Bu2 and Aq2. For the organic extract (AcF) and the aqueous extract (AqF) of leaves, their weight was 0.5 g and 13 g respectively.

Antibacterial activity

Results obtained for antibacterial activity of all fractions are reported in (Table 1). None of the different fractions was effective against *Escherichia coli*, *Citrobacter freundii*, *Klebsiella pneumonia*, and *Serratia sp*. However, various antimicrobial effects have been obtained against both Gram-positive and Gram-negative bacteria as well as against phytopathogenic fungus. Analysis of the data revealed that organic fractions from methanol and ethanol root extracts exhibited high inhibition zones against one or more of the tested microorganisms, compared to aqueous fractions which showed the lowest activity. Similar results were obtained with the ethyl acetate and butanol fractions from the methanol and ethanol extracts of roots. *Staphylococcus aureus*, strains showed high sensitivity to the investigated fractions. The range of the zone of the inhibition was from 7 to 20 mm (Table 1). Maximum inhibition zone of 20 mm was obtained with aqueous fraction (Aq1), organic fractions (Ac1 and Ac2) against *Staphylococcus aureus* and *Bacillus cereus* respectively.

Table 1: Antibacterial activity of root and leaf extracts

Test Bacteria	Inhibition Zone in mm									
	Ac1	Bu1	Aq1	Ac2	Bu2	Aq2	AcF	AqF	C ⁻	C ⁺
<i>Staphylococcus aureus</i>	-	-	20 ± 3.5	7 ± 1.5	-	10 ± 1.5	10 ± 1.0	20 ± 1.0	-	20
<i>Escherichia coli</i>	-	-	-	-	-	-	-	-	-	25
<i>Bacillus cereus</i>	20 ± 1.5	16 ± 1.5	-	20 ± 1.5	17 ± 2.0	12 ± 2.5	10 ± 0.6	-	-	20
<i>Citrobacter freundii</i>	-	-	-	-	-	-	-	-	-	nt
<i>Enterobacter faecalis</i>	-	-	-	10 ± 1.00	-	-	-	-	-	nt
<i>Klebsiella pneumoniae</i>	-	-	-	-	-	-	-	-	-	nt
<i>Serratia sp.</i>	-	-	-	-	-	-	-	-	-	nt
<i>Acinetobacter bowie</i>	15 ± 2.0	10 ± 1.5	-	15 ± 1.0	10 ± 1.0	-	10 ± 0.6	20 ± 1.5	-	nt
<i>Proteus sp.</i>	-	-	-	-	-	-	-	-	-	nt
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	-	10 ± 0.6	-	20

C⁻: Negative control (Methanol). C⁺: positive control (Gentamycin 10 µg/disc). - indicates no inhibition. Results are the mean ± SD of triplicate determinations. nt: Not tested.

Low activity was exhibited for the organic fraction (Ac2) from root extract on *Staphylococcus aureus* and *Enterobacter faecalis*, and their inhibition zones were 7 and 10 mm respectively. Inhibition zones of 16 and 17 mm were obtained with the organic fractions (Bu1 and Bu2) against *Bacillus cereus*, and low activity of 10 mm was seen with the same fractions on *Acinetobacter bowie*.

For the leaf extracts, organic and aqueous fractions (AcF and AqF) were effective only on *Acinetobacter bowie* and *Staphylococcus aureus* and the corresponding inhibition zones were 18 and 20 mm. However, low activity of 10 and 9 mm was obtained for organic fraction against *Acinetobacter bowii* and *Bacillus cereus*, and for aqueous fraction on *Staphylococcus aureus* and *Pseudomonas aeroginoa*.

Our results indicated high sensitivity of *Staphylococcus aureus*, *Acinetobacter bowii* and *Bacillus cereus*. Extracts of *Carthamus caeruleus* were more active than those of *Carthamus lanatus*^[33] and *Carthamus tinctorius*.^[6]

The Minimum Inhibition Concentrations (MIC) of the root extracts aqueous fraction (Aq1) was of 1.3 mg/ml

for *Staphylococcus aureus*, while the ethyl acetate fractions Ac1 and Ac2 had a MIC of 0.47 mg/ml and 0.2 mg/ml on *Acinetobacter bowie* respectively. For *Bacillus cereus*, the MIC values were 0.12 mg/ml, 0.2 mg/ml, 0.68 mg/ml and 0.29 mg/ml corresponding respectively to different organic extracts Ac1, Ac2, Bu1 and Bu2.

For the aqueous leaf extract (AqF), a MIC of 12.5 mg/ml was obtained on *Staphylococcus aureus*. In this study, it was observed that the aqueous fractions Aq1 and AqF had a higher MIC than the organic extracts. The ethyl acetate fraction Ac1 and Ac2 showed a higher inhibitory activity. This difference is attributed to the solubility of the active component in different solvents.^[34] For the antifungal assay, results obtained of all fractions are reported in (Table 2).

The organic fractions (Ac1, Ac2 and Bu2) and the aqueous fraction (Aq2) exhibited maximum antifungal activity with inhibition zones of 25 mm, 17 mm, 18 mm and 20 mm on *Candida albicans* respectively.

Root and leaf extracts were tested on phytopathogenic fungi in order to determine their potent effect

Table 2: Antifungal activity of root and leaf extracts against pathogenic fungi

Fungus strains	Inhibition Zone in mm									
	Ac1	Bu1	Aq1	Ac2	Bu2	Aq2	AcF	AqF	C ⁻	C ⁺
<i>Candida albicans</i>	25 ± 2.0	10 ± 1.5	-	17 ± 2.6	18 ± 2.0	20 ± 1.5	-	-	-	14.3 ± 0.5
<i>Aschochyta rabiei</i>	12 ± 1.5	11.3 ± 3.9	-	11 ± 1.2	-	-	-	-	-	22.5 ± 3.5
<i>Fusarium oxysporum albidinis</i>	10.6 ± 0.6	-	-	11.3 ± 2.5	8.3 ± 1.1	-	-	-	-	22.5 ± 3.5
<i>Fusarium Var coeruleum</i>	11 ± 2.1	-	-	10.3 ± 0.6	10 ± 1.0	-	-	-	-	11.6 ± 1.5

C⁻: Negatif control (Methanol). C⁺: Amphotericin B (10 mg/ml) as positive control for *Candida albicans* and Digrain₄ [Dichlorvos (125 g/l), Malathion (100 g/l)] as positive control for plant pathogenic fungus. - indicates no inhibition. Results are the mean ± SD of triplicate determinations.

to treat plant diseases caused by fungi for new pesticide development. The fractions Ac1 and Bu1 inhibited the growth of the tested phytopathogenic fungi. The ethyl acetate fraction (Ac1) inhibited the growth of *Aschochyta rabiei*, *Fusarium Var coeruleum* and *Fusarium oxysporum albidinis* and the inhibition zones were 12 mm, 11 mm and 10.6 mm respectively. Buthanol fraction (Bu2) exhibited low activity of 10 mm on *Fusarium Var coeruleum* and 8.3 mm on *Fusarium oxysporum albidinis*. The organic and aqueous fractions of leave extract did not express any activity on the tested fungus strains.

The root extracts were more active than the leaf extracts against the tested microorganisms. This agrees with my own ethnobotanic investigation^[35] near the herbalists and the population of the Sétif. In traditional medicine, roots are used for the treatment of the burns and gave good results in the case of the diabetic patients.

CONCLUSIONS

This plant is an endemic species. The roots of this botanical genus are intensively used as a medicinal plant in the region of Sétif. The extracts showed significant effects on bacteria: *Staphylococcus aureus*, *Bacillus cereus* and *Acinetobacter baumannii*; and human pathogenic fungus: *Candida albicans*. *Carthamus* sp could be used as a potential source of natural antimicrobial agents with beneficial therapeutic effects.

Conversely, good antifungal activities were observed on phytopathogenic fungi. Our current research offers the possibility of developing strategies for controlling plant pathogens with natural extracts or bioactive metabolites of medicinal plants. Further, phytochemical studies are ongoing to define the chemical structure and characteristics of bioactive compounds especially present in the roots of this botanical genus.

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