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# Antimicrobial and antioxidant activities of acetone extract of *Ammodaucus leucotrichus* Coss. & Dur. seeds

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## Abstract-

The present study reports the antimicrobial and antioxidant activities of acetone extract of *Ammodaucus leucotrichus* seeds collected from Béchar provinve (Algeria).
The antioxidant activity was evaluated by 2, 2'-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging compared to the synthetic antioxidant (ascorbic acid). The acetone extract showed excellent scavenging activity with an $IC_{50}$ value of 0.28 mg/mL. The antimicrobial activity was determined using disc diffusion method. The acetone extract at a dose of 2 mg/disc, showed significant antibacterial activity against two bacterial strains (*Bacillus subtilis* and *Escherichia coli*) and two yeast species (*Candida albicans* and *Saccharomyces cerevisiae*).

**Key words:** *Ammodaucus leucotrichus* Coss. & Dur., Acetone extract, Antimicrobial activity, Antioxidant activity.

## Résumé

La présente étude indique les activités antimicrobiennes et antioxydantes de l'extrait acétonique des graines d'*Ammodaucus leucotrichus* collectés de la zone de Béchar (Algérie).
L'activité antioxydante a été évaluée par 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) comparé à l'acide ascorbique (antioxydant synthétique).

L'extrait acétonique a montré une excellente activité antioxydante avec une valeur de 0,28 mg/mL de l'$IC_{50}$. L'activité antimicrobienne a été déterminée en utilisant la méthode de diffusion de disque. L'extrait acétonique à une dose de 2 mg/disc a montré une activité antibactérienne significative contre les deux souches bactériennes (*Bacillus subtilis* et *Escherichia coli*) et les deux espèces de levure (C*andida albicans* et *Saccharomyces cerevisiae*).

**Mots-clés :** *Ammodaucus leucotrichus* Coss. & Dur, Extrait acétonique, Activité antimicrobienne, Activité antioxydante.

## 1.- Introduction

*Ammodaucus leucotrichus* Coss. & Dur. (*Apiaceae*) is a plant that plays an important role in traditional medicine in North African countries, especially in the Algerian Sahara (Boulos, 1983). The seeds of this plant are used to treat diseases related to the digestive system, to ease stomach-ache, for vomiting and pain (Hammiche and Maiza, 2006). In addition, leaves of this plant are used to aromatize tea and the powder of this plant is an appreciated spice in the Djanet area (Benchelah et al., 2000).

A number of studies on various plants reported that some plant extracts have antioxidant activity and benefits the human health in playing an important role neutralizing free radicals, which can cause several disorders of the immune system and gene expression (Halliwell, 1995; Pourmorad et al., 2006; Safaei-Ghomi et al., 2009; Sharma and Bhat, 2009). For these reasons, plant extracts can be used to protect organisms and cells from damage induced by oxidative stress, the latter being considered a cause of ageing, degenerative diseases and cancer (Adiguzel et al., 2009). Moreover, some plant extracts show antimicrobial activity against a wide range of microorganisms including fungi and antibiotic resistant bacteria. They can affect both Gram-positive and Gram-negative bacteria in addition to yeasts and filamentous fungi (Nantitanon et al., 2007).

This work is the first report on the antimicrobial and antioxidant properties of the acetone extract obtained from *Ammodaucus leucotrichus* seeds.

## 2.- Materials and methods

### 2.1.- Seed collection and preparation

*Ammodaucus leucotrichus* seeds were collected from Béchar district located at the South west of Algeria (31.6166°N, -2.21669°W).

### 2.2.- Preparation of acetone extract

The seeds of the studied plant (100 g) were submitted to hydrodistillation for 3h using a Clevenger type-apparatus. After the isolation of the essential oil, the powdered materials were dried at 45 °C for 24 h. Then, 20 g of the dried materials were loaded to a Soxhlet apparatus and extraction was carried out with acetone (400 mL) at 90 °C for 3h. The remaining acetone was evaporated by placing the samples in a vacuum drier under reduced pressure. The viscous extracts were stored in a refrigerator at 4 °C until use (Singh et al., 2007).

## 2.3.- Antioxidant activity using DPPH radical scavenging assay

The antioxidant activity of the acetone extract was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical 2, 2'-Diphenyl-1-picrylhydrazyl (DPPH) (Politeo et al., 2006). One milliliter of various concentrations of the extract (0.02 to 0.5 mg/ mL) in ethanol was added to 1 mL of a 0.004 % ethanol solution of DPPH. The mixture was strongly shaken and left to stand at room temperature for 30 min in the dark. The absorbance was measured at 517 nm against a blank (Brand et al., 1995). The radical-scavenging activity was expressed as percentage of inhibition (I %) according to the following formula:

$$I\ (\%) = (A\ control - A\ sample)/A\ control \times 100$$

where A control is the absorbance of the DPPH solution without extract, and A sample is the absorbance of sample with DPPH solution. The concentration providing 50% inhibition ($IC_{50}$) was reported as the amount of antioxidant required to decrease the initial DPPH concentration by 50%.

## 2.4.- Antimicrobial activity

### 2.4.1.- Tested microorganisms

The acetone extracts were individually tested on different microorganisms, including Gram-positive bacteria; *Bacillus subtilis* and Gram-negative bacteria; *Escherichia coli* and on two yeast species *Candida albicans* and *Saccharomyces cerevisiae.*

All the used microorganisms were provided by the "Laboratoire de Biologie des Systèmes Microbiens (LBSM), Ecole Normale Supérieure de Kouba, Alger, Algeria".

### 2.4.2.- Disk-diffusion assay

The disk diffusion method was used for the determination of the antimicrobial activity. Target microorganisms were cultured on Muller Hinton agar medium. The inocula were suspended in sterile saline water and

diluted according to 0.5 Mc Farland standard and then spread on solid media plates. Sterile filter paper disks (5.5 mm in diameter) were impregnated with 10 µL of extract and placed in the center of the inoculated Petri dishes then remained 2 h at 4°C. The cultures were incubated at 37 °C for 24 h. Each experiment was carried out in three repetitions.
The antimicrobial activities were evaluated by measuring the inhibition zone diameters (millimeter) surrounding each disk (Ozcan et al., 2010).

## 2.5.- Statistical analysis

The data were presented as mean ± standard deviation of three replicates. Statistical analyses were performed using a one-way analysis of variance. Statistical significance was declared at $P < 0.05$.

## 3.- Results and Discussion

## 3.1.- Antioxidant activity

The DPPH test is the oldest indirect method for determining the antioxidant activity of various samples. DPPH is a stable nitrogen-centered free radical, the color of which changes from purple to yellow upon reduction by either the process of hydrogen or electron donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers (Brand et al., 1995). Antioxidant activities of all samples and standard ascorbic acid are presented in Table 1 in which lower $IC_{50}$ values indicates higher antioxidant activity. The $IC_{50}$ value of the ascorbic acid was 0.45 mg/mL, whereas $IC_{50}$ value of acetone extract of seed was lower at 0.28 mg/mL. This is the first study showing that the antioxidant capacity of the acetone extract of seeds of *A. leucotrichus* is higher than the synthetic antioxidant agent.

**Table 1.** Antioxidant capacities of the acetone extract of *A. leucotrichus* seeds

| **Extract and control** | **DPPH[a] (mg $mL^{-1}$)** |
|---|---|
| Acetone extract of seed oil | 0.28 ± 0.21 |
| Ascorbic acid | 0.45 ± 0.11 |

[a]$IC_{50}$ values of DPPH assay

The results of this work indicate that extracts obtained from *A. leucotrichus* seeds showed capacity to donate hydrogen; therefore they present scavenging activity of DPPH. This activity might be due to the presence of

hydroxyl groups existing in the chemical compounds detected in the samples, which could react with free radicals to stabilize and terminate radical chain reactions (Das and Pereira, 1990; Shimoi et al., 1996; Matkowski, 2008).

## 3.2.- Antimicrobial activity

Antimicrobial activity of the acetone extract obtained from *A. leucotrichus* seeds are shown in Table 2.

**Table 2.** Antimicrobial activities of acetone extract using a disc diffusion method

| Microorganisms | Inhibition zone/mm (acetone extract) |
|---|---|
| *Bacillus subtilis* (Gram positive bacteria) | 8.50 ± 0.1 |
| *Escherichia coli* (Gram negative bacteria) | 7.50 ± 0.14 |
| *Candida albicans* | 9.54 ± 0.47 |
| *Saccharomyces cerevisiae* | 7.17 ± 0.65 |

The acetone extract at a dose of 2 mg/disc showed significant antibacterial activity against Gram negative and Gram positive bacteria, and also against the yeasts *Candida albicans* and *Saccharomyces cerevisiae*.
This antimicrobial activity could be related to the structural configuration of the constituent components and their functional groups and possible synergistic interactions between them (Dorman and Deans, 2000). The chemical structures of molecules in the acetone extract could play an important role in the antimicrobial activity by disturbing the cell structures making them more permeable, which will lead to cell death (Farag et al., 1989; Daw et al., 1994). Some reports showed that the Gram-negative bacteria are more sensitive to extracts than Gram-positive bacteria due to their outer membrane barriers; the Gram-positive bacteria have only an outer peptidoglycan layer which is not an effective permeability barrier while Gram-negative bacteria have an outer phospholipids membrane (Burt, 2004; Arias et al., 2004).

## 4.- Conclusion

The results of this work are the first report giving the antimicrobial and antioxidant properties of the acetone extract obtained from *A. leucotrichus* seeds. The acetone extract of seed seemed to have more effective antioxidant properties equivalent to or higher than those of synthetic antioxidants and possessed effective antimicrobial activity against tested microorganisms.

On the basis of the results of this work, *A. leucotrichus* seeds can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical applications.

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