Universal Impact Factor 0.9285:2012; 1.2210:2013

Index Copernicus
ICV 2011: 5.09
ICV 2012: 6.42

NAAS Rating
2012 : 1.3;2013:2.69

Received on:
9th March 2014

Revised on:
15th March 2014

Accepted on:
16th March 2014

Published on:
1st June 2014

Volume No.
Online & Print
52 (2014)

Page No.
52 to 56

Life Sciences Leaflets is an international open access print & e journal, peer reviewed, worldwide abstract listed, published every month with ISSN, RNI Free-membership, downloads and access.

BIOLOGICAL ACTIVITIES OF PLANTS COLLECTED IN THE ALGERIAN SAHARA

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ABSTRACT:

Nine extracts (dichloromethane, methanol, water) of three plants: *Ammodaucus leucotrichus* C. et D. (Apiaceae), *Cymbopogon schoenanthus* (L.) Spreng. (Poaceae), *Matricaria pubescens* (Desf.) Schultz. (Asteraceae) collected in the Algerian Sahara were screened for antibacterial, antifungal, cytotoxic activity, as well as inhibitory activity towards two key enzymes PLA and elastase.

KEY WORDS: *Ammodaucus leucotrichus, Cymbopogon schoenanthus, Matricaria.*

INTRODUCTION:

In the Algerian Sahara the population is scattered and part of it remains nomadic. The healthcare structures are not very effective; accessibility is the main problem due to limited means of transport as well as extreme weather conditions, vastness of the region. Therefore the population has fallen back on

its ancestral knowledge of everyday remedies. To date, 80 wild indigenous medicinal plants have been identified and their vernacular Tamahaq and Arabic names, their distribution, the parts used, the modes of preparation and routes of administration reported in our preceding papers (Hammiche *et al.*, 2006; Maiza *et al.*, 2006). The present study was aimed at evaluating biological activities of *Ammodaucus leucotrichus* C. et D., *Cymbopogon schoenanthus* (L.) Spreng. and *Matricaria pubescens* (Desf.) Schultz.

A. leucotrichus is an endemic saharian species, whose local names are "Akaman" (Tamahaq) and "Oum draiga" (Arabic). *C. schoenanthus* is a tropical-afro-asiatic species called "Tiberimt" (Tamahaq) and "Lemmad" (Arabic). *M. pubescens* is a North-African endemic species, whose local names are "Aynasnis" (Tamahaq) and "Ouazouaza or Guertoufa" (Arabic).

In Algeria these species are currently used for treating various illnesses. (Hammiche, 2006; Maiza, 2006). *A. leucotrichus* (seeds and aerial parts) is used in stomach diseases, vomits, allergies, and is also emmenagogue, abortive and aphrodisiac. *C. schoenanthus* (whole plant) is used to treat rheumatism, fever, digestive diseases (aerophagia, flatulence). *M. pubescens* (aerial parts) is used in dysmenorrhoea, muscle contraction, conjonctivitis (tiny globose flower soaked in water and applied in situ), scorpion stings.

In Morroco, *A. leucotrichus* is recommanded in treatment of cardiac diseases (Jouad *et al.*, 2001). In Saudi Arabia, *C. schoenanthus* is known for its analgesic and antipyretic properties (Mohsin *et al.*, 1997). Previous phytochemical investigations described the presence of ammolactone and perillaldehyde in *A. leucotrichus* (Muckensturm *et al.*, 1997), thienyl-hexadien-isobutylamide, decadien-isobutylamide, herniarin in *M. pubescens* (Greger *et al.*, 1983), γ-elemene, calemenene, cadalene, caryophyllene epoxide, β-sitosterol, β-eudesmol, elemol and α-eudesmol in *C. schoenanthus*: (Dawidar *et al.*, 1990).

METHODOLOGY:

Plant material

A. leucotrichus, *C. schoenanthus* and *M. pubescens* were collected first time at El Golea (Algeria) in May 2000. The plant material was authenticated by J.P. Lebrun (Museum National d'Histoire Naturelle, Paris) and voucher specimens (Hv 684 for *A. leucotrichus*, Hv 677 for *C. schoenanthus*, Hv 644 for *M. pubescens*) were deposited at the "Laboratoire de Botanique d'Alger", Algeria.

Extracts

The aerial parts (soft twigs and leaves) of *A. leucotrichus*, and *M. pubescens* and the whole plant of *C. schoenanthus*, were air-dried and extracted successively with dichloromethane, methanol and water.

Studied activities

a. Antimicrobial and antifungal activities:

Antimicrobial and antifungal activities were determined by the diffusion-disk method (Bauer et al., 1966) on cultures of two bacterial strains: *Staphylococcus aureus* Gram (+), *Escherischia coli* Gram (-) and a

yeast *Candida tropicalis*. *S. aureus* and *E. coli* were grown on Mueller-Hinton medium, and *C. tropicalis* on agar enriched with Bactopeptone and Dextrose (20‰). Aliquots of each extract (1mg) were transferred, in 10 µl of solvent, to a 6.0 mm diameter paper disk. Disks were air-dried and applied onto solid agar plates seeded with micro-organisms. Each sample was screened in duplicate. After 24 h incubation at 37°C for *S. aureus* and *E. coli*, and 27°C for *C. tropicalis*), antibiotic and antifungal activities were determined by measuring the inhibition zone diameter.

b. *In vitro* cytotoxicity assay :

KB cells (human buccal epidermal carcinoma) were provided by Rhône-Poulenc-Rorer. For the bioassay we used the method of Arisawa (Arisawa *et al.*, 1997) with slight modifications. The cell suspension (3.10^3/ml) was placed in 96-well tissue culture microplates. Samples of extracts were dissolved in 0.2% ethanol and added to the cell suspension at different concentrations. After 72 h incubation, KB cells were counted by using neutral red as dye and absorbances were measured at 550 nm in a microplate reader (Ceres 900-Bio-tek Instruments), against blanks (without drug). Assays were made in triplicate.

c. *Enzymatic assays :*

Enzymatic studies were performed using a microplate reader. PLA_2 inhibition was evaluated by measuring hydrolysis of lecithin by PLA_2 of bee venom (*Apis mellifera*) using red phenol according to Lobo de Araùjo *et al.*,(1987), in 96-well microplates. Bee venom PLA_2 (0.2 µg) was incubated with 100 µg of each extract in 20 µl of DMSO, for 1h30 at room temperature. Then substrate was added (200 µl) for a final concentration of extract of 0.5 mg/ml and absorbance read at 550 nm, after 5 min. Percent inactivation was determined by comparison to a vehicle control without drug (DMSO). Elastase inhibition was evaluated measuring amidolysis of N-succinyl-alanyl-alanyl-alanyl p-nitroanilide (Suc(Ala)3pNA) (Sigma) by porcine pancreatic elastase (PPE) (BIOSYS) at 410 nm. 95 µl aliquots of 40 nM PPE in 200 µl pH 8 buffer and 20 µl of extract solution (100 µg) in DMSO were mixed at 25 °C for 30 mn prior to addition of 10 µl of substrate solution (30 µmoles of Suc(Ala)3pNA in 1ml of DMSO) and plates were red after 10 mn.

RESULTS AND DISCUSSION:

Results of the different bioassays are reported in tables 1 (antimicrobial and antifungal activity), 2 (cytotoxic activity) and 3 (PLA_2 and elastase inhibition).

Of the three extracts of each plant, the dichloromethane and the methanol extracts appear the most active in all bioassays. Of particular interest were the dichoromethane extract of *A. leucotrichus* which exhibits marked antibacterial and antifungal activity and the extracts (dichloromethane and methanol) of *M. pubescens* whose inhibition of PLA_2 activity suggests a possible use as an antiinflammatory drug. On the other hand, if the aqueous extracts are devoid of any antimicrobial or antifungal activities they display noticeable elastase inhibition. These results give an additional interest to these plants used in traditional

medicine. Thus it would be interesting to identify the substance(s) responsible for these activities and to enlarge the traditional water-soluble components use to ethanolic extracts.

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Table 1: Antimicrobial and antifungal activity of the extracts. Zone of inhibition (mm)

Strains	*S. aureus*	*E. coli*	*C. tropicalis*
AI	14	8	12
AII	9	0	7
CI	11	0	0
CII	9	0	0
MI	15	0	0
MII	10	0	0
S*	30	34	15

A: *A. leucotrichus*; C : *C. schoenanthus*, M : *M. pubescens*.
I: dichloromethane , II: methanol.
S* Standards: oxacillin (5µg) for *S. aureus*; cefotaxime (30 µg) for *E. coli*; amphotericin B (2.5 µg) for *C. tropicalis*.

Table 2: Cytotoxic activity of the extracts towards KB cells. % Inhibition

Concentration	10 µg/ml	5 µg/ml
AI	100	88
AII	44	0
AIII	43	22
CI	98	94
CII	64	27
CIII	0	0
MI	94	79
MII	29	18
MIII	17	7

A: *A. leucotrichus*; C : *C. schoenanthus*, M : *M. pubescens*.
I: dichloromethane , II: methanol, III: water extract.
Standard: adriamycin ID_{50} : 0.015 µg/ml

Table 3: Inhibition of PLA_2 and elastase. % Inhibition at 0.5 mg/ml

Enzyme	PLA_2	Elastase
AI	74	22
AII	74	49
AIII	25	78
CI	20	59
CII	0	49
CIII	17	52
MI	96	50
MII	96	63
MIII	6	61

A: *A. leucotrichus*; C : *C. schoenanthus*, M : *M. pubescens*.
I: dichloromethane , II: methanol, III: water extract.