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Comparative Evaluation of the Antiradical and Antimicrobial Activities of Organic Extracts of Algerian *Pulicaria arabica* (L.) Cass. with Reference Products

Nadia Djermane[1], Noureddine Gherraf[2], Khellaf Rebbas[3], Rabah Arhab[1]

[1]*Department of Nature and Life Sciences, Faculty of Exact Sciences and Nature and Life Sciences, Larbi Ben Mhidi University, Oum El Bouaghi 04000, Algeria*
[2]*Laboratory of Natural Resources and Development of Sensitive Environments, Larbi Ben Mhidi University, Oum El Bouaghi 04000, Algeria*
[3]*Department of Natural and Life Sciences, Faculty of Sciences, University of M'sila, Algeria*

ABSTRACT

This study is to assess the antimicrobial and antiradical activities of organic extracts from the species Pulicaria arabica (L.) Cass. We used four clinical strains and four reference strains, to assess the antimicrobial activity of organic extracts of P. Arabica (L.) Cass. Evaluation of the antiradical activity is done by spectroscopic assay using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. Measurement of the antiradical activity of three plant extracts was carried out using the DPPH test.

The anti-radical tests of ours extracts were assessed using the free radicals DPPH catching method. The results showed clearly, that the methanol extract (EMeOH) had the best anti-radical ability followed by chloroform extract (EDCM) and lastly the hexane extract (In-Hex). The antimicrobial tests were evaluated against reference pathogenic strains and others isolated from pathological materials, using agar diffusion method. The results indicated that the apolar extracts (hexane and chloroform) had inhibitory activity against a number of microorganisms higher than that of the polar methanolic extract.

Antimicrobial and antiradical activities of organic extracts from the species Pulicaria arabica (L.) Cass has shown that these extracts have considerable inhibitory activity against certain germs and good potential of DPPH free radical scavenging, but these activities are generally lower than those obtained with the reference products (antibiotics, antifungal and ascorbic acid).

Keywords: *Pulicaria arabica,* Organic extracts, Antimicrobial activity, Antiradical activity

INTRODUCTION

Nowadays, many pharmaceutical and academic laboratories are recurring to plants to look for active substances [1]. This special interest is motivated by the increasing resistance of some bacteria to antimicrobials, as well as the multiplicity and severity of diseases caused by oxidative stress.

These reasons increase the research for new active substances, from plants origin as sources of antimicrobial agents, to remove or, even eradicate cases of antimicrobial resistance, in order to take under control new infections of bacterial or fungal origin, or as sources of antioxidant compounds for preventing oxidative stress and its associated syndromes such as diabetes, Alzheimer, rheumatism and cardiovascular diseases [2,3].

Several Studies directed by many researchers, in order to assess the biological properties of members of the genus *Pulicaria*, reveal that most of them look for the evaluation of their antimicrobial and antioxidant activities.

This study aims to evaluate the *in vitro* antiradical and antimicrobial activities of the crude extracts of the species *Pulicaria arabica* (L.) Cass, which is a Saharan endemic plant, widespread in Africa (Algeria, Morocco, Tunisia, Libya and Sudan), and in Asia (Afghanistan, Cyrus, Egypt, Iran, Iraq, Palestine, Jordan, Lebanon, Syria, Turkey, Turkmenistan and Pakistan), where it is used in traditional medicine to treat swelling and painful boils [4-7].

MATERIALS AND METHODS

Vegetal material and extracts preparation

The whole plant was harvested in July 2011 at the flowering period, from M'sila province, located in the southeast of Algeria. The plant was identified by Dr. K. Rebbas. The vegetal material was dried in shelters, at room temperature and then transformed into a powder. 20 g of the powder were placed under maceration for 3 days, in 200 ml of three different solvents (methanol, chloroform and n-hexane). The aim is to separate compounds according to their polarity. The three macerates that obtained are evaporated to dryness using a rotary evaporator. The obtained extracts are then stored at 4ºC, until testing antimicrobial and antiradical activities.

Evaluation of the antimicrobial activity using the agar diffusion method

The microbial strains and antibiotic discsused in the test

We used four clinical strains: *Staphylococcus aureus*, *Salmonella typhi*, *Citrobacter freundi*, and *Candida albicans*(yeast), and four reference strains (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 43300, *Staphylococcus aureus* ATCC 25923), to assess the antimicrobial activity of organic extracts of *P. arabica*. The reference strains were obtained from the collections of Pasteur Institute in Algiers. While clinical strains were isolated from human samples, from hospitals and medical laboratories in the provinces of Oum El Bouaghi and Constantine in Algeria.

The antibiotics used to perform the antibiogrammeare: AML (25 µg), AMC (30 µg) 3.SXT (25 µg) KZ (30 µg), CTX (30 µg), GN (10 ug) for the Gram (-) strains.

P (10 ug), L (10 ug), VA (30 µg) OX (5 µg), GN (10 ug) for Gram (+) strains.
Nystatin (10 mcg/ml) for yeast.

Protocol

The antimicrobial activity of the organic extracts of the plant was performed, using the agar medium diffusion method, following the recommendations of the National Committee for Clinical Laboratories Standards (NCCLS, 2003; 2004). Microbial suspensions of different microorganisms to test (of 0.5 McFarland), were inoculated on Petri dishes of Muller Hinton agar. Disks impregnated with 10 µl of reference antibiotic disks extracts, used as positive control, were placed on the MHA agar, previously inoculated with different microorganisms. After pre-diffusion of 30 min at room temperature, the Petri dishes were incubated for 24 h at 37ºC for bacteria and 48 h at 30ºC for yeast, (The operation was performed in triplicate).

The antimicrobial activity of our extracts was demonstrated by the presence of a halo of inhibition, around the disks impregnated by the different extracts.

For the antibiotic sensitivity test, the interpretation was carried out, following the recommendations of the antibiogramme committee of the French Society of Microbiology [8].

Evaluation of the antiradical activity

Spectroscopic dosage using the DPPH method

The measurement of the antiradical activity, of three plant extracts was performed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) test [9,10]. A series of concentrations (1 M, 10^{-1} M, 10^{-2} M, 10^{-3} M, 10^{-4} M, 10^{-5} M) of the plant extracts, or ascorbic acid (reference antioxidant), was prepared in ethanol, 1 µl of each extract solutions (solvent only for the test white), were mixed in 1000 µl of ethanolic solution of DPPH (0.004%) and placed in the darkness. The kinetics of the reaction was followed for 5 min, by measuring the absorbance using spectrophotometer at 517 nm every 30 s.

Measurement of DPPH inhibition rate

The free radicals DPPH catching rate was calculated according to the following equation:

$$I\% = [(\text{White Abs} - \text{test Abs})/\text{control Abs}] \times 100$$

Where: White Abs: absorbance of negative control; test Abs: Sample absorbance.

RESULTS AND DISCUSSION

Extraction efficiency

The amount of crude extracts obtained (20 g of powder with 200 ml of organic solvents of increasing polarity), varies depending on the solvent. The methanol is the solvent which provided the highest yield of crude extract, conversely, the hexane give the lowest yield (Table 1).

Table 1: Solvents extraction efficiency

Extract	Extraction efficiency (%)
Méthanolic	8.50
Chloroformic	2.52
n-Hexanic	1.64

Antimicrobial activity

Antibacterial activity

The results of the antimicrobial activity obtained with plant extracts and with antibiotics are recorded in Tables 2 and 3

Table 2: Effet organic extracts on bacterial growth

Strains	Vegetal extract (8 mg/ml)					
Gram positive Bacteria	EMeOH		EDCM		En-Hex	
S. aureus ATCC 43300	0	IA	19.33 ± 1.15	A	0	IA
S. aureus ATCC 25923	0	IA	17.00 ± 1.73	A	0	IA
S. aureus	0	IA	21.66 ± 0.58	A	0	IA
Gram negative Bacteria	EMeOH		EDCM		En-Hex	
E. coli ATCC 25922	10.00 ± 0.00	A	11.33 ± 0.58	A	11.00 ± 0.00	A
P. aeruginosa ATCC 27853	12.00 ± 1.00	A	0	IA	0	IA
Klebseilla pneumoniae	0	IA	0	IA	11.66 ± 1.15	A
Salmonella typhi	0	IA	0	IA	13.00 ± 0.50	A
Citrobacter freundi	0	IA	0	IA	10.33 ± 0.58	A

A: Active, IA: Inactive, EMeOH: Methanolic extract, EDM: Chloroformic extract, En-Hex: N-hexanic extract

Table 3: Effect of antibiotics on bacterial growth

Strains	Reference Antibiotics									
Gram positive Bacteria	P		L		VA		OX		GN	
S. aureus ATCC 43300	0	R	0	R	18	S	0	R	20	S
S. aureus ATCC 25923	0	R	0	R	18	S	0	R	20	S
S. aureus	0	R	0	R	27	S	0	R	22	S
Gram negative Bacteria	AML		SXT		KZ		CTX		GN	
E. coli ATCC 25922	21	S	29	S	25	S	30	S	20	S
P. aeruginosa ATCC27853	0	R	0	R	0	R	18	S	18	S
Klebseillapneumoniae	0	R	0	R	15	S	28	S	16	S
Salmonella typhi	29	S	34	S	29	S	35	S	20	S
Citrobacter freundi	0	R	0	R	0	R	0	R	16	I
Strains	Reference Antibiotics									
Gram positive Bacteria	P		L		VA		OX		GN	
S. aureus ATCC 43300	0	R	0	R	18	S	0	R	20	S
S. aureus ATCC 25923	0	R	0	R	18	S	0	R	20	S
S. aureus	0	R	0	R	27	S	0	R	22	S
Gram negative Bacteria	AML		SXT		KZ		CTX		GN	
E. coli ATCC 25922	21	S	29	S	25	S	30	S	20	S
P. aeruginosa ATCC27853	0	R	0	R	0	R	18	S	18	S
Klebseillapneumoniae	0	R	0	R	15	S	28	S	16	S
Salmonella typhi	29	S	34	S	29	S	35	S	20	S
Citrobacter freundi	0	R	0	R	0	R	0	R	16	I

R: Resistant, S: Sensitive, I: Intermediate P: Pénicilline, L: Lincomycine, VA: VincomycineOX: Oxacilline, AML: Amoxicilline, SXT: Cotrimoxazol, KZ: Céfazoline, CTX: Céfotaxime

After 24 h of incubation of petri dishes at 37ºC, we notice that all organic extracts (EMeOH, EDCM and In-Hex) of *Pulicaria arabica* showed an inhibitory activity against at least one of the tested microbial strains. The highest activities were recorded with the chloroformic extract (EDCM) against Gram positive species, the largest inhibition surface was observed for clinical *Staphylococcus aureus*, followed by collection staphylococci. Whereas no activity was observed against these organisms by the methanolic and the n-hexane extracts.

For the Gram negative species, the inhibitory activity on these germs is more significant with the hexanic extract (four germs between five tested). However, these microorganisms have not shown the same sensitivity. *Salmonella typhi* revealed the most sensitive species. Therefore, we can conclude that the intensity of the effects on the growth of the tested germs varies depending on the type of microorganism, Gram the extraction solvent used.

From the results of comparative anti biogramme, we generally observe that antibiotics showed large inhibiting zones compared to those obtained by the crude extracts of the plant. Various parameters help to explain this difference in efficacy between different antibiotics and extract i.e. the degree of purity, concentration and toxicity.

Antifungal activity

The results of the antifungal activity of the various extracts on *Candida albicans* strain (Diameters of inhibition zones) are recorded in Figure 1 and Table 4.

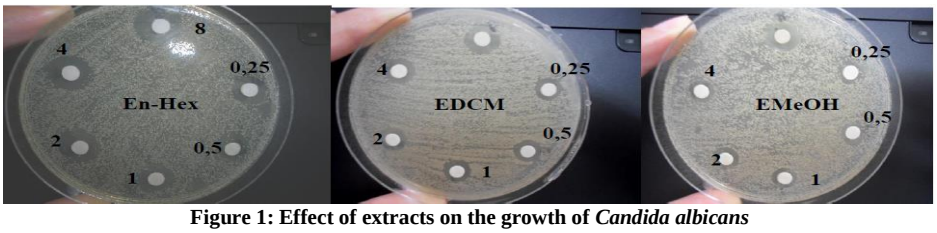

Figure 1: Effect of extracts on the growth of *Candida albicans*

Table 4: Diameters of zones of inhibition of organic extracts of *Pulicaria arabica* on *Candida albicans*

Strain	Extracts and Reference ATF	Diametre of the inhibition zone (mm)					
		Pulicaria arabica extracts Dilutions (mg/ml)					
		8	4	2	1	0.50	0.25
Candida Albicans	En-hex	14.66 ± 0.57	13.00 ± 0.00	12.33 ± 0.00	11.00 ± 0.57	10.6 ± 1.00	10.00 ± 1.00
	EMeOH	10.66 ± 1.15	10.33 ± 0.57	10.00 ± 0.57	9.66 ± 0.57	9.33± 1.52	9.00 ± 0.57
	EDCM	12.66 ± 0.57	11.33 ± 1.00	10.33 ± 0.57	10.00 ± 1.00	9.66 ± 0.57	9.00 ± 0.57
	Nystatine	17 mm					
ATF: Antifungal, EMeOH: Methanol extract, EDM: Chloroform extract, In-Hex: n- hexane extract, each value represents the average of three trials ± Standard Deviation (SD)							

After 48 h incubation at 30ºC, we observe that all tested organic extracts exert inhibitory activity against *Candida albicans*, this activity increases gradually as the concentration extracts is increasing in impregnated discs. So the strain tested is sensitive to all the extracts in a dose-response relationship. *Candida albicans* is also more sensitive to a polar extracts the polar extracts. We can therefore conclude that the extraction using nonpolar solvents (n-hexane and dichloromethane) permit well concentration of antifungal active substances, compared with polar solvents (Methanol).

The antifungal activity of the same plant used in our study was already justified [5], which tested the essential oil extracted from different parts of this plant, on some fungal strains: *Fusarium solani* f.sp. *cucurbitae*, *Fusarium oxysporum* f *lycopersici*, f.sp. *niveum Fusarium oxysporum*, *Phytophthora cactorum*, *Alternaria solani* and *Rhizoctonia solani*.

The result of the comparative anti fongigramme carried out on *Candida albicans* showed that nystatin has an anti-*Candida albicans* effect than n-Hexane, Diclorométhane and Methanolextracs. This difference in effect can be due to the degree of purity of nystatin in comparison with the crude extracts which are generally characterized by a large number of different compounds.

Antiradical activity

The results of the antiradical activity of various organic extracts from *P. arabica* and reference antioxidant (I%: percentage of free radical DPPH inhibition) are given in Figure 2.

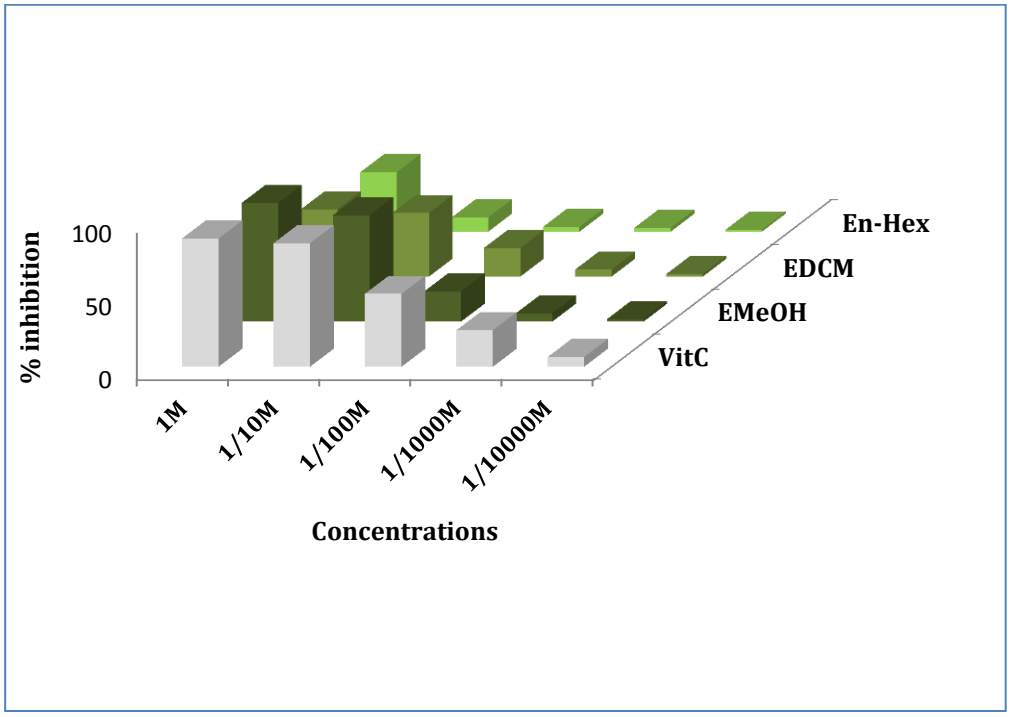

Figure 2: Percentage of DPPH radical inhibition of ascorbic acid (Vit C) and the organic extracts from *Pulicaria arabica*

According to the results, we observe that the catching capacity of free radical DPPH showed by methanolic extract (EMeOH) is important (I=80.91%), it remains significantly higher than that of the chloroform extract (DCM) and the hexane (In-Hex) extract, but relatively lower than that of ascorbic acid (I%=87.52). Furthermore, we observe that the ability of free radical DPPH trapping increases gradually as the concentration of the extracts or the reference antioxidant increases.

The high antiradical capacity of EMeOH of *P. arabica* could be explained by the presence of phenolic compounds. Similar results were found by other authors on extracts of plants belonging to the same family of our plant, have shown that the methanol extract of the aerial part of these plants (*Cynara cardunculus* L., *Tagetes minuta* and *Galinsoga parviflora*) had great potential to catch free radicals DPPH [11,12].

Moreover, results reported by Algabr et al. [13] on the antiradical activity of the extract from a species of the same genus of our plant (*Pulicaria jauberti*) showed that the scavenging activity of DPPH radicals was in order according to the polarity of the solvent, AcOEt extract (96.87%)>n-BuOH extract (63.62%)>$CHCl_3$ extract (33.78%).

From these results, it is possible to conclude that the polar extracts were more active than the a polar extracts that is to say the polar solvents are better suitable to concentrate anti-free radical active substances than a polar solvents.

CONCLUSION

The study of antimicrobial and antiradical activities of organic extracts from the species *Pulicaria arabica* (L.) Cass has shown that these extracts have considerable inhibitory activity against certain germs and good potential of DPPH free radical scavenging, but these activities are generally lower than those obtained with the reference products (antibiotics, antifungal and ascorbic acid). These results can be considered as research initiators, further phytochemical studies must be carried out, followed by chromatography to purify and identify anti-free radical and antimicrobial active substances of this plant.

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