



EVALUATION OF BIOLOGICAL ACTIVITIES OF *MATRICARIA PUBESCENS* FROM ALGERIA

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Abstract: *Matricaria pubescens* is a plant of the asteraceae family that grows wild in Algeria and throughout North Africa especially in the desert. It is commonly used as folk medicine to cure a variety of ailments in various preparations, aerial parts of the plant were sampled from two different sites in Algeria, the biological activities of the methanolic extracts and essential oils have been evaluated namely antioxidant, anti-inflammatory, antidiabetic, antibacterial, and antifungal activities, besides a safety bioassay, The essential oils of the plant have shown significant efficacy against the studied bacteria, and a strong antifungal activity against *Candida albicans*. Methanolic extracts and essential oils have shown noticeable anti-inflammatory activity. The essential oils showed good antidiabetic activity and weak antioxidant power. Meanwhile *Matricaria pubescens* essential oils noted significant cytolytic effect that may hamper its systemic use, it also noteworthy to mention the variability between the samples confirming the differences in the chemical patterns.

Keywords: Essential oil, Methanolic extracts, Biological activities, *Matricaria pubescens*, toxicity.

INTRODUCTION

From the dawn of civilization, human beings have been using plants to treat diseases; According to the World Health Organisation (WHO) 80% of the world's population relies on medicinal plants for their primary health care need¹. In Algeria, nearly 2/3 of the population use phytotherapy, mainly owing to the natural availability of plants and their affordable cost. Apart from their few side effects and good impacts on human health, plant-derived pharmaceutical products have piqued the interest of scientists throughout the world for many years. In fact, ethnomedicine use can be a rich source of chemicals for the treatment of a variety of health problems and infectious diseases in the pharmaceutical environment. Medicinal plants are thought to be a storehouse

of a variety of bioactive chemicals with various medicinal properties. Anti-inflammatory, antiviral, anticancer, antimalarial, and analgesic activities are among the many therapeutic benefits linked with medicinal plants².

Matricaria pubescens (Desf) Sch a member of the Asteraceae family that grows primarily in the Sahara is one of the medicinal plants native to North Africa. It is used for a variety of therapeutic, dietary, and cosmetic purposes. *Matricaria* is derived from the word matrix, which means "female matrix"³. regarding phytotherapy it is commonly used to treat rheumatism, dermatosis, dysmenorrhea, asthma, allergy, eye infection and scorpion stings. The plant contains secondary metabolites that are kind of micromolecular organic compounds produced during the growth and development of plants with

adaptation to the external environment. It is estimated that they could reach more than 100,000 including terpenoids, phenols, alkaloids, polyacetylenes⁴. For the Asteraceae family, the plants are known to produce isochlorogenic acid, sesquiterpene lactones, pentacyclic triterpene alcohols, various alkaloids, acetylenes (cyclic, aromatic, with vinyl end groups), tannins, and terpenoid essential oils that never contain iridoid^{5&6}. The pharmacological and therapeutics virtues of *Matricaria* may be attributable to the panel of the secondary metabolites produced by the plant. The objective of the current study is the evaluation of the biological activities of essential oils and methanolic extracts of *Matricaria pubescens* (Desf) Sch from several samples collected from two sites in Algeria, in order to confirm or refute the therapeutic uses purported by the local population. The studied activities are antioxidant activity, anti-inflammatory activity, antidiabetic activity, antibacterial activity and antifungal activity. On the other hand, the safety of the plant is assessed through a cytotoxicity assay.

MATERIALS AND METHOD

Plant material

Aerial parts and the flowers of the plant were sampled from the month of November 2015 to May 2016 from two sites in the southern east of Algeria namely: Biskra and Ouargla. The samples from 1 to 5 were taken from the site Biskra, harvested respectively in November 2015, January, February, March, April 2016, whereas the samples from 6 to 9 constitute the samples harvested from Ouargla in January, February, March, April 2016. The essential oils are extracted by hydrodistillation from the aerial parts of the *Matricaria pubescens* for 3 continuing hours using an extraction apparatus standardized by the European Pharmacopoeia. The operation was repeated several times for each sample. The essential oil yield is determined in mL/Kg of the dried vegetal material, the yield for the samples of Biskra was 2.23 ± 0.99 , and 1.51 ± 0.68 for the samples of Ouargla. The obtained essential oil was then stored at 4°C protected from light.

Regarding methanolic extracts, a test portion of 100 mg of flowers of *Matricaria* is extracted with 80 mL of methanol heated at reflux for 1 hr, and then the extract is filtered

and adjusted to 100 mL. The extraction yield (g/100g) and extract concentration (g/5 mL) respectively for the samples of Biskra was 16.23 ± 3.32 and 0.14 ± 0.03 , whereas they were 26.79 ± 4.82 and 0.2 ± 0.03 for the samples of Ouargla.

Reagents

- Methanol, Sigma Aldrich SZBE2100V, Antioxidant activity.
- Trichloroacetic acid, Biochem Chemopharma B76039, Antioxidant activity.
- Potassium ferricyanide, Fluka Chemiko 396340/121300, Antioxidant activity.
- Iron trichloride, Prolabo K151, Antioxidant activity.
- Dextrose, Sigma Aldrich SZBF0480V, Preparation of Alsever's solution and Preparation of potato dextrose agar (PDA) and potato dextrose broth (PDB) culture media.
- Sodium citrate, Biochem Chemopharma B6132043-1104-1, Preparation of Alsever's solution.
- Citric acid, Riedel-de Haën 11080, Preparation of Alsever's solution.
- Sodium chloride, Sigma Aldrich BCBK5916V, Preparation of Alsever's solution.
- Alpha amylase, Sigma Aldrich BCBS0008V, Antidiabetic activity.
- Starch, BDH Laboratory reagents 30264, Antidiabetic activity.
- Dinitrosalicylic acid, Sigma Aldrich MKBL2573V, Antidiabetic activity.
- Tween 80, Biochem Chemopharma B9005645-1104-1, Antibacterial and antifungal activity.
- Agar agar, Cheminova, Preparation of PDA and culture medium.
- Sodium monohydrogen phosphate, Analar Normapur VWR chemicals Prolabo 13G090023, Preparation of the phosphate buffer.
- Sodium dihydrogen phosphate, Biochem Chemopharma B10039324, Preparation of the phosphate buffer.
- DMSO, Honeywell Riedel-de Haën SZBG2600H, Preparation of dilutions of methanolic extracts.

Laboratory equipment

- Spectrophotometer, Jasco V-530 a double beam spectro controlled by software: The Spectra Manager
- pH meter, Adwa AD8000
- Water bath, GFL 1086
- Centrifuge, EBA 21 Hettic
- Densitometer, DensiCHEKTM Plus VITEK® BIOMERIEUX
- Autoclave, Cominox SterilClave 18 B - 18 liters
- Microplate reader, sun rights / basic Tecan, Minimum Inhibitory Concentration (MIC).

Biological material

- *Escherichia coli* T. Escherich, 1885, Gram negative bacilli, 25922.
- *Staphylococcus aureus* Rosenbach, 1884, Gram positive cocci, 27923.
- *Pseudomonas aeruginosa* (Schroeter, 1872) Migula, 1900, gram negative bacilli Oxidase positive, 27853.
- *Candida albicans* Berkhout, 1923, non-pathogenic yeast.

Biological media

- Mueller-Hinton liquid, Zara lab 30/2016, Antibacterial activity.
- Mueller-Hinton solid, Zara lab 32/2016, Antibacterial activity.
- Potato dextrose agar, locally made, antifungal activity.
- Potato dextrose broth, locally made, antifungal activity.

Reference standards

- Ascorbic acid, Standard provided by Roche, Antioxydant activity.
- Acarbose, Standard provided by Novapharm (Algeria), Antidiabetic activity.
- Sodium Diclofenac, Standard provided by Laboratory Salem (Algeria), Anti-inflammatory activity
- Gentamicin, Standard provided by FRATER-RAZES(Algeria), Antibacterial activity
- Fluconazol, Standard provided by Novapharm (Algeria), Antifungal activity.

METHODS

Antioxidant activity

DPPH radical scavenging assay

The antioxidant power of essential oil and methanolic extracts of *Matricaria pubescens*

was assessed by the method which uses DPPH (1,1-diphenylpicrylhydrazyl) as a relatively stable free radical^{7&8}. In this assay, the violet-colored DPPH is reduced to a yellow compound: diphenylpicrylhydrazine, whose color intensity is inversely proportional to the reducing capacity of the antioxidants present in the medium^{9&10}. The reaction is carried out in a total volume of 2.5 mL containing 2 mL of 0.1 mM DPPH dissolved in methanol. The samples of essential oils or methanolic extracts were prepared by dissolution in absolute methanol, these so-called stock solutions of 0.05mg/mL will then be diluted to obtain the final concentrations^{11&12}. Ascorbic acid is used as a reference antioxidant, in fact, a stock solution of 0.05 mg/mL is prepared, and then dilutions are made to obtain final concentrations from 1 to 10 µg/mL. The samples are left in the dark for 60 minutes, and the discoloration compared to the negative control containing only the DPPH solution. Measurement is made at 515 nm. The antioxidant activity (AA) is estimated according to the following equation¹³:

$$AA = \frac{\text{abs } 515 \text{ nm}(\text{control}) - \text{abs } 515 \text{ nm}(\text{test})}{\text{abs } 515 \text{ nm}(\text{control})} * 100$$

Ferric reducing antioxidant power assay (FRAP)

The reducing power of iron (Fe³⁺) in the extracts is determined according to the method described by OYAIZ (1986). The technique is based on the reduction of ferric iron to iron salt (Prussian blue) by antioxidants that give the blue color. 1mL of the methanolic extract (solution stock of 0.5 mg/mL) or essential oil (solution stock of 5 mg/mL) at various concentrations from 10 µg/mL to 100µg/mL for methanolic extracts, and from 100 µg/mL to 1000 µg/mL are mixed with 0.5mL of 0.2 M phosphate buffer solution (pH 6.6) and 0.5 mL of potassium ferricyanide K₃Fe(CN)₆ (1%) solution. The whole is incubated in a water bath at 50° C. for 20 min and then left to cool, 0.5 mL of 10% trichloroacetic acid is added to stop the reaction. The tubes are centrifuged at 3000 rpm for 10 min. An aliquot (0.5 mL) of the supernatant is combined with 0.5mL of distilled water and 0.1mL of an aqueous solution of FeCl₃ (ferric chloride) at 0.1%. The absorbance (abs) of the reaction medium is read at 700 nm against a similarly prepared blank, made from distilled water. The positive control is

represented by a reference standard of an antioxidant; ascorbic acid, whose absorbance was measured under the same conditions applied to the samples. An increase in absorbance corresponds to an increase in antioxidant activity¹⁴. The reducing power of iron is expressed by IC₅₀ which corresponds to the concentration of the sample giving an absorbance of 0.5.

Anti-inflammatory activity

The anti-inflammatory activity is determined *in vitro* by membrane stabilization method described by Shinde *et al.*, 1999^{15&16}. In fact the study was conducted using human red blood cells (HRBC) owing to the similarity between the globular membrane and the lysosomal membrane and therefore, it could be used for the evaluation of the membrane stabilizing effect by the plant methanolic extract or essential oil. Alsever's solution is prepared by dissolving 2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride in distilled water and the obtained solution is sterilized in the autoclave. Human blood is mixed with an equal volume of sterilized Alsever's solution then centrifuged at 3000 rpm for 10 min. The pellet is washed three times with isosaline solution (0.9%, pH=7.2), then the red cell suspension is prepared by solubilizing the pellet with isosaline solution in (1v/9v) proportions. A reaction mixture (4.5mL) is prepared by mixing 1 mL of phosphate buffer (pH= 7.4), 2 mL of hyposaline solution (0.45%), 1 mL of sample (plant essential oil or methanolic extract) /standard (all at 1 mg/mL) and 0.5 mL of red cell suspension¹⁷. The reaction mixture without the vegetal sample is used as control and phosphate buffer as blank. The mixtures are incubated at 37°C for 30 minutes and then centrifuged again. The hemoglobin content in the supernatant solution is estimated by spectrophotometry at 560 nm.

Calculation of the inhibition percentage:

$$\%I = \frac{(\text{Abs of Control} - \text{Abs of Treated Sample})}{\text{Abs of Control}} * 100$$

Antidiabetic activity

Bioassay for alpha-amylase inhibition^{17,18}

A starch solution (1%) is obtained by stirring 1 g of potato starch in 100 mL of 20 mM sodium phosphate buffer (0.1324 g of

NaH₂PO₄H₂O and 0.2788 g of Na₂HPO₄) with 6.7 mM Sodium chloride (0.039 g) at pH 6.9. An alpha-amylase solution is prepared by mixing 0.0253 g of alpha-amylase in 100 mL of cold distilled water (1 unit/mL). Then, 100 µL of control, standard or sample (extracts or essential oils at 100 ug/mL) were added to 900 µL of an alpha-amylase solution, followed by incubation at 37°C for 10 minutes. After incubation, 500 µL of the starch solution is added; the incubation at 37°C for 10 min ensued. Afterward, 500 µL of Dinitrosalicylic acid reagent are added and the mixture is placed in a boiling water bath for 5 min. After cooling in an ice bath, 3 mL of distilled water are added and the absorbance is measured at 540 nm against a blank. The generation of maltose is quantified by the reduction of 3,5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid, which is detectable at 540 nm. In the presence of β-amylase inhibitors, less maltose is produced and consequently absorbance decreased¹⁹.

Percentage inhibition is determined using the following equation:

$$\% \text{ Inhibition} = \frac{(Ac - (As1 - As0))}{Ac} * 100$$

Where Ac is the absorbance of the control reaction that contains all reagents except the standard or sample, As1 is the absorbance in the presence of a standard or sample (plant essential oil or extract) with enzyme and starch solution, As0 is the absorbance in the presence of a standard or sample with neither enzyme nor starch solution.

Antibacterial activity

The bacteria chosen are of the ATCC type. The preparation of bacterial inoculum is usually carried out following several steps. Initially, samples kept frozen or refrigerated should be activated in liquid Mueller-Hinton medium, after 6–8 hours at 35°C, an aliquot is transferred to Mueller-Hinton medium. After 24 h at 35 °C, axenic culture colonies can be dissolved in 5 mL of sterile saline solution (8.5 g/L NaCl) and measured using a 0.5 McFarland densitometer (corresponding to 1-2 x 10⁸ CFU/mL)²⁰. The culture medium consists of liquid Muller Hinton with 0.5% tween 80.

The technique is usually performed in U-plates with 96 well²¹. 20 µL of essential oil (0.82ug/L) is added to the first well that contains 170 µL of Mueller-Hinton broth (tween 80: 0.5%) the other wells already

contain 95 µL of Mueller-Hinton broth (tween 80: 0.5%). After homogenization of the first well, 95 µL of the mixture from the first well is transferred to the second well and so on, the 95 µL of the last well are eliminated. At the end 5 µL of a bacterial suspension of 3.5×10^7 CFU/mL is added to each well. Results are read after an incubation period of 24 hrs at 35°C. The Minimum inhibitory concentration (MIC) value is the lowest concentration of the natural product that visually inhibits microbial growth. From the wells without visible bacterial growth, 10 µL of solution is removed and streaked on Mueller-Hinton agar, then incubated for another 24 hrs/35°C to determine the minimum bactericidal concentration (MBC). The absence of colony forming units (or growth less than 0.1% of the initial inoculum) indicates that the essential oil is bactericidal. The percentage inhibition of bacterial growth by essential oils of the plant can also be calculated using a spectrophotometer in comparison with positive (Gentamicin) and negative control wells.

Antifungal activity

Preparation of culture medium

Potato dextrose agar

Potato infusion is prepared by boiling 200 g of sliced potatoes (washed but unpeeled) in water for 30 minutes, letting the obtained broth decant, then filtering it through cotton. Distilled water is added to reach a final volume of one liter. 20 g of dextrose and as much powdered agar-agar are dissolved in it before sterilization by autoclave at 100 kPa for 15 min.

Potato dextrose broth

A similar culture medium is prepared identically to Potato dextrose agar, but omitting the agar-agar and adding 5 mL of tween 80 per liter. A pathogenic strain of *Candida albicans* is subcultured on the culture medium (Potato dextrose agar + tween 80 at 0.5%) and incubated at 37° C for 48 h. Using physiological water, a solution of *Candida albicans* is prepared with a density of 0.5 McFarland using a densitometer and left resting for 3 minutes. The fungal suspension is diluted 1/10 in Potato dextrose broth. The wells of the microtiter plate are filled with 85 µL of Potato dextrose broth (PDB) except or the first well filled with 170 µL of PDB and 20 µL of the essential oil (0.82 µg/L), 95 µL of the mixture

from the first well is transferred into the second well and so on, the 95 µL of the last wells should be discarded and 5 µL of the fungal suspension added to each well. Regarding the control wells, they contain 5 µL of fungal suspension and 95 µL of PDB. An antifungal agent (Fluconazol) is added to the fungal suspension for a positive control and the culture medium with the fungal suspension as a negative control. The plate is incubated without shaking at 35°C for up to 70 hrs, checking for visible growth in the wells after 24 and 48 hrs. The natural product showing antifungal activity will prevent any discernible growth of *Candida albicans*²².

Test of erythrocytes toxicity

Hemocompatibility is an important parameter to assess the safety of the plant material in the human body. Oxyhemoglobin has a strong absorption peak at 541 nm resulting from lysed red blood cells (RBCs). Whereas, for extracts that do not induce hemolysis, this peak will be negligible. The analysis of the hemolytic activity of methanolic extracts and essential oils of *Matricaria* is carried out as described in the work of Lee, Powers and Baney (2004).

Blood is collected in a heparinized tube from a healthy donor. Then it is centrifuged at 2800 rpm for 15 min. The plasma (supernatant) is discarded and the pellet is washed with a phosphate buffer solution and centrifuged, the solution is eliminated, this step is repeated two other times. The suspension of the red blood cell obtained is diluted 1/20 with a solution of the saline phosphate buffer. Different concentrations of methanolic extracts and essential oils are prepared in saline phosphate buffer. The initial concentrations prepared are (100 mg/mL and 200 mg/mL). In hemolysis tubes, 2970 µL of the prepared red blood cell suspension are placed with 30 µL of the extract or essential oil. The final concentrations will be (1 mg/mL and 2 mg/mL). The tubes are incubated at 37°C while shaking for 120 min. A quantity of 500 µL is taken at 30, 60 and 120 min and to which is added 1.5 mL of saline phosphate buffer solution, the tubes are gently shaken and placed in an ice bath to stop the reaction then centrifuged at 2800 rpm for 15 min.

The absorbance is measured using a UV-Visible spectrophotometer, against a blank containing saline phosphate buffer at a

wavelength of 545 nm. The negative control is prepared under the same conditions and contains 2770 µL and 30 µL of saline phosphate buffer solution. While that the positive control consists of a completely haemolyzed tube prepared by mixing 250 µL of the erythrocytes suspension and 4750 µL of distilled water in the absence of any plant material. The hemolysis rate is calculated as a percentage of the total hemolysis, after 30, 60 and 120 min of incubation, according to the following formula:

$$\% \text{hemolysis} = \left(1 - \frac{At - Ae}{At} \right) * 100$$

At is the absorbance of total hemolysis

Ae is the absorbance of the sample (extract or essential oil)^{16&23&24}.

Statistical analysis was conducted using Microsoft Excel 2010.

RESULTS AND DISCUSSION

Results of the antioxidant activity

The overproduction of free radicals in the body and the deficit of the endogenous defense system are an important component in the pathophysiology of several conditions (cardiovascular, neurological diseases and neoplastic processes). Currently research aims to strengthen these endogenous defenses with natural substances from plants, which are endowed with antioxidant properties. The growing interest in the beneficial effects of the antioxidant on health has led to the development of a large number of assays to determine the antioxidant capacities of natural extracts. Two methods were employed:

scavenging of the free radical DPPH (antiradical capacity), chelation and reduction of the ferric ion (reductive capacity)²⁵.

DPPH assay

The results of the antioxidant activity related to essential oils and methanolic extracts show that *Matricaria* has a weak activity compared to the antioxidant activity of ascorbic acid. Furthermore, the methanolic extracts have a higher activity than that of the essential oils. It is also noted that the IC50 of the reference namely ascorbic acid by DPPH is 3.94. The result of the antiradical activity is 190.32 ± 54.45 µg/mL for the methanolic extracts (table1). While it is 1068.69 ± 182.18 µg/mL for essential oils (table2). The antioxidant power of the essential oils is shown to be very low, much lower than that of the methanolic extracts, however High variability is noted regarding the antioxidant activity between the samples with high differences shown as an instance between the sample 8 and 9 regarding the methanolic extracts, where the antioxidant activity of the sample 8 is almost half that of the sample 9, this difference does not apply to the essential oils where the samples 8 and 9 showed comparable antioxidant activities. The methanolic extracts of *Matricaria pubescens* from Biskra have a higher antiradical power than that of *Matricaria pubescens* from Ouargla, meanwhile that the essential oils of *Matricaria pubescens* from Ouargla have a higher antiradical activity than that of *Matricaria pubescens* from Biskra.

Table1: Summary of the biological activities of the methanolic extracts of *Matricaria pubecens*

Samples	Antioxidant activity		Anti-inflammatory activity (%)	Antidiabetic activity (%)	
	IC 50 DPPH	IC 50 DPPH			
BISKRA	1	136.87	18.1	47.41	69.32
	2	108.61	27.12	52.71	65.12
	3	168.64	19.17	52.87	68.68
	4	208.66	17.69	48.36	67.64
	5	258.59	18.85	43.16	62.85
	6	188.29	18.83	48.95	52.14
	7	249.56	33.94	48.07	54.62
	8	133.23	26.5	46.37	50.67
	9	260.45	22.55	47.72	55.21
AVERAGE BISKRA	176.27± 59.19	20.19±3.92	48.90±4.05	66.72±2.69	
AVERAGE OUARGLA	207.88± 59.04	25.45± 6.46	47.77±1.07	53.16±2.12	
TOTAL AVERAGE	190.32±54.45	22.53± 5.57	48.4±2.99	60.69±7.51	

Table2: Summary of the biological activities of the essential oils of *Matricaria pubescens*

Samples	Antioxidant activity		Anti-inflammatory activity (%)	Antidiabetic activity (%)	Antibacterial activity						Antifungal activity		
	IC 50 DPPH	IC 50 FRAP			Escherchia Coli		Pseudomonas aeruginosa		Staphylococcus aureus		Candida albicans		
					MIC µg/mL	MBC µg/mL	MIC µg/mL	MBC µg/mL	MIC µg/mL	MBC µg/mL	MIC µg/mL	MFC µg/mL	
BISKRA	1	874.52	85.72	48.22	23.25	41.2	41.2	20.6	41.2	20.6	20.6	5,41	10,81
	2	992.15	97.25	50.43	27.55	20.75	20.75	41.5	41.5	20.75	20.75	5,42	5,42
	3	1212.79	118.5	48.53	28.3	41.8	41.8	41.8	41.8	41.8	41.8	2,67	2,67
	4	1432	70.61	48.32	21.29	20.8	41.6	20.8	41.6	10.4	10.4	21,35	21,35
	5	1192.76	77.51	48.03	24.26	40.9	40.9	40.9	40.9	10.22	10.22	1,35	21,5
OUARGLA	6	918.51	70.25	48.99	21.95	20.75	20.75	20.75	41.5	20.75	20.75	21,7	21,7
	7	1098.51	68.25	49.6	25.55	20.75	20.75	20.75	41.5	20.75	20.75	10,91	21,82
	8	921.28	60.59	50.09	25.57	41.35	41.35	41.35	41.35	20.67	20.67	5,23	5,23
	9	975.74	74.35	48.89	32.14	20.47	40.95	20.47	40.95	5.11	10.23	10,82	10,82
AVERAGE ISKRA		1140.84 ± 215.45	89.92 ± 18.8	48.71 ± 0.98	24.93 ± 2.94	33.09	37.25	33.12	41.4	20.75	20.75	7.24	12.35
AVERAGE OUARGLA		978.51 ± 84.22	68.36 ± 5.77	49.39 ± 0.56	26.30 ± 4.24	25.83	30.95	25.83	41.32	16.82	18.1	12.16	14.9
TOTAL AVERAGE		1068.7 ± 182.18	80.34 ± 17.84	49.01 ± 0.85	25.54 ± 3.41	29.86	34.45	29.88	41.36	19	19.57	9.43	13.48

FRAPs assay

IC 50 of ascorbic acid measured by FRAP is 2.35. The results of the antioxidant activity by the FRAP method show that the methanolic extracts have a significant higher activity compared to essential oils concurring with the results obtained in the DPPH assay. The iron-reducing activity of the methanolic extracts is $22.53 \pm 5.57 \mu\text{g/mL}$, as for essential oils, it is $80.34 \pm 17.84 \mu\text{g/mL}$. The antioxidant power of the essential oils of *Matricaria pubescens* is shown to be very low concurring to the results noted for DPPH assay. The methanolic extracts of *Matricaria pubescens* from Biskra have a higher reducing power than that of from Ouargla. Contrarily, the essential oils of *Matricaria pubescens* from Ouargla have a higher reducing power than that from Biskra, meanwhile that the methanolic extracts show higher reducing power than that of the essential oils. The current results are shown to have higher activities comparing to the study of Metrouh et al where the extracts of the root of *Matricaria pubescens* were shown to have an iron reducing power of 4380 ± 20 for the aqueous extract, 4240 ± 10 for the acetone extract (50%), 4260 ± 20 for the methanol extract (50%), 4140 ± 10 for ethanol extract (50%). Besides, according to Drissi and Saadi, the extract of *Matricaria pubescens* has an antiradical activity with DPPH= $237.62 \pm 0.66 \mu\text{g/mL}$ ²⁶. Furthermore, Gherboudj has even evaluated the antioxidant activity on two

extracts and also on two isolated compounds from *Matricaria pubescens*, finding that the ethyl acetate extract has an activity of 9.79 ± 0.11 , the butanol extract has an activity of 33.07 ± 0.47 , Luteolin-7-O-β-D-glucoside has an activity of 3.98 ± 0.02 and 4'-O-Methylisoscutearein-7-O-[6''-O-acetyl-β-D-glucosyl-(1→2)-β-D-glucoside] has an activity of 15.69 ± 0.13 ²⁷. On the other hand, Dehimat showed that the 50% ethanolic extract has an antiradical power of 14.19 ± 1.15 and the 70% methanolic extract has an antiradical power of $18.33 \pm 4.14 \mu\text{g/mL}$ ²⁹. Furthermore, for the purpose of comparing between plants, a study conducted on *Matricaria chamomilla* L. collected from Egypt, showed a significant higher antioxidant activity with EC₅₀ (the moles of phenolic compounds divided by moles of DPPH• necessary to decrease by 50% the absorbance of DPPH) of 0.0022 ± 0.0005 , and antiradical power of 455 ± 35.7 regarding its methanolic extracts²⁸. The EC₅₀ value was $26.7 \mu\text{g/mL}$ for samples of *Matricaria recutita* L obtained from United Arab emirates, while that Iranian chamomile showed very high EC₅₀ ($5.52 \pm 0.15 \text{mg/mL}$), these comparisons permit to suggest that *Matricaria pubescens* has lower antioxidant activity than other species of *Matricaria*²⁸.

Anti-inflammatory activity

The results of the anti-inflammatory activity by the membrane stabilization method

show that the methanolic extracts and the essential oils of the plant have a lower activity than that of Sodium Diclofenac (69% for 1 mg/mL).

The percentage of membrane stabilization of the methanolic extracts $48.4 \pm 2.99\%$ (table 1) is noted to be comparable to that of the essential oils $49.01 \pm 0.85\%$ (table 2). The variabilities of the anti-inflammatory activity of both methanolic extracts and the essential oils in the samples are noted to be low. The percentages of membrane stabilization of the methanolic extracts and of the essential oils are shown to be lower than that of Sodium diclofenac 1 mg/mL = 69%. In a parallel study, *Stachys tibetica* oil was evaluated for its anti-inflammatory activity by the HRBC membrane stabilization method, and the protection percentage of the oil was 66.8%, 68.4% and 72.3% at 100, 200, and 400 g/mL, respectively¹⁶. Metrouh et al showed through an in vivo assay the anti-inflammatory activity of the alkaloids extracted from *Matricaria pubescens*³⁰.

In a parallel study the methanolic extracts of Chamomile showed significant anti-inflammatory activity represented by the bioassays of rat paw oedema against carrageenan and arachidonic acid and inhibition of writhing induced by acetic acid³¹. Besides, the anti-inflammatory activity was attributed to flavonoids and mainly to Apigenin³²

Antidiabetic activity

The percentage inhibition of α -amylase activity of methanolic extracts of *Matricaria pubescens* is $60.69 \pm 7.51\%$ (table 1), while it is $25.54 \pm 3.41\%$ for essential oils (table 2), showing higher activity for the methanolic extracts. The percentage inhibition of α -amylase activity regarding methanolic extracts of *Matricaria pubescens* from Biskra is higher than the activity of methanolic extracts from Ouargla. The variability of the antidiabetic activity within the samples of the essential oils and the methanolic extracts is noted to be low. Besides, the percentage inhibition of the α -amylase activity of the essential oils from Ouargla is higher than that from Biskra. The activities of methanolic extracts and essential oils of *Matricaria* are lower than the activity of Acarbose = 81.51%. In a similar study the aqueous extract of *M. pubescens* was shown to lower blood glucose levels in Streptozotocin - induced diabetic rats. In additionally the

histopathological tissues of the liver and pancreas showed to be enhanced³³. Besides, an in silico study of phytochemical compounds of *M. chamomilla* showed high antidiabetic potential for: anthecotulide, quercetin, chlorogenic acid, luteolin, and catechin as a result of their binding affinities towards both PTP1B and aldose reductase protein³⁴.

Antibacterial activity

The results of the antibacterial activity of essential oils are presented in the following table. The results show that the essential oils present variability in the inhibition of the growth of the studied strains. *Matricaria pubescens* essential oils are all bactericidal, inversely; the methanolic extracts have no antibacterial activity on the studied strains.

The MICs of gentamicin tested on the *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherchia coli* strains are respectively: 1 $\mu\text{g/mL}$ (0.5-2 $\mu\text{g/mL}$), 0.5 $\mu\text{g/mL}$ (0.12-1 $\mu\text{g/mL}$) and 0.5 $\mu\text{g/mL}$ (0.25-1 $\mu\text{g/mL}$). Aligianis et al.³⁵ suggested a classification of antimicrobial activity of plant products. Based on MIC results as follows: Potent inhibitors: MIC less than 500 $\mu\text{g/mL}$, Moderate inhibitors: MIC between 600 and 1500 $\mu\text{g/mL}$; Weak inhibitors: MIC greater than 1600 $\mu\text{g/mL}$.

Regarding *Escherchia coli*, the essential oils showed potent antibacterial activity with MIC and MBC respectively of 29.86 ± 10.8 and 34.45 ± 9.69 $\mu\text{g/mL}$. The essential oils are bactericidal since $\text{MBC/MIC} < 32$. Essential oils of *Matricaria pubescens* from Ouargla showed higher antibacterial activity (MIC = 25.83 ± 10.35 $\mu\text{g/mL}$) against *Escherchia coli* compared to essential oils from Biskra (MIC = 33.09 ± 11.24 $\mu\text{g/mL}$). Likewise, for *Pseudomonas aeruginosa*, *Matricaria pubescens* essential oils showed potent antibacterial activity with MICs of 29.88 ± 10.91 $\mu\text{g/mL}$, while that MBC was 41.36 ± 0.28 $\mu\text{g/mL}$. It is noteworthy to note that the essential oils are shown to have bactericidal activity because $\text{MBC/MIC} < 32$. The essential oils of *Matricaria pubescens* from Ouargla showed higher antibacterial activity (MIC = 25.83 ± 10.35 $\mu\text{g/mL}$) compared to the essential oils from Biskra (MIC = 33.12 ± 11.34 $\mu\text{g/mL}$).

Similarly, *Matricaria pubescens* essential oils showed potent antibacterial activity against *Staphylococcus aureus*, with MIC of 19.00 ± 10.47 $\mu\text{g/mL}$ and MBC 19.57 ± 9.18 $\mu\text{g/mL}$. It is by the way bactericidal since $\text{MBC/MIC} < 32$.

Essential oils of *Matricaria pubescens* from Ouargla showed greater antibacterial activity (MIC= $16.82 \pm 7.8 \mu\text{g/mL}$) against *Staphylococcus aureus* compared to essential oils of *Matricaria pubescens* from Biskra (MIC = $20.75 \pm 12.86 \mu\text{g/mL}$). *Staphylococcus aureus* is a major human pathogen capable of colonizing numerous tissues and organs, thus causing a wide variety of infectious diseases. The resulting complexity of staphylococcal pathogenesis pose a persistent challenge, especially given the growing resistance to antibiotics and the emergence of serious invasive infections³⁶. The essential oils showed significant better activity against Gram-positive bacteria comparing to Gram-negative bacteria ($p=0.031$). The higher resistance of Gram-negative bacteria can be explained by the fact that the outer membrane of Gram-negative bacteria forms an aril against many substances, including antibiotics³⁷ besides the periplasmic enzymes that are capable of degrading external molecules³⁸. Additionally, Gram-negative bacteria are disposed with efflux pumps that reduce cellular levels of antibiotics³⁹. The obtained results revealed the in vitro antibacterial properties of the essential oils of *Matricaria pubescens*, leading the path to additional studies related to their application in the antibiotic treatment of infectious diseases.

Essential oil of chamomile showed lower MIC values (12.5–10), the MIC for methanolic extracts were respectively 11.57, 13.71, 14 and 14.14 against *Escherichia coli*, *Salmonella*

typhi, *Bacillus cereus* and *Staphylococcus aureus*³¹.

Antifungal activity

The results showed no antifungal activity given by the methanolic extracts, while that the MIC value of the essential oils of the plant against *Candida albicans* in the PDB culture medium was $9.42 \pm 7.56 \mu\text{g/mL}$ and that the minimum fungicidal concentration (MFC) was $13.48 \pm 8.11 \mu\text{g/mL}$. The essential oils are fungicidal because $\text{MFC/MIC} < 32$.

Essential oils of *Matricaria pubescens* from Biskra have greater antifungal activity (MIC= $7.24 \pm 8.08 \mu\text{g/mL}$) compared to essential oils from Ouargla (MIC= $12.16 \pm 6.89 \mu\text{g/mL}$). The antifungal activity of essential oils is comparable to Fluconazole (MIC ≤ 8). In a similar study regarding chamomile the MIC of the methanolic extracts against *Candida albicans* was 17.71³¹

Results of erythrocytes toxicity

The capacity of essential oils and methanolic extracts to induce hemolysis was studied for two concentrations 1 and 2 mg/mL²⁴. The essential oils of *Matricaria pubescens* caused 90% hemolysis after 120 min of incubation for the two experimental concentrations 1 and 2 mg/mL. The average percentages of hemolysis caused by the methanolic extracts of *Matricaria pubescens* have not exceeded whatsoever 11.00% (table 3, figure1).

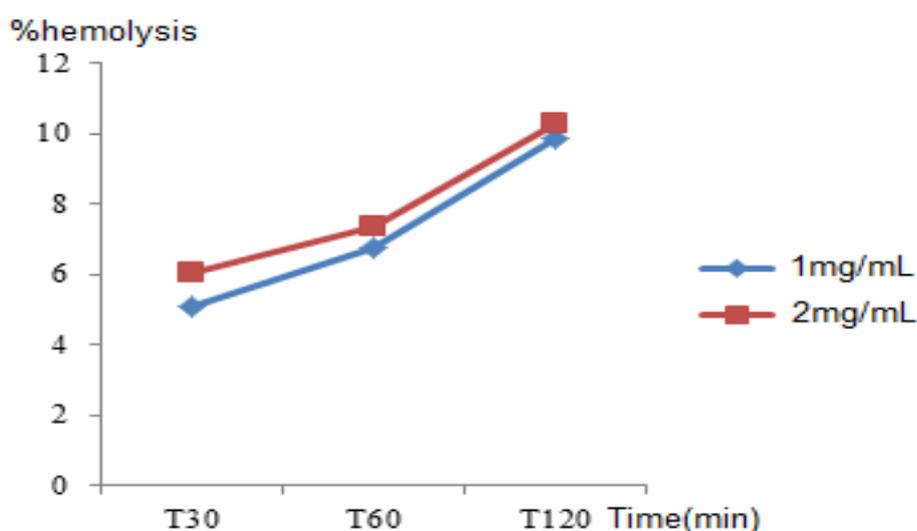


Fig. 1: Evolution of erythrocytes lysis as a function of time in the presence of methanolic extracts of *Matricaria pubescens*.

In fact, the hemolysis caused by the methanolic extracts is $9.87 \pm 1.87\%$ at a concentration of 1 mg/mL after 120 min of incubation and $10.25 \pm 3.57\%$ at a concentration of 2 mg/mL after 120 min of incubation, on the other hand, hemolysis caused by essential oils of *Matricaria pubescens* is shown to be high reaching $91.30 \pm 2.34\%$ at 1 mg / mL after 120 min of incubation and $90.71 \pm 4.21\%$ for 2 mg/mL (Table 3, figure 2). The difference of hemolysis by the essential oils of *Matricaria pubescens* at the concentration of 1 mg/mL is significant between 30 min and 60 min, and also between 60 min and 120 min ($p < 0.001$) and ($p = 0.05$) respectively. Parallely, it is significant between 30 and 60 min ($p < 0.001$) and not significant between 60 and 120 min ($p = 0.36$) for the concentration of 2 mg/mL. Adekunle et al evaluated the effect of beta ocimene (which is a principle compound in the essential oil of *Tagetes minuta*, showing that it was highly toxic to the eggs of *Meloidogyne incognita*, revealed by the inhibition 72-79% of eggs hatch within 14 days. This Inhibition was found to be directly proportional to oil concentration⁴⁰. Similarly, juvenile *Meloidogyne incognita* incubated in *T. minuta* oil were lysed within 72 hrs.

From the obtained results, the popular uses of *Matricaria pubescens* for infectious diseases whether bacterial or fungal especially to treat gynecologic, or skin infections may be justified owing to the proved high antibacterial and antifungal potential of the essential oil, besides the treatment of some inflammatory affections as rheumatism relies on *Matricaria pubescens* especially in some secluded areas in the south of Algeria could be more or less questioned due to the moderate antiinflammatory proprieties of both the methanolic extracts and essential oils shown in the current study, on the other hand the current study noted a significant antidiabetic activity even not equal to the reference, which is not that common in use within the local population. The use of plant by intra parenteral injection should be proscribed due to the high hematotoxicity of plant material and mainly to the essential oil, this data would limit the use of the plant to local application and oral uptake.

The effectiveness of the essential oils of *Matricaria pubescens* in the antibacterial and antifungal activity and the effectiveness of the essential oils and the extracts in the antidiabetic activity must be confirmed by in vivo assays due to the problem of transposition to the biological and physiological complexity of the in vivo medium.

Table 3: the percentages of the hemolytic activities of the methanolic and essential oils of *Matricaria pubescens* at 1mg/mL and 2mg/mL.

Site	N°	Methanolic extracts						Essential oils					
		Incubation 30 minutes		Incubation 60 minutes		Incubation 120 minutes		Incubation 30 minutes		Incubation 60 minutes		Incubation 120 minutes	
		1mg/mL	2mg/mL	1mg/mL	2mg/mL	1mg/mL	2mg/mL	1mg/mL	2mg/mL	1mg/mL	2mg/mL	1mg/mL	2mg/mL
Biskra	1	4.35	4.14	7.21	7.06	8.14	16.16	35.82	69.10	89.80	91.46	90.26	92.74
	2	5.53	4.46	7.00	7.36	9.90	8.25	31.83	72.50	94.72	91.34	92.71	93.27
	3	1.83	4.69	5.19	7.06	8.61	7.80	32.87	72.31	80.27	87.50	93.61	89.42
	4	3.67	6.02	5.15	6.84	7.95	8.07	34.73	72.94	94.72	99.45	95.63	99.45
	5	6.75	5.87	6.89	6.00	13.76	7.70	34.89	82.12	80.27	87.78	88.20	84.75
Ouargla	6	4.3	8.84	6.60	8.84	9.45	16.57	36.09	68.57	87.13	90.61	89.09	90.61
	7	6.48	5.87	6.52	5.98	11.52	8.21	37.42	69.23	83.37	87.54	89.95	88.60
	8	7.11	6.85	8.32	8.51	10.74	9.05	35.03	68.31	85.20	72.89	91.57	87.07
	9	5.55	7.48	8.15	8.35	8.80	10.48	38.58	69.25	82.80	88.59	90.72	90.52
Average		5.06±1.69	6.02±1.52	6.78±1.10	7.33±1.04	9.87±1.87	10.25±3.57	35.25±2.08	71.59±4.35	86.47±5.58	88.57±6.95	91.30±2.34	90.71±4.21

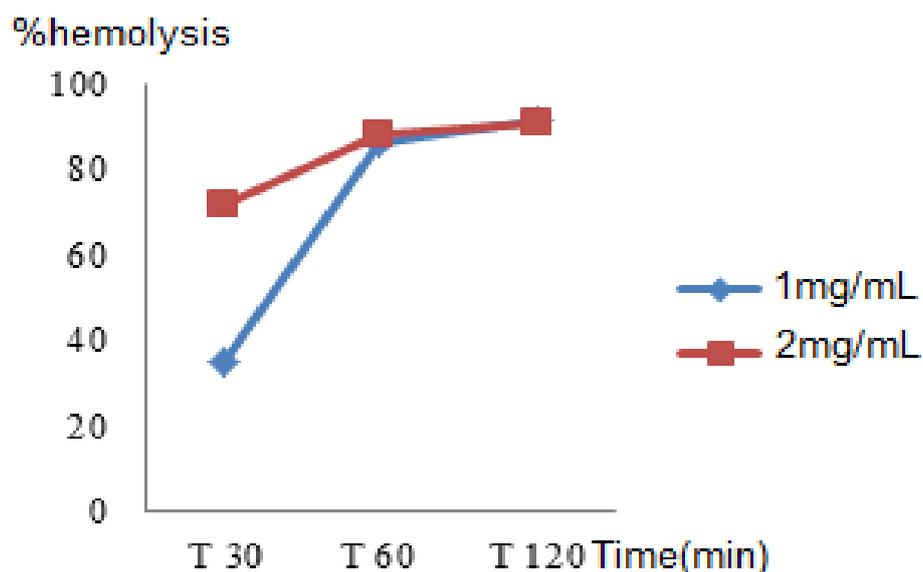


Fig. 2: Evolution of erythrocytes lysis as a function of time in the presence of essential oils of *Matricaria pubescens*.

Conclusion

In order to assess the claims of the local population regarding the use of a common plant *Matricaria pubescens* for its therapeutic virtues, some of the biological activities of methanolic extracts and essential oils, namely antioxidant, anti-inflammatory, antidiabetic, antibacterial and antifungal activities, besides the cytotoxicity were assessed through in vitro assays. Samples were collected from two sites then essential oils and methanolic extracts obtained.

The essential oils of the plant have shown significant efficacy against some species of bacteria as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and an antifungal activity against *Candida albicans*. The antidiabetic activity of the essential oils noted to be good although lower than that of the reference drug, while that methanolic extracts and essential oils elicited moderate anti-inflammatory activity, besides, the essential oils of the plant have given weak antioxidant power, on the other hand *Matricaria pubescens* essential oils showed to have significant cytolytic toxicity. These results may justify some of the therapeutic uses of the plant by the local population.

Considerable variabilities were noted mainly regarding the antioxidant, antifungal and antibacterial activities between the samples indicating different chemical patterns.

Conflict of Interest

The authors report no conflict of interest.

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نشرة العلوم الصيدلانية جامعة أسيوط



تقييم الأنشطة البيولوجية لماتريكاريا بوبسينس من الجزائر

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ماتريكاريا بوبسينس نبتة من عائلة الأستيرياسيا التي تنمو في برية الجزائر وفي جميع أنحاء شمال إفريقيا. يشيع استخدامها كدواء شعبي لعلاج مجموعة متنوعة من الأمراض في مختلف المستحضرات ، تم أخذ عينات من الأجزاء الهوائية من النبتة من موقعين مختلفين في الجزائر ، وتم تقييم الأنشطة البيولوجية للمستخلصات الميثانولية والزيوت الأساسية وهي مضادات الأكسدة ومضادات الالتهاب ، ومضاد لمرض السكر ، ومضاد للبكتيريا ، ومضاد للفطريات ، إلى جانب اختبار حيوي للأمان وعدم السمية ، أظهرت الزيوت الأساسية للنبتة فعالية كبيرة ضد البكتيريا المدروسة ، ونشاطًا قويًا مضادًا للفطريات. أظهرت المستخلصات الميثانولية والزيوت الأساسية نشاطًا ملحوظًا في مقاومة الالتهابات. كما أظهرت الزيوت الأساسية نشاطًا جيدًا مضادًا لمرض السكر بالمقابل ضعف في خاصية مقاومة الأكسدة . وفي نفس السياق ، أبانت الزيوت الأساسية على قدرة كبيرة على انحلال الخلايا ، وتجدر الإشارة أيضًا إلى التباين بين العينات التي تؤكد الاختلافات في الأنماط الكيميائية.