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CHEMICAL STUDY AND ANTIBACTERIAL ACTIVITY IN VITRO OF THE ESSENTIAL OIL OF *CHRYSANTHEMUM CORONARIUM L.*

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Abstract

Many aromatic plants, sometimes considered as weeds, have very interesting biological properties whose application extends to various fields such as medicine, pharmacy, cosmetology and agriculture. This work consists of evaluating the antibacterial activity of the essential oil of *Chrysanthemum coronarium* L., spontaneous collected in the region of Khenifra – Morocco. After extracting the essential oils from the aerial part *Chrysanthemum coronarium* L., by hydrodistillation using an "Alembic" pilot extractor, the chromatography analysis was carried out by gas chromatography coupled with gas spectrometry. mass (GC / MS) for the determination of chemical compositions. The results showed that the essential oil of *Chrysanthemum coronarium L.*, is characterized by the presence of Chrysanthenone (17.02%), Camphor (15.38%), and Pulegone (20.68%), The three major compounds were obtained with a percentage of (53.08%). On the other hand, the antimicrobial power of the essential oil of *Chrysanthemum coronarium* L. has been studied in vitro against bacterial strains: Three Gram-negative bacteria, namely Klebsiella pneumoniae (ATCC 4352), Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853). And Three Gram-positive bacteria namely Micrococcus luteus (ATCC 533), Staphylococcus aureus (ATCC 6538), Staphylococcus epidermidis (ATCC 12228). All of these results obtained only constitute a first step in the search for biologically active substances of natural origin.

Keywords: *Chrysanthemum coronarium L.; essential oil; chemical analysis; GC-MS; antimicrobial activities.*

Introduction

Aromatic medicinal plants, the traditional and medical use of which have long been known, are attracting increasing interest from researchers in the therapeutic field. They provide a wide range of inexhaustible active compounds with antifungal, antibacterial and antiviral properties and have been reported as potential sources of new antimicrobial compounds, food preservation agents and substitution treatments for infectious diseases **[1-4]**. Morocco with its mild and sunny climate is endowed with a very rich plant biodiversity with an avalanche of aromatic and medicinal plants **[5]**. These plants by their great diversity, represent an important reservoir of products, in particular essential oils, having various activities and consequently, being able to have multiple commercial applications, as well in the perfumery and the food industry as in the pharmaceutical and biomedical fields **[6-8]**. The diversity of climatic and ecological conditions in Morocco as well as its meeting position between European and Saharan flora make it a true floristic crossroads of undeniable diversity and complexity **[9]**. This diversity results in a great flora richness and forest areas which cover nearly 9 million hectares, ie a cover rate of 12.7% of the national territory and an average afforestation rate of 8% **[10-11]**. In recent years, increasing consumer demand for natural products without preservatives has led industries to consider incorporating natural substances **[12]**. Thus, essential oils are starting to have a lot of interest as a potential source of bioactive natural molecules **[13]**. They are the subject of study for their possible use as bio-insecticide, antifungal, antimicrobial and anti-viral ..., and therefore as a natural preservative which protects from various alterations **[14-15]**.

Material and methods

Plant Material

The studied species was collected from the Middle Atlas central in Morocco, particularly in the region of Khenifra, and the species was identified by an expert botanist.

Essential oil extraction

The extraction of the essential oil was carried out by hydrodistillation using an "Alambic" pilot extractor for 2 hours, in a laboratory at the EST-Khenifra (Morocco). The amount of 10 kg of dry plant material was used with a volume of 50 liters of water. The temperature of the device is set at 100 ° ± 5 ° C, to promote maximum evaporation of water and essential oil without destroying it. This method is recommended by the European Pharmacopoeia. The recovered oil is dehydrated with sodium sulfate (Na_2SO_4), then weighed to calculate the yield, then and stored in opaque and hermetically sealed bottles at 4 ° C ± 1. **[16]**.

Chemical composition

GC-MS analysis of the oils was performed on an Agilent HP-6890 gas chromatograph (Agilent Technologies) with an HP-5MS 5% phenylmethylsiloxane capillary column (30 mx 0.25 mm, film thickness 0.25 µm) equipped with an Agilent HP-5973 selective mass detector in electronic impact mode. Operation under the conditions described below: initial temperature 38 ° C, maximum temperature 250 ° C, equilibration time 5 min, ramp 6 ° C / min, final temperature 250 ° C, input: split less, pressure 6.75 psi, purge rate 1 ml / min and 1 µl of sample were injected for analysis, gas type: helium, column: capillary. The components of the oils were identified by matching their mass spectra with those of the computer library (NIST mass spectra library). The percent composition was calculated from the sum of the peak areas of the total oil composition **[17]**.

Antimicrobial activities

The antimicrobial activity of *Chrysanthemum coronarium L.* has been studied in vitro against bacterial strains: Three Gram-negative bacteria, namely Klebsiella pneumoniae (ATCC 4352), Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853). And Three bacteria Gram-positive, namely Micrococcus luteus (ATCC 533), Staphylococcus aureus (ATCC 6538), Staphylococcus epidermidis (ATCC 12228).

The diameters of the zones of inhibition of the different fractions of EO were determined by the method of Ainane et al. (2014) **[18]**, from 24-hour cultures (10^5 -10^6 CFU / mL). Flood seeding was done from the obtained inoculum, solubilizing the colonies in sterile distilled water and agar. The obtained inoculum (1 mL) is poured into Petri dishes

containing Mueller Hinton's agar. The excess inoculum was subsequently aspirated, and the dishes were dried in an oven (37 ° C). After 15 min of drying, the wells were cut out using Pasteur pipettes (6 mm thick end). Then, each EO fraction (50 µL) and gentamycin (50 mL) (control) were distributed into each well. After diffusion, the cultures were incubated in incubators at 37 ° C for 24 h. The inhibition halos were measured with a caliper. The activity is considered zero for a diameter of the inhibition zone less than or equal to 8 mm; low for a diameter of the inhibition zone between 8 and 14 mm, medium for a diameter of the inhibition zone between 14 and 20 mm; strong for a diameter of the inhibition zone greater than or equal to 20 mm.

For determination of the minimum inhibitory concentration (MIC), a medium consisting of sterile Mueller Hinton broth and a sterile solution of Tween 80 was prepared in order to obtain a homogeneous distribution of the EO fractions in the medium, and to maximize their miscibility in the medium. For the determination of the MIC, 10 test tubes were used. The first eight were used to prepare concentration ranges (80; 40; 20; 10; 5; 2.5; 1.25 and 0.625 mg / mL) of each EO fraction; the last two containing the positive and negative controls. In the test tubes containing each fraction of EO and the positive control, 10 µL of the inoculum was introduced. After 24 hours incubation of the tubes at 37 ° C, the MIC corresponding to the lowest concentration of essential oil capable of inhibiting bacterial growth after 18 to 24 hours of contact, was determined **[19]**. The concentration ranges used for the determination of the MIC were used to measure the CMB. Samples were taken from the control tube and from each of the tubes without a bacterial pellet, then deposited "streaked" on MHA agar. The inoculated dishes were incubated for 24 h at 37 °C.

Results and discussion

Essential oil

The average yield of essential oil of *Chrysanthemum coronarium L.* was calculated as a function of the dry plant matter obtained from the aerial parts of the plant studied. The essential oil yield obtained is 0.18% (v/w).

The analysis of the results of the chemical composition carried out by gas chromatography coupled with the mass spectrometry of essential oil of *Chrysanthemum coronarium L.* studied is mentioned in Table 1. Analysis of the results showed the chemical composition of the essential oil of *Chrysanthemum coronarium L.*, The main major compounds identified are: Chrysanthenone (17.02%), Camphor (15.38%), and Pulegone (20.68%)), The three major compounds were obtained with a percentage of (53.08%).

The results of Alvarez-Castellanos et al. (2001) **[20]**, show that the main compounds identified in the oil were camphor (29.2%), -pinene (14.8%), β-pinene (9.5%) and lyratyl acetate (9.8%).

The study carried out by Andriani Basta et al. (2007) **[21]**, on the essential oils of the flower heads of Chrysanthemum coronarium, collected at two different sites in Greece, shows the existence of Fifty-six constituents, among which the oxygenated monoterpenes made the greatest contribution. The main constituents of the sample (A) were trans-chrysanthenyl acetate (13.2%), trans-chrysanthenyl isovalerate (10.2%) and cis-chrysanthenyl acetate (9.9 %), while those in sample (B) were camphor (15.7%), cis-chrysanthenyl acetate (9.1%) and trans-chrysanthenyl acetate (7.8%) .

The Spanish study carried out by Alvarez-Castellanos et al. (2003) **[22]**, on a group of nine populations of *Chrysanthemum coronarium L.* cultivated in south-eastern Spain aims to study the effect of the application of fertilizers on the yield of the flower heads and the essential oil composition. An increase in flower head yield was obtained in the fertilized plots while this was not the case for the essential oil content. Camphor was the main compound identified in this oil (13.9 / 26.9%) and it was contained in greater amounts (2.32 / 5.47% more) when the flower heads came from fertilized plants. The accumulation of germacrene D in the oil, on the other hand, was adversely affected by the fertilization treatment.

Antimicrobial activities

The results of the antibacterial evaluations of all the methods applied to the essential oil of *Chrysanthemum coronarium L.* are expressed in terms of diameters (Φ) of zones of inhibition measured around the discs, of the minimum inhibitory concentration (MIC), of the minimum

bactericidal concentration (MBC) and ratio (MBC / MIC). All the values obtained are listed in Table 2. From the results obtained, it can be concluded that the essential oil of *Chrysanthemum coronarium L.* has interesting antimicrobial activities against the strains studied. The diameters of zones of inhibition Φ obtained from the disk diffusion method vary from 15.81 mm to 6.24 mm. All strains have different sensitivities, it grew in more or less moderate and acceptable activities. The minimum MIC inhibitory concentrations vary from 83.47 to 296.72 µL / mL and the minimum bactericidal MBC concentrations vary from 89.34 to 367.41 µL / mL. In general, therefore, the essential oil of *Chrysanthemum coronarium L.* exhibits remarkable activities against all the strains studied. According to the MBC/MIC ratio, the essential oil *Chrysanthemum coronarium L.* has bactericidal effects against all the strains studied (Table 3). In general, it has also been established in numerous studies that the activity of an essential oil is related to the majority compounds and the possible synergistic effects between the constituents.

Conclusion

In this work, we studied the chemical composition and the antimicrobial activity of the aerial part of the essential oil of *Chrysanthemum coronarium L.*, collected by the central Atlas in Morocco in particular in the region of khenifra, Eight chemotypes were identified in the crude oil, The results showed that the essential oil is characterized by the presence of Chrysanthenone (17.02%), Camphor (15.38%), and Pulegone (20.68%), The three majority compounds were obtained with a percentage of (53.08%). The antimicrobial activity of *Chrysanthemum coronarium L.* essential oil is remarkable against the six bacteria tested. The findings show that this species' essential oil has a significant antimicrobial activity, which is mostly attributable to its major constituents. On the other hand, the factors that impact active molecules are numerous; variability may be explained by a variety of factors such as geographic location, climate, attitude, the portion used, and the extraction method chosen. The sum of his findings raises the possibility of developing formulations based on *Chrysanthemum coronarium L.* essential oils instead of synthetic preservatives in the agrofood, pharmaceutical, and cosmetic industries.

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Table 1. Chemical composition of the essential oil of *Chrysanthemum coronarium L.*

Pic	RT	Compound name	%
1	11.60	Eucalyptol	1.43
2	14.03	1,6-Dimethylhepta-1,3,5-triene	0.48
3	14.55	β-Thujone	0.72
4	**14.99**	**Chrysanthenone**	**17.02**
5	**15.80**	**Camphor**	**15.38**
6	16.44	endo-Borneol	0.82
7	**19.27**	**Pulegone**	**20.68**
8	23.07	Verbenone	3.58

Table 2. Parameters of antibacterial activity of essential oil of *Chrysanthemum coronarium L.*

Strains	*Applied methods*	*Values*
Klebsiella pneumoniae	**Φ (mm)**	8.04 ± 0.95
	MIC (µL/mL)	135.58
	MBC (µL/mL)	144.72
	MBC/MIC	1.06
Escherichia coli	**Φ (mm)**	10.14 ± 0.61
	MIC (µL/mL)	83.47
	MBC (µL/mL)	89.34
	MBC/MIC	1.07
Pseudomonas aeruginosa	**Φ (mm)**	13.30 ± 0.47
	MIC (µL/mL)	164.25
	MBC (µL/mL)	191.53
	MBC/MIC	1.16
Micrococcus luteus	**Φ (mm)**	14.38 ± 0.29
	MIC (µL/mL)	100.01
	MBC (µL/mL)	101.85
	MBC/MIC	1.01
Staphylococcus aureus	**Φ (mm)**	15.81 ± 0.45
	MIC (µL/mL)	120.36
	MBC (µL/mL)	124.75
	MBC/MIC	1.03
Staphylococcus epidermidis	**Φ (mm)**	6.24 ± 0.51
	MIC (µL/mL)	296.72
	MBC (µL/mL)	367.41
	MBC/MIC	1.23

Table 3. Bactericidal or bacteriostatic nature of the essential oil of *Chrysanthemum coronarium* L.

Strains	*Effect*
Klebsiella pneumoniae	Bactericidal
Escherichia coli	Bactericidal
Pseudomonas aeruginosa	Bactericidal
Micrococcus luteus	Bactericidal
Staphylococcus aureus	Bactericidal
Staphylococcus epidermidis	Bactericidal