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CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *CHAMAEMELUM NOBILE* (L.) ALL.

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Abstract

The chemical composition of the essential oil of *Chamaemelum nobile* (L.) All. was studied for the first time on a local scale collected from the region of khenifra in Morocco. The extraction of essential oil was carried out by hydrodistillation, the average yield obtained at a rate of about 0.44%. The quantitative analysis of this essential oil was carried out by gas chromatography coupled with mass spectrometry made it possible to identify 72 compounds including Verbenone (33.74%), Pulegone (26.45%), and 3-Cyclohexen-1- one, 2-isopropyl-5-methyl- (8.90%) were obtained as majority compounds with a percentage of (69.09%). The evaluation of the antimicrobial property of essential oil of *Chamaemelum nobile* (L.) All. was carried out against Gram-positive bacteria: *Staphylococcus epidermidis* (CECT 231), *Listeria monocytogenes* (CECT 934), and *Listeria innocua* (CECT 910), and Gram-negative bacteria: *Escherichia coli* (CECT 515), *Yersinia enterocolitica* (CECT 4315), and *Pseudomonas aeruginosa* (CECT 108). The *in vitro* antibacterial tests carried out revealed significant bacterial activity against all the strains studied, in particular with bactericidal affects.

Keywords: *Extraction; Chamaemelum nobile (L.) All.; Essential oil; GC-MS; Antimicrobial activity.*

Introduction

Plants are capable of producing very diverse natural substances. In fact, alongside primary metabolism, it accumulates secondary metabolisms, including essential oils widely used by humans in fields as different as pharmacology or the food industry [1]. In general, aromatic and medicinal plants in particular are characterized by two metabolisms. The primary metabolism provides the basic constituents, and the secondary metabolism produces metabolites in small quantities but of great importance in applications to various fields, in particular of pharmaceutical and cosmetic interest [2]. Essential oils or vegetable essence are part of this group of metabolites along with alkaloids and phenols. Essences in plants are synthesized and secreted through particular cells or organs where they remain localized [3]. They can be stored in all organs of the plant. These specialized histological structures are located on or near the surface of the plant and vary according to the botanical families [4]. The extraction of essential oils is certainly the most delicate and important phase of the process. It aims to capture the most subtle and fragile products produced by plants. Many processes are used for the extraction of these substances, hydro-distillation is the most simple and practical process [5].

The essential oils obtained are products that are generally not very polar and have a density for the most part lower than that of water [6]. Essential oils are known to be endowed with anti-insecticidal, antimicrobial and antiseptic properties. Many of them have antitoxic, antivenom, antiviral, antioxidant and antiparasitic properties. More recently, they have also been recognized as having anticancer properties. The biological activity of an essential oil and to relate to its chemical composition and the possible synergistic effects between its components. Certain pathogenic microbial species are less and less sensitive to antibiotics and develop multiple resistance to them [7]. In contrast, chemical compounds with broad spectrum antibacterial and antifungal efficacy without phenols, aldehydes, alcohols and terpene ketones. Apart from their widely used antimicrobial effect today, essential oils have always been the subject of other biological studies [8]. These studies have shown their anti-inflammatory, anti-parasitic, antioxidant activities, etc. The antioxidant power of its oils developed as a substitute in food preservation, it is above all the phenols and polyphenols which are responsible for this power [9].

Material and methods

Plant Material and Sample Collection

Roman chamomile (*Chamaemelum nobile* (L.) All.) belongs to the Asteraceae family, it is a perennial herbaceous plant more commonly called "chamomile" for short, the plant is used in culinary, medicinal (particularly herbal tea) and cosmetic use. It should not be confused with two other medicinal plants resembling it and also locally called "chamomile": little chamomile *Matricaria recutita* and feverfew *Tanacetum parthenium*. This species is native to the regions of the Atlantic coast of Europe (Portugal, Spain, France, United Kingdom, Ireland) and North Africa (Morocco, Algeria). It is therefore not native to Italy, contrary to what its quality of "Roman" might suggest. In this study, *Chamaemelum nobile* (L.) All. was collected from the Khénifra-Morocco region. These species were verified by a botanist at the Khénifra Forest Research Center - Morocco.

Essential oil extraction

The essence of chamomile (*Chamaemelum nobile* (L.) All.) was obtained by hydro-distillation of the aerial parts in fractions of 600 g for 3 hours using a Clevenger type extractor. The operation of the condensation of the vapors charged with essential oil carried out by a refrigerant and are collected in a separating funnel. The phases less dense than water are collected and dried over anhydrous sodium sulfate (Na_2SO_4) before analysis. Then the essential oil obtained is stored in glass vials protected from light in refrigerants at 4 degrees Celsius for later uses [10]. The "Y" yield of essential oil is the ratio of the volume V of the essential oil extracted to the weight W of the plant material. It is expressed in mL of distillate per 100 g of dry matter (percentage volume / mass: v / w%) [11].

$$Y(\%) = \frac{V}{W} \times 100$$

Analysis of the chemical compositions of essential oils

The gas chromatograph coupled with mass spectrometry (GC-MS) is the most widely used chemical analysis for the characterization of essential oils. The analyzes were carried out at the National Center for Scientific and Technical Research in Rabat (CNRST) Morocco. The apparatus used in a column of type VB-5 (5% phenyl Methyl polysiloxane) has the following characteristics: (film thickness: 0.25 µm; internal diameter: 0.25 mm; length: 30 m). The operating conditions are: injection volume is 1 µl; the temperature of the split injector: 220 ° C°; the carrier gas: He at 0.3 ml / min; the scan mode is full scan; the temperature programming from 40 ° C (2 min) to 180 ° C at a rate of 4 ° C / min then reaches 300 ° C for 2 min at 20 ° C / min; the temperature limit varies from 20 to 260 ° C and that of the ion source is 200 ° C; the ionization energy is equal to 70 ev. The temperature of the auxiliary zone is 300 ° C and the scan range varies from 30 to 500 (M / Z). The identification of the retention indices of the various constituents is carried out from their mass spectra in comparison with those of standard compounds from the NIST computerized data bank [12].

Antimicrobial activities

- **Bacterial strains used:**

The evaluations of the essential oil properties of *Chamaemelum nobile* (L.) All. was carried out against Gram positive bacteria such as: *Staphylococcus epidermidis* (CECT 231), *Listeria monocytogenes* (CECT 934), and *Listeria innocua* (CECT 910); Gram negative bacteria such as: *Escherichia coli* (CECT 515), *Yersinia enterocolitica* (CECT 4315), and *Pseudomonas aeruginosa* (CECT 108).

- **Antibacterial test:**

The diameters of the zones of inhibition of the different fractions of EO were determined by the method of Hayes et al. (2002) [13] from 24-hour cultures (105 -106 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 fraction of EO (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 [14].

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.

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 [15].

Results and discussion

The average essential oil yield was calculated based on the dry plant material obtained from the aerial parts (stems, leaves and flowers) of the plant studied. The essential oil yield obtained is given in Table 1. The average essential oil yield of *Chamaemelum nobile* (L.) All. was calculated based on the dry plant matter of the aerial part of the plant. The species sample of *Chamaemelum nobile* (L.) All. provided a rate of around 0.44%.

Few studies have been carried out on *Chamaemelum nobile* (L.) All. Therefore we compared the results obtained with other species of the genus *Chamaemelum*. For example, in the study carried out by Darriet et al. (2011) [16], Harras et al. (2014) [17] and Elouaddari et al. (2019) [18], the distillation of *Chamaemelum mixtum* (L.) provided 0.47% essential oil. Similarly, chamomile (*Matricaria recutita* L., *Matricaria chamomilla*) contains according to McKay et al. (2006) [19] from 0.24 to 2% essential oil. So we can conclude that the factors influence the yield are numerous, the harvest season, the origin of the plant, the parts of the plant used and the extraction processes.

Analysis of essential oil of *Chamaemelum nobile* (L.) All.

The analysis of the results of the chemical composition carried out by gas chromatography coupled with the mass spectrometry of essential oil of *Chamaemelum nobile* (L.) All. studied is listed in Table 2.

Analysis of the results given in Table 2 showed the chemical composition of the essential oil of *Chamaemelum nobile* (L.) All. The main majority compounds identified are: Verbenone (33.74%), Pulegone (26.45%), and 3-Cyclohexen-1-one, 2-isopropyl-5-methyl- (8.90%). The majority compounds were obtained as with a percentage of (69.09%). The plant contains many biologically active compounds. The oil has been little studied, its composition is quite variable according to the geographical region of production, the harvest season and the duration of the distillation. The results of a comparative study of the essential oil of *Chamaemelum nobile* (L.) All. obtained by Antonelli et al. (1998) [20] from two different regions of northern Italy examined by gas chromatography coupled with mass spectrometry by GC-MS show no significant difference in the composition of the two oils which were characterized by the presence with a high content of isobutyl angelate respectively (36.3-38.5%), and of 2-methylbutyl angelate isobutyl angelate (18.2-20.30) thus the presence of other Esther. The results obtained by Farkas et al. (2003) [21] carried out in the Slovak Republic on the essential oil of *Chamaemelum nobile* (L.) All. show the presence of the following chemotype: angelates (63.4%) and isobutyrates (19.6%) as main components among the esters: acetates (3.5%), methacrylates (5.7%), tiglates (0.5%) and 2-methylbutyrates (0.8%) were other important components.

The terpenoid fraction was only 4.5% of the oil. The main components of the oil analyzed were 2-methylbutyl angelate (14.4%), isobutyl angelate (21.6%), 3-methylamyl angelate (8.4%) and 2-methyl-2-propenyl angelate (9.1%). The other constituents of the oil exceeding 5% were 2-methylbutylisobutyrate (5.2%), isoainyl agelate (5.5%), and (E) -methyl-2-isobutyrate -butenyl (5.1%). As shown in the review of Lawrence (1998) [22], there are significant differences in the qualitative and quantitative composition of oils from *Chamaemelum nobile* (L.) All. of different origin. These differences may be the result of ontogenetic variation, cultured difference, and extrinsic conditions such as climate and altitude.

Antimicrobial activities

The results of antibacterial evaluations of all methods applied to the essential oil of *Chamaemelum nobile* (L.) All. are expressed in terms of the diameters (Φ) of zones of inhibition measured around the discs, the minimum inhibitory concentration (MIC), the minimum bactericidal concentration (CMB) and the ratio (CMB / MIC). All the values obtained are listed in Table 3.

It can be concluded that from the results obtained, the essential oil of *Chamaemelum nobile* (L.) All. possess interesting antimicrobial activities against the strains studied. The diameters of zones of inhibition Φ obtained from the disk diffusion method vary from 11.28 to 16.10 mm, *Staphylococcus epidermidis* (CECT 231), *Listeria monocytogenes* (CECT 934), *Yersinia enterocolitica* (CECT 4315), and *Pseudomonas aeruginosa* (CECT 108) have strong activity, while *Escherichia coli* (CECT 515) and *Listeria innocua* (CECT 910) have medium activity. The minimum MIC inhibitory concentrations vary from 26.67 to 70.11 µL / mL and the minimum bactericidal CMB concentrations vary from 30.02 to 71.37 µL / mL. So in general the essential oil of *Chamaemelum nobile* (L.) All. exhibits remarkable activities with respect to all the strains studied. According to the

CMB / CMI report, the essential oil of *Chamaemelum nobile* (L.) All. possesses bactericidal effects with respect to all the strains studied (Table 4). 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

As part of our contribution to the enhancement of medicinal plants from the flora of Morocco, the essential oil extracted from the aerial part of *Chamaemelum nobile* (L.) All. was first studied locally. The qualitative and quantitative analysis made it possible to identify respectively 72 constituents among which Verbenone (33.74%), Pulegone (26.45%), and 3-Cyclohexen-1-one, 2-isopropyl-5-methyl- (8.90%) were obtained as majority compounds with a percentage of (69.09%), the structures of which were established by GC-MS. The *in vitro* antibacterial tests carried out revealed significant bacterial activity against all the strains studied, in particular bactericidal activities. The results obtained showed that the essential oil of *Chamaemelum nobile* (L.) All. has interesting prospects, and can be the subject of several biological applications. At the end of this work, it seems important to continue the search for essential oils from medicinal plants and also other extracts and purified molecules. It is also recommended to study other activities *in vitro* and *in vivo*.

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Table 1. Yield of essential oil.

Species	Yield
Chamaemelum nobile (L.) All.	0.44 %

Table 2. Chemical composition of the essential oil of *Chamaemelum nobile* (L.) All.

Peak	RT	Composants	(%)
1	7.72	α-pinene	0.09
2	7.77	Dextro-α- pinene e	0.10
3	8.09	3-Carene	0.12
4	8.56	Hydrazinecarboxylic acid, ethyl ester	0.06
5	9.17	α-Myrcene	0.32
6	9.25	β-Terpinen	0.18
7	9.40	β- pinene	0.28
8	9.72	Propanamide, 2-hydroxy-	0.06
9	10.33	3-Octanol	0.62
10	10.83	Propanoic acid, 2-methyl-, 2-methylbutyl ester	0.07
11	10.91	6-Isopropenyl-3-methoxymethoxy-3-methyl-cyclohexene	0.05
12	11.05	D-Limonene	0.54
13	11.59	Eucalyptol	2.33
14	12.45	p-Mentha-3,8-diene	0.43
15	13.85	Oxirane, 2-butyl-3-methyl-, cis-	0.03
16	14.57	neo-iso-Dihydrocarveol	0.08
17	14.95	Chrysanthenone	0.29
18	15.76	Isobornyl thiocyanoacetate	0.30
19	15.99	p-Menthone	2.23
20	16.48	dl-Isopulegol	0.99
21	16.87	(14-methyl-(Z)-8-hexadecen-1-ol	1.04
22	17.23	Levomenthol	0.03
23	17.30	3,7-dimethyloct-2-en-1-ol	0.08
24	18.63	2,3,4,5,6,7-hexahydro-1H-indene	0.06
25	19.96	p-Menth-4(8)-en-3-one	0.26
26	20.19	p-Menthan-3-one	0.02
27	20.55	Pulegone	26.45
28	20.82	3-Cyclohexen-1-one, 2-isopropyl-5-methyl-	8.90
29	20.87	Menthol	1.49
30	21.08	Isopiperitenon	1.11
31	21.28	Menthyl acetate	0.10
32	21.51	6-ethenyl-6-hydroxy-5-methylbicyclo[3.2.0]heptan-2-one	0.50
33	21.64	Cyclohexasiloxane, dodecamethyl-	0.39
34	21.75	Hexahydrothymol	0.09
35	21.88	trans-p-Menth-8-ene	0.13
36	21.99	Thymol	0.09
37	22.39	trans-1-Isopropenyl-4-methylcyclohexane	0.67
38	23.91	Verbenone	33.74
39	24.11	α-Ionol	0.11

40	24.24	(-)-α-Bourbonene	0.07
41	24.83	Methyleugenol	0.45
42	25.42	Caryophyllene	1.59
43	26.24	11H-Benzo[a]cyclopenta[d]cycloocten-11-one, 4-acetyltetradecahydro-	0.20
44	26.35	(+)-α-himachalene	0.13
45	26.57	Humulene	2.44
46	27.25	Longifolene-(V4)	0.10
47	27.35	Cycloheptasiloxane, tetradecamethyl-	0.48
48	27.97	(-)-β-himachalene	0.61
49	28.18	p-Isopropylphenetole	0.11
50	28.33	Isolongifolene, 4,5,9,10-dehydro-	0.09
51	28.47	Mint furanone	0.17
52	28.56	α-Cadinene	0.09
53	28.85	3a-methyl-2,3,4,5-tetrahydrocyclopenta[a]naphthalen-3-ol	0.10
54	29.50	Dihydrojasmone	0.06
55	30.21	Elemicin	0.09
56	30.44	Terrein	0.13
57	30.66	(3E)-4,4-dimethyl-3-(3-methylbut-3-enylidene)-2-methylidenebicyclo[4.1.0]heptane	0.08
58	30.96	Caryophyllene oxide	0.53
59	31.40	Isoaromadendrene epoxide	0.08
60	31.77	Calarene epoxide	0.55
61	32.27	Aromadendrene oxide-(2)	0.05
62	32.45	γ-Muurolene	0.09
63	32.59	Cyclooctasiloxane, hexadecamethyl-	0.22
64	33.14	Ar-tumerone	1.15
65	34.18	Curlone	0.19
66	36.27	(5β,7β,10β)-3,11-Eudesmadien-2-one	0.14
67	37.12	Cyclononasiloxane, octadecamethyl-	0.14
68	37.88	2-Pentadecanone, 6,10,14-trimethyl-	0.04
69	41.07	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	0.17
70	41.12	Cyclodecasiloxane, eicosamethyl-	0.11
71	41.21	Dibutyl phthalate	0.10
72	48.23	Tetracosamethyl-cyclododecasiloxane	0.19
Total			**95.17**

Table 3. Parameters of antibacterial activity of essential oil of *Chamaemelum nobile* (L.) All.

Strains	Applied methods	*Chamaemelum nobile* (L.) All.
Staphylococcus epidermidis	**Φ (mm)**	15.65 ± 0.34
	CMI (µL/mL)	26.67
	CMB (µL/mL)	33.01
	CMB/CMI	1.23
Listeria monocytogenes	**Φ (mm)**	14.76 ± 0.53
	CMI (µL/mL)	28.10
	CMB (µL/mL)	30.02
	CMB/CMI	1.06
Listeria innocua	**Φ (mm)**	11.28 ± 0.81
	CMI (µL/mL)	50.39
	CMB (µL/mL)	50.40
	CMB/CMI	1.00
Escherichia coli	**Φ (mm)**	12.6 ± 0.72
	CMI (µL/mL)	63.15
	CMB (µL/mL)	65.43
	CMB/CMI	1.03
Yersinia enterocolitica	**Φ (mm)**	14.45 ± 0.25
	CMI (µL/mL)	70.11
	CMB (µL/mL)	71.37
	CMB/CMI	1.01
Pseudomonas aeruginosa	**Φ (mm)**	16.1 ± 1.44
	CMI (µL/mL)	52.61
	CMB (µL/mL)	53.03
	CMB/CMI	1.00

Table 4. Bactericidal or bacteriostatic character of essential oil.

Strains	*Chamaemelum nobile* (L.) All.
Staphylococcus epidermidis	Bactericidal
Listeria monocytogenes	Bactericidal
Listeria innocua	Bactericidal
Yersinia enterocolitica	Bactericidal
Escherichia coli	Bactericidal
Pseudomonas aeruginosa	Bactericidal