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Chemical Composition and Antimicrobial Activities of the Oil Essential Oil of Mentha Rotundifolia

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Abstract:- Essential oils extracted from aromatic or medicinal plants have recently proved different applications in various fields (manufacture of medicines, perfumes and foodstuffs). The objective of this study is to evaluate the antimicrobial effect of essential oils extracted from the plant Mentha Rotundifolia against pathogenic strains. Essential oils are extracted by the "Clevenger" hydrodistillation technique. The results obtained show that the yield of the essential oils is of the order of 1.58% and the chemical composition of the essential oils is determined by the gas chromatography (GC) technique, the products obtained with their proportion are: 2- isopropylidenecyclohexanone (11.99%), eucarvone (11.42%), gamma.-Muurolene (8.61%), 2-isopropyl-5-methyl-3-cyclohexen-1-one (6.83%) and p-menthane- 1,2,3-triol (6.72%).
For antibacterial activity, the essential oil of Mentha Rotundifolia is shown to be effective against bacteria: Porteus mirabilis (susceptible with a diameter of 5, 6), Acinobacter baumannii (sensitive with a diameter of 1.6), Salmonella sushi (susceptible with a diameter of 1.4), Escherichia Coli (sensitive with a diameter of 1.2), Klibesilla pneumoniae (sensitive with a diameter of 1.1), Citrobacter freundii (intermediate with a diameter of 0.8) and Staphylococcus aureus (1 cm).
While the bacterium Pseudomenas aeruginosa (0cm) showed resistance against the inhibitory effect of essential oils of Mentha Rotundifolia.

Keyword: Essential Oils, Mentha Rotundifolia, Pathogenic Strains, Hydrodistillation.

I- INTRODUCTION

Mentha species are grown in several countries around the world for the production of essential oils (Sutour et al., 2010, Ladjel et al., 2011). According to the flora of Algeria, this genus is represented by five main species: Mentha rotundifolia, Mentha longifolia, Mentha spicata, Mentha aquatica and Mentha pulegium (Quezel and Santa, 1962).

M. Rotundifolia (L.) Huds (MR), commonly called "green apple" is a perennial herb with wild growth. It is widely distributed in northern Algeria in the sub-humid areas, along the rivers in the plains and mountains where it is known as "Timija or Timarssat" (Brada et al., 2006), and it is widely used, for example; A leaf decoction is made for topical application to treat furunculosis and abscesses, to reduce fever and as a mouthwash for dental pain (Brahmi et al., 2014b). In addition, the plant would treat bronchitis, cough and ulcerative colitis.
It is also taken as a tonic, used as a stimulant, stomach, carminative, analgesic, choleretic, antispasmodic, sedative, and hypotensive as well as a common spice (Ladjel et al., 2011).
This oxygenated monoterpene has very interesting biological effects. It presents cardiovascular effects (hypotensive activity, vasodilator, bradycardia), activity on the sympathetic nerve centers (relaxant, stimulant, depressant), antibacterial and antifungal properties, and also acts as an agent delaying the reproduction of the malaria vector Anopheles stephensis (Damien et al., 2003, Tripathi et al., 2004).

Piperinity oxide is also of interest for the synthesis of heterocycles, pyrazoles, pyrazolines and allylic alcohols (Ghoulami et al., 2001).
The composition of essential oils among species of the genus Mentha has demonstrated a chemical diversity due to geographical environmental factors (Beghidja et al., 2007, Brada et al., 2007, Hussain et al., 2010, Kumar et al., 2011; Baser et al., 2012, Sitzmann et al., 2014, Kasrati et al., 2015).
A research in the literature reveals some studies on the biology activities of MPE and MRE conducted on plants harvested outside Algeria.
The importance of the essential oils of the plant M. Rotundifolia is illustrated in many scientific works,
describing the chemical composition of the plant, contain the following compounds: trans-Piperitone epoxide with a percentage of 30.2% and Piperitone oxide with a percentage 8.7%. (F. Brahmi et al., Industrial Crops and Products xxx (2016).

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The objective of this work is to study the antimicrobial effect of the essential oils extracted from the leaves of Mentha rotundifolia grown in the region of Allal Tazi - Kenitra province on pathogenic germs from our microtheca and to determine the chemical composition of Mentha rotundifolia essential oils, growing wild in this region and highlighting a possible variability in the chemical composition of these oils.
This study is part of the broader context of enhancing the biodiversity of Moroccan aromatic plants for their medicinal and food properties.

II- MATERIALS AND METHODS

A-Plant material

The harvest of the plant was carried out between March and September while its flowering is between June and August. Only the aerial part (leaves) was dried in the open air, in the shade and at room temperature (25 ° C).

B-Hydro distillation

Extraction of the essential oil was carried out by hydrodistillation in a "Clevenger" type apparatus. The plant, once dried, is decorticated with its leaves and then put into the 1 liter flask with a quantity of 100 g added with 500 ml. distilled water.

The whole is boiled for a period of 3 hours. Three distillations were performed to recover a volume of 1ml of the essential oil. The recovery of essential oils is made by cooling the refracting material.
The resulting extract is dissolved in ethanol solvent and placed in a cryotube and stored in the dark in a refrigerator at 4 ° C. [21].

C-Microorganisms studied

The bacteria studied are: *Porteus mirabilis, Acinetobacter baumannii, Salmonella Typhi, Escherichia coli, Klebseilla pneumoniae, Citrobacter freundii, Staph aureus and Pseudomenace aeruginosa* were chosen for their high frequencies to contaminate foodstuffs and for their pathogenicity and The bacteria tested are cultured on their specific media for their recovery, the minimal inhibitory concentrations (MIC) of the essential oil of Mentha rotundfolia are determined according to the method reported by Remmal et al. (1993) and Farah et al. (200 I).d for their pathogenicity

a- Determination of the minimum inhibitory concentration:

The determination of the minimum inhibitory concentration (MIC) of the essential oils was carried out according to the method reported by (Remmal et al., And Satrani et al). Due to the immiscibility of the essential oil with water and therefore with the culture media, emulsification was carried out using a 0.2% agar solution in order to promote the seed / compound contact.

In order to find the minimum inhibitory concentration of essential oils, a series of dilutions of this oil is prepared as follows: 1/10, 1/25, 1/50, 1
/ 100.1 / 200, 1/300 and 1/500 in an agar solution. In test tubes, each containing 13.5 ml of Mueller solid medium, are added 1.5 ml of each of the dilutions so as to obtain the lowest final concentrations of the essential oil which inhibits any visible bacterial growth. naked eye including 1/10, 1 / 25.1 / 500, 1/50, 1/100, 1/200, 1/1300 1/500 (v / v), then the contents of each tube is poured into a petri dish after stirring. Controls, containing the culture medium and the 0.2% agar solution, are also prepared after the incubation time (16 to 20 hours) at 37 ° C.

b-Determination of The minimum bactericidal concentration (MBC)

Is determined following the inoculation of a sample of the non-growth plates on Mueller-Hinton agar. The lowest concentration of essential oil resulting in the death of 99.99% of the bacteria after incubation at 37 ° C corresponds to the minimum bactericidal concentration CMB. Each test was repeated three times for confirmation.

D-Gas Chromatography (GC)

This is a separation technique with an apparatus that contains a column exists in the oven consists of separated volatile constituents in a mixture, their use depends on 3 parameters that are: sensitivity; reproducibility; lifetime.
We begin by introducing the sample which takes place in three phases

Spray of the sample.

Transfer of the sample to the column.

Initialization of the gas chromatography (GC) oven program.
GC Mechanism
Analytes cross the column

They are separated according to two criteria: volatility and polarity.

Separation of compounds:

As the elution progresses; the compounds are quantified in the detector.

The retention time (TR) is the passage time of an analyte in the column.

The chromatogram is the representation of the intensity in the detector during the retention time **(TABLE.1).**

E- Results and discussion

a- Chemical composition of essential oils

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The results of the analysis of the essential oil extracted from the plant Mentha rotundifolia are grouped in the following table showed the presence of chemical substances.

TR Retention time	Area	% of the chemical element exists in the plant	Name of the chemical compound
4.574	541806	0.61	alpha.-Pinene
5.434	437468	0.49	beta.-Terpinene
5.545	729177	0.82	beta.-Pinene
5.630	1212828	1.37	beta.-Myrcene
5.913	612906	0.69	1-Octen-3-ol
6.203	660823	0.75	(+)-4-Carene
6.418	741751	0.84	D-Limonene
6.542	1042369	1.18	beta.-trans-Ocimene
6.727	3280721	3.71	p-Cineole
7.025	1083124	1.23	gamma.-Terpinene
7.301	363321	0.41	p-Mentha-3,8-diene
7.485	1523508	1.72	5-Isopropyl-2-methylbicyclo[3.1.0]hexan-2-ol
7.936	2206188	2.50	Octen-1-ol, acetate
8.984	3330074	3.77	2,5,5-Trimethyl-3,6-heptadien-2-ol
9.551	4395660	4.97	4-Terpineol
9.916	769957	0.87	alpha.-Terpinol
10.167	481075	0.54	p-Cymen-8-ol
10.975	5114067	5.78	p-Menthen-3-one $$ Pulegone
11.195	808752	0.91	Piperitone 1-oxide
11.291	2691757	3.04	Pipertone, oxide $$ 1,2-Epoxy-p-menthane-3-one $
11.346	6036983	6.83	2-Isopropyl-5-methyl-3-cyclohexen-1-one
11.509	424007	0.48	Isopulegylacetate $$ 2-Isopropenyl-5-methylcyclohexyl acetate
12.076	341043	0.39	.beta.-Elemene,
12.668	4008337	4.53	Caryophyllene
12.872	377012	0.43	beta.-Farnesene
12.957	10095277	11.42	**Eucarvone**
13.058	1510506	1.71	(+)-Epi-bicyclosesquiphellandrene
13.278	10580593	11.99	**2-Isopropylidenecyclohexanone**
13.380	5936941	6.72	p-Menthane-1,2,3-triol
13.535	1218692	1.38	Grindelene
13.671	7610067	8.61	gamma.-Muurolene
13.948	1034780	1.17	o-Menth-8-ene, 4-isopropylidene-1-vinyl-
14.155	478750	0.54	delta.-Cadinene
14.452	236749	0.27	1-Isopropyl-4,7-dimethyl-1,2,4a,5,6,8a-hexahydronaphthalene
14.493	370836	0.42	Calamenene
15.586	960833	1.09	Cubenol
15.790	799013	0.90	delta.-Cadinol
16.483	967320	1.09	alpha.-Cadinol

Table1: Composition of essential oil of Mentha rotundifolia

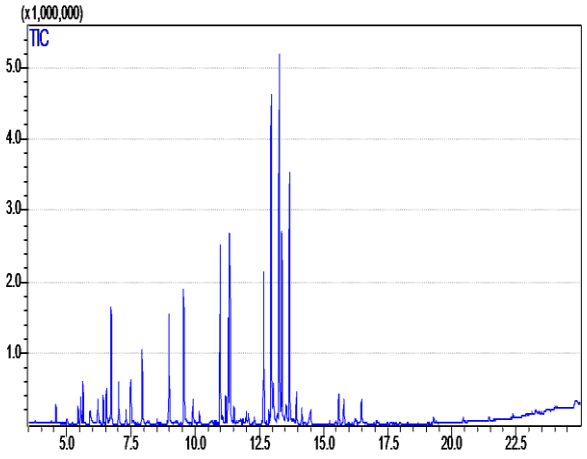

Figure 1: gas chromatography graph

The study of the literature reveals differences in the qualitative and especially quantitative composition of the essential oil Mentha rotundifolia. Indeed, we have seen that the contents of the different compounds are far from fixed and sometimes vary a lot from one study to the next. In order to compare the results obtained and to try to understand these variations in *Mentha rotundifolia L.*

- ✓ Climatic conditions:
- ✓ The age of the plant:
- ✓ The harvest period
- ✓ Desiccation:
- ✓ Conservation of the plant

Analysis of the chemical composition of the essential oil, derived from the plant Mentha rotundifolia, grown in the region of Sidi Allal Tazi province of Kenitra. by gas chromatography, showed us the presence of the two dominant elements: 2-Isopropylidenecyclohexanone with a percentage of 11.99% and Eucarvone with a rate of 11.42%. in comparison, these percentages are totally different from those of the essential oil from the same plant grown in Miliana, which is located in the south of Dahra whose major compounds are piperitone oxides: 31.4% Piperitone oxide has been reported as the major constituent of the essential oil of M. rotundifolia from Greece (Kokkini, Papageorgiou, 1988) and Germany (Van Os, Hendriks, 1975). The variation of the chemical composition of the essential oils of M. rotundifolia and revealed the existence of particular chemotypes with as major compounds menthyl acetate (Kokkini, Papageorgiou, 1988), dihydrocarvone (Hendriks et al., 1976), 2,4 (8), 6-p-menthatrien-2,3-diol (Pino et al., 1999) and pulegone (Il Idrissi, Bellakhdar, 1989). The difference in composition observed on the essential oils investigated is likely to be related to abiotic factors such as the climate specific to the regions of origin of the samples, geographical factors such as altitude and the nature of the soil.

b-Biological test of the essential oil of Mentha Rotundifolia

Table 2: Biological effect of HE of Mentha rotundifolia.

	Gram	Types	Name of Bacteria	Inhibition diameter (mm)	Effect
Bacteria	Gram (-)	bacilli	*Escherichia Coli*	11	S
			Pseudomonas aeruginosa	0	R
			Klibesillapneumoniae	10	S
			Citrobacterfreundii	8	R
			Salmonella sushi	14	S
			Proteus mirabilis	56	S
		Coccies	*Acintobacterbaumanni*	16	S
	Gram (+)	Coccies	*Staphylococcus aureus*	10	S

S: sensitive

R: resistant

The results obtained show that all the bacteria tested are inhibited by the amount of Essential Oil used.

The biological test gave us a strong sensitivity of Acintobacter baumanni with a diameter of 1.6 cm, followed by Salmonella (1.4 cm). For Escherchia Coli, Staphylococcus aureus and Kliebsella pneumoniae showed approximately the same sensitivity, the first has an inhibition diameter of 1.1
cm, the second and the third both have an inhibition diameter of 1 cm. The inhibitory effect of Mentha

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rotundifolia oil that has been recorded on Citrobacter freundii is 0.8cm. Whereas for Pseudemonas aeruginosa, the essential oil applied in the antimicrobial activity was not successful (**TABLE 2**)

Table 3: Different concentrations of HE and their effects on bacterial strains

Dilutions / Souches	Témoin	1/10	1/25	1/50	1/100	1/200	1/300	1/500
Escherichia Coli	++	--	--	--	++	++	++	++
Acintobacterbaumanni	++	--	--	--	--	++	++	++
Staphylococcus aureus	++	--	--	--	--	++	++	++
Pseudomenas aeruginosa	++	--	--	--	++	++	++	++
Klibesiellapneumoniae	++	--	--	--	++	++	++	++
Salmonella sushi	++	--	--	--	++	++	++	++
Citrobacterfreundii	++	--	--	--	++	++	++	++
Proteus mirabilis	++	--	--	--	++	++	++	++

(+) Growth
(-) Inhibition

The minimum inhibitory concentrations (MIC) of the Mentha Rotundfolia essential oil are determined according to the method reported by Remmal et al. (1993) and Farah et al. (200 I).

The search for the minimum inhibitory concentration showed that the dilution of 1/200 is the minimum inhibitory limit, it is the lowest concentration which showed an effective antibacterial effect against the bacterial strains tested namely Acinobacter baumanni (16mm), Staphylococcus aureus (10mm). For Klibesiella pneumoniae (10mm), Salmonella sushi (14mm), Escherichia Coli (11mm); Proteus mirabilis (56mm) and Pseudomenas aeruginosa (0) the first signs of inhibition started with the concentration of 1/100 which could block the development of these types of bacteria, And the total inhibition of all bacterial species did not is revealed only with a concentration of 1/50.
Pour en finir par la déclaration que cette concentration est la CMI de l'huile essentielle de la plante Mentha rotundifolia**.(TABLE3).**

CONCLUSION:

The chemical analysis of the essential oils of Mentha rotundifolia by gas chromatography showed a strong diversification at the level of the chemical constituents with very variable contents in the plant.
The chemical component:
2-Isopropylidenecyclohexanone is the most dominant with a rate of 11.99%, followed by Eucarvone with a percentage of 11.42%, gamma.-Murolene with a percentage of 8.61%, 2-Isopropyl-5-methyl-3- cyclohexen -1-one (6.83%) and p-Menthan-1,2,3-triol (6.72%).
The remaining substances found have a rate of less than 6%. The qualitative analysis of this essential oil of Mentha rotundifolia has shown a great interest in the microbiological field by its inhibitory effect.
The results obtained proved its bacteriostatic efficacy against all microorganisms tested with the exception of Pseudomonas aeruginosa. The minimum inhibitory concentration used is of the order of 1/200. The strain Proteus mirabilis was the most sensitive.

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