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CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITIES OF THE ESSENTIAL OIL OF WILD ALGERIAN *MARRUBIUM VULGARE L*

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ABSTRACT

This study describes the chemical composition and antimicrobial activity of essential oil of *Marrubium vulgare* growing wild in Mascara region. This plant is very used in Algeria and widely used by local people for its medicinal properties. We proposed to determine the physicochemical, organoleptic and chemical identification of these components. The essential oil from the leaves of *Marrubium vulgare* was obtained by hydrodistillation and analyzed by GC and GC/MS . Other parameters such as refractive index, optical rotation; density, polarimetric deviation;freezing point and Solubility in ethanol are also measured. 99,01% of the total peak areas were identified. The main constituents of the essential oil were the hydrocarbon and oxygen compounds: Sulfamethoxypyridazine n 14.44%, Eugenol 10,50%,o- Undecylenic Acid 10,48% _-Citronellol 9,78% ,Silvex8,38% ,Germacrene-D 8,21,pinene 7,30% Carbromal4,87%.The antibacterial activity of the oil was determined using the agar diffusion method and it was found that it was active against Escherichia coli (ATCC 25923), Salmonella typhimurium ATCC14028), Staphylococcus aureus (ATCC-29213) ,Bacillus subtilis (ATCC-6633) ,*Escherichia coli (G-)*(ATCC (35218) and Pseudomonas aeroginosa (ATCC 27853).

Keywords*: Marrubium vulgare*, Essential oil, antimicrobial activity, GC/SM.

INTRODUCTION

The application of Medicinal plants, which are well known for their pharmacological activity, contain many substances that exhibit radical scavenging properties .such bioactive plant components may increase the stability of foods and, at the same time, improve their health properties associated with anti-cancer antiallergic and anti-inflammatory activities of polyphenols in the human body [(1)]. *Marrubium vulgare* (Lamiaceae) is a plant traditionally used for the treatment of diabetes in Algeria. Compositional analysis of the aqueous infusion revealed the presence of fifteen metabolites, all belonging to the class of polyphenols. Particularly, seven flavonoids have been detected [(2)],The plant is reported to possess hypoglycemic[(3)], vasorelaxant, antihypertensive, analgesic ,anti-inflammatory [(4)], antioxidant activity[(5)], antioedematogenic activityand many other reported biological activities.Phytochemicals present in the plant include caryophyllene oxide, trans-aryophyllene[(6)]

Experimental

Plant material: Leaves of *Marrubium vulgare* were collected from Ain fares situated in the North West of Algeria in may 2012. This plant was identified by botanists of Faculty science. A voucher specimen is deposited in the Herbarium of the Department of Botany and Ecology at the Agronomic Institute under code number 2011-562458.The leaves were shade, dried, and stored in a tightly closed container for further use. The essential oils were obtained by hydro-distillation from the plant material using a Clevenger –type apparatus for 3h. The essential oil was dried over anhydrous Na_2SO_4 and stored in a scaled vial in the dark; at 4°C [(7)].The essential oil yield was calculated on a dry weight by gravimetric method.

Analysis of the essential oils: In the essential oil these some parameters are measured the refractive index, density, polarimeter deviation; point of freezing, solubility in ethanol at 90^0C; and the acidity. The analyses of the volatile constituents were run on a Hewlett-Packard GC-MS Gas Chromatograph (FID) detector system GC: 5890 series II, MSD 5972) the fused-silica HP-5MS capillary Colum (30 m X 0.25 mm id, film thickness of 0.25 (µm) was directly coupled to the MS. The carrier gas was helium, with a flow rate of 1.2 ml/min. Injector port 250°C and oven temperature was programmed as flows :isotherm at 50°C for 1 min, then increased to 280°C at a rate of 5°C/min and subsequently held isothermal for 20 min.Detector280°C, volume injected: 0.1 Ol of 1 % solution(diluted in hexane) and split ratio:1:50. Ionization voltage:70 ev ion source temperature 280°C, mass range: 40-300;mass units scan time 1,5 Sec .Software

adopted to handle mass spectra and chromatograms was a skin station. The extract composition percentage was calculated from the GC peak area. For retention indices (RI) determination, a hydrocarbon series was chromatographed together with the essential oil on a polar columns, and their retention times were used to convert GC retention values to RI by linear interpolation with those of authentic compounds and literature data [(8),] and also by computer matching them with the NIST/EPA/NIH MASS SPECTRAL LIBRARY data with those of the published data by Adams [(9, 10)] .

Microbial strains: Microbial strains: Antimicrobial activity was carried out according to the disc diffusion assay, tested in vitro against: Escherichia coli (ATCC 25923), Salmonella typhimurium ATCC14028),*Staphylococcus aureus*(ATCC-29213) *Bacillus subtilis* ATCC-6633 , *Escherichia coli* (G-) ATCC 35218 and *Pseudomonas aeroginosa* (ATCC 27853).

The bacterial suspensions were adjusted to1×10^7 CFUmL− (equivalent to 0.5 McFarland).

Antimicrobial activity: Antimicrobial tests were carried out using the disc diffusion method. The muller-hinton mutriet agar and dimethyl sulfoxide (DMSO) solutions (in ratio 1:25 v.v-1) were vortexes for 2 min and immediately 20 ml were poured into sterile Petri dishes (90 mm diameter) and left to set for 30 min. Paper discs (6 mm diameter) were impregnated aseptically with 3 µl of essential oil at final concentrations of 1-20µ g/ml and placed on the inoculated agar surfaces. After aerobic incubation for 24 hours at 37°C, the antimicrobial activity was estimated by measuring the diameters of inhibition zone12. The control test by aqueous DMSO alone showed no toxicity in the concentrations used for these bacteria. The antibacterial minimum inhibitory concentrations (MICs) were performed according to the Mueller-Hintonbroth microdilution method in 96 multiwell microtiterplate. The essential oils were dissolved in the aqueous DMSO and the initial concentration was 25µ g/ml The initial test concentration was serially diluted two fold .Each well was inoculated with 5 µg/mlof suspension containing 10^7 CFU/ml of bacteria and incubated for 24hours at 37°C. The MIC of the tested material was determined as the lowest concentration at which no visible growth of the microorganism had occurred. Each test was carried out in triplicate.

RESULTS AND DISCUSSION

Physicochemical analysis showed an essence yellow, with Pleasant odor .the essential oil yield obtained by the hydro-distillation of dry plant was 0,33%.Determination density was obtained by double weighing d = 0.978, the optical activity =+6.5 by polarimetry and the refractive index n = 1.4654 by an interferometric method. The chromatograms of the essential oil had numerous peaks and many of them were overlapping. Gas Chromatographic analysis was performed under some specific conditions the compounds are listed along with their percent constituents Table 1.

Table 1. Physicochemical composition of *Marrubium vulgare*

Specification	***Marrubium vulgare***
Density D20	0.987
Refractive index	1.4665
Optical activity N20	+6.5
Solubility in ethanol90(%)	1 : 3
Freezing Point (°C)	-18
Acidité	0.41

Essential oils more then 23 components were separated on HP- Column and of them were identified as major component representing of 99,01% of the total Table 2 The identification of chromatogram component demonstrated that this species was characterized by its high rate: Sulfamethoxypyridazine 14.44%, another important constituent were Sulfamethoxypyridazine n 14.44%, Eugenol 10,50%,o- Undecylenic Acid 10,48% _-Citronellol 9,78% ,Silvex8,38% ,Germacrene-D 8,21,pinene 7,30% Carbromal4,87%.Chemical analysis of essential oil showed that its major products were Carbromal 4.87 2-Pentene, 3,4-dimethyl-, (Z)- 4.52%; tau.-Cadinol 3.70%; Camphor 2,29% .Other components were present with smaller percent. By other findings. The chemical constituents of the essential oil from aerial parts of *Marrubium vulgare*, collected in Algeria, were analyzed by GC and GC/MS. The oil yield of the dried plant aerial parts, obtained by hydrodistillation, was 0.04 % (w/w)[(11).] Compounds, accounting for 82.46 % of the oil, were identified. The major constituents were: 4,8,12,16-Tetramethyl heptadecan-4-olid (16.97 %), Germacrene D-4-ol (9.61 %),α- pinéne (9.37 %) Phytol (4.87 %), Dehydro-sabina ketone (4.12 %), Piperitone (3.27 %), δ – Cadinene (3.13 %), 1-Octen-3-ol

(2.35 %) and Benzaldehyde (2.31 %)(12) .the essential oil of *marrubium vulgare* l.from poland , The main components of the oil were as follows: E-caryophyllene,germacrene D, bicyclogermacrene, and α-humulene. The oil from the flowering plants was shown to have the highest contents of germacrene D (43.36%). Other constituents (Ecaryophyllene – 44.54%, bicyclogermacrene -20.6%, and α-humulene – 5.79%) were found in higher amounts in the oil from vegetative stage plants (13).

Of this essential oil appears to include a bacterial membrane damage that occurs when the essential oil passes through the cell wall and cytoplasmic membrane, and disrupts the structure of their different layers of polysaccharides, fatty acids and phospholipids (14).

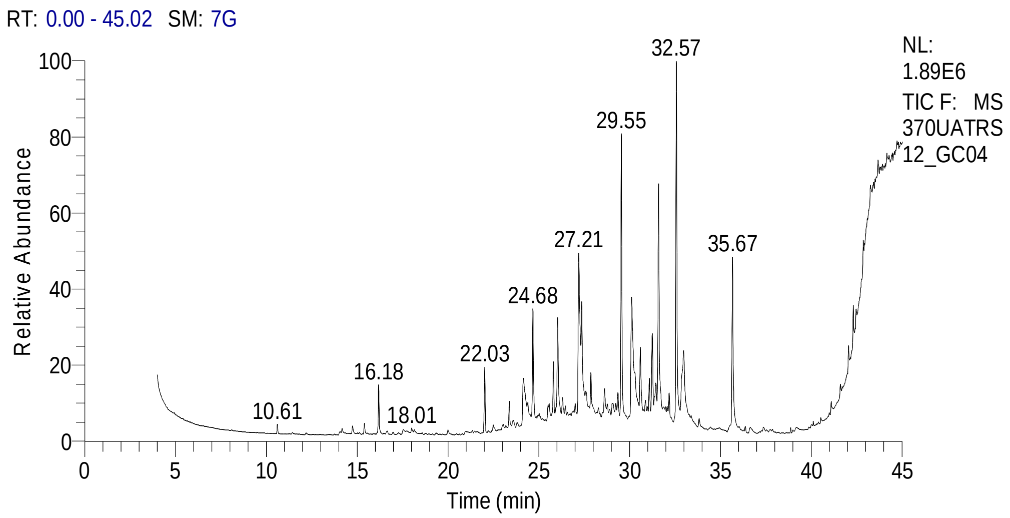

Table 2. The major identified components in essential oil from *Marrubium vulgare* analyzed by GC-MS technique with retention indices on HP-5MS capillary Column

N	RT	Name	%
1	10.61	Pinene	7.30
2	14.18	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	0.61
3	14.75	Clorophene	0.43
4	15.41	à-CAMPHOLENE ALDEHYDE	0.40
5	16.18	Cypentil	1.77
6	17.55	Octadecane	0.33
7	18.01	Bromomethane	0.50
8	22.03	Camphor	2.29

9	23.38	Pentalin	0.97
10	24.16	odecanedihydrazide	3.05
11	24.68	(2-Pentene, 3,4-dimethyl-, (Z)-	4.52
12	25.81	Diacetonalcool-	1.99
13	26.04	Carbromal	4.87
14	27.21	Octadecane, 1-isocyanato-	9.78
15	27.87	Torreyol	1.90
16	29.55	Eugenol	10.50
17	30.12	Silvex	7.72
18	30.60	camphor	2.87
19	31.25	tau.-Cadinol	3.70
20	31.60	Undecylenic Acid	10.48
21	32.58	Sulfamethoxypyridazine	14.44
22	32.98	Germacrene-D	8.37
23	35.67	Diethylstilbestrol	0,20
Total			**99,01%**

Staphylococcus aureus (inhibition zone:12 mm, MIC:14.5 µg/ml *Salmonella typhi* (inhibition zone : 16mm, MIC:14.60 µg/ml Escherichia coli ATCC 25922 (inhibition zone: 15.5mm, MIC: 10.20µg/ml *Bacillus subtilis* (ATCC-6633) (inhibition zone 13.5mm) and *Escherichia coli* ATCC 35218 (inhibition zone:15.30 mm, MIC: 12.5 µg/ml. Our results from *Marrubium vulgare* essential oil found in Algeria may be regarded as Sulfamethoxypyridazine chemotype and its broad spectrum of activity against bacteria supports the traditional uses of this plant as disinfectant (table 3). The oil could be exploited to treat topical infections. In front of these encouraging results, further studies on a larger specter of antimicrobial strains activity will be necessary. Actually, the isolation of the essential oil components are under progress.

Table 3. Inhibition zone (mm) using direct contact technique in agar medium and MIC (µg/mL) for the essential oil using microdilution method in 96 multiwall microliter plate

Microorganism	Diameter of inhibition zones (mm)	MIC (µg/mL)

Pseudomonas Aeruginosa (G-) ATCC 27853	14.5±0.14	25.50
Escherichia coli ATCC 25922	15.5± 10.20	0.18
Salmonella typhimurium TCC14028	16.0 ± 0.33	14.60
Escherichia coli (G-) ATCC 35218	15.3± 0.10	12.50
Staphylococcus aureus (G+) ATCC 25923	12 ± 0.03	14.50
Bacillus subtilis ATCC-6633	13.5±0.8	15.00

CONCLUSIONS

Essential oil of *M. vulgare* from Algeria had significant differences in the chemical composition as compared to the same essential oil from other region, which can be attributed to several factors. The results demonstrated that the major components of the essential oil were: Sulfamethoxypyridazine 14.44%, Eugenol 10,50%,o- Undecylenic Acid 10,48%, Citronellol 9,78% ,Silvex8,38% ,Germacrene-D 8,21,pinene 7,30% Carbromal4,87%. The antibacterial activity of the oil was determined; it was found that it was active against all bacteria tested.

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