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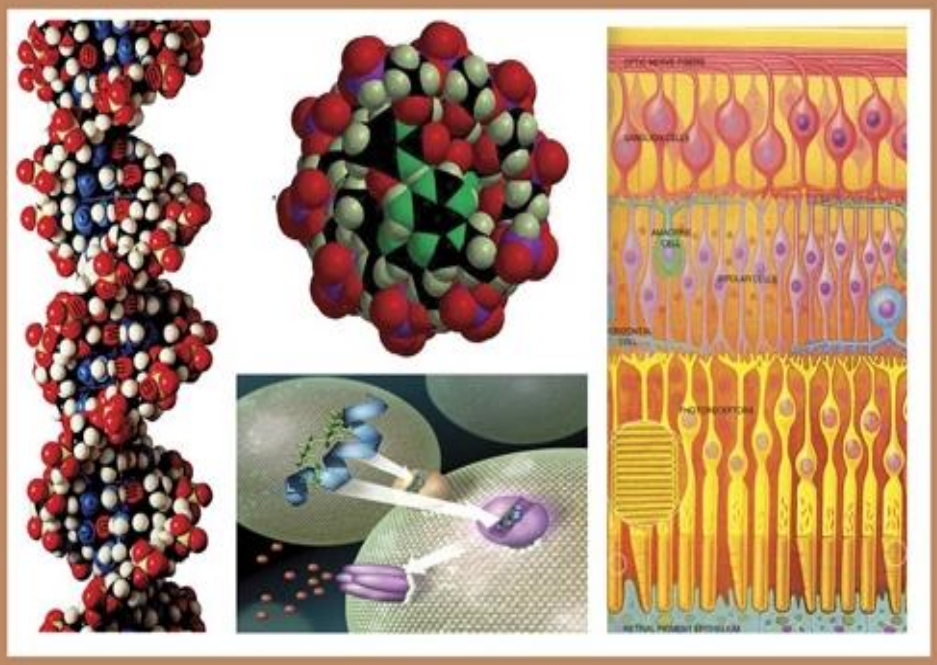

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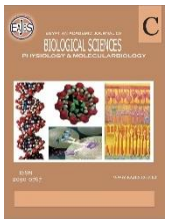

Valorization of Essential Oil of *Rosmarinus Officinalis* L. from El Bayadh Region-Algeria

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ABSTRACT

Our work focuses on endorsing the biological effects of *Rosmarinus Officinalis* L essential oils from the arid zone in Algeria by studying its chemical composition, and biological activities; in vitro and in vivo. The chemical compounds of the essential oil have been identified by gas chromatography and mass spectrometry. Thirty-two constituents, accounting for 99.97% of the total oil composition were identified. the main compounds of the essential oil were Eucalyptol (70,90%), Borneol (16.63%), α-Pinene (10,52%) β- Pinene (5,77%), Camphor (2,15 %), and α-terpineol (1,45%), respectively. The antioxidant activity of the hydro distilled oil was studied using DPPH to determine IC50 of 18.04µg/ml. The antibacterial activity of essential oils was evaluated against five microorganisms using the diffusion disc method on bacteria: *Candida Albicans* ATCC 10231, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853), *Staphylococcus aureus* ATCC 25923, *Bacillus* ATCC 11778, diameters vary between 6 and 19 mm. The toxicity was tested on Winstar rats; for a dose less than or equal to 2000mg/kg no toxicity signs were shown. In vivo, the Anti-Inflammatory properties were evaluated using the Induction of edema by carrageenan, the essential oil has a great effect at the concentration of 600mg /kg.

INTRODUCTION

Rosmarinus officinalis L is a small evergreen that grows wild in most Mediterranean countries (Hèthelvi *et al*., 1987) it is an evergreen, generally erect, rounded shrub with aromatic, needle-like, grey-green leaves and tiny, two-lipped, pale blue to white flowers. *Rosmarinus* is native to dry scrub and rocky grows on loam soil with good drainage in an open, sunny position (Rebeiro *et al*., 2015).

Rosemary oil has been widely used, for centuries, as an ingredient in cosmetics, soaps, perfumes, and deodorants, both for flavoring and preservation of food products (Valentini *et al*., 1997). Rosemary oil has also many therapeutic and antiseptic effects (Palevitch *et al*., 1991). In this context, our aim is to study the phytochemical composition of *Rosmainus officinalis* essential oil (REO), its toxicity determination, and its antioxidant, antimicrobial and anti-inflammatory potencies (in vitro and in vivo activities).

MATERIALS AND METHODS

Extraction of Essential Oil:

The aerial part of *Rosmarinus officinalis L* was harvested in the month of March 2020 from Brezina (El Bayadh) located in south west Algeria. The aerial parts collected were dried at room temperature and obscurity. The essential oil was extracted by hydrodistillation using an apparatus of Clevenger type for 4H by mixing 200g of *Rosmarinus officinalis L* in1500 ml of distilled water.

Analysis of the Essential Oil:

The evaluation of the chemical composition of essential oils and the identification of the main components were carried out by GC and GC-MS analysis. GC / MS analyzes were performed with a Varian CP-3800 gas chromatograph equipped with a DB-5 capillary column (30 m × 0.25 mm, 0.25 µm coating thickness) and a Varian Saturn 2000 ions. The analytical conditions were as follows: injector and transfer line temperatures 220 ° C and 240 ° C respectively; programmed over temperature from 60 ° C to 240 ° C at 3 ° C / min; helium carrier gas at 1 ml/min; 0.2 µL injection (10% hexane solution); 1:30 division ratio. The identification of the constituents was based on the comparison of retention times with those of authentic samples, comparing their linear retention indices in relation to the hydrocarbon series, and in computer with mass spectra of commercial and household libraries constructed from pure substances and components of known oils and data from MS literature Data (Adams, 2007); (Davies, 1990).

Antioxidant Study:

DPPH Radical Scavenging Assay:

The free radical-scavenging activity was determined spectrophotometrically by the DPPH˙ assay according to the following described method. Two ml of various concentrations of essential oil of *Rosmarinus officinalis L* was added to 0.4 ml solution of DPPH radical in ethanol (Blois MS., 1958). The mixture was blended and kept in the dark for 30 min and the absorbance of the resulting solution was measured at 517 nm. A sample represents the absorbance of the tested compound. Ethanol was used as a control while, acid Gallic, and acid ascorbic used antioxidant standards for comparison of the activity.

The scavenging capability of DPPH radical was calculated using the following equation: DPPH scavenging effect (%) = [(A Control – A Sample)/A Control] X 100 Where: A control is the initial concentration of the DPPH**.** The results were recorded as 50% inhibition concentration (IC50).

Antimicrobial Study:

This biological activity was evaluated against five strains of bacteria, three with Gram-negative (*Candida Albicans* ATCC 10231, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853), two strains of Gram-positive (*Staphylococcus aureus* ATCC 25923, *Bacillus* ATCC 11778).

Antibiotic Susceptibility Test:

Susceptibility to a panel of antimicrobial agents was determined by the standardized disk diffusion assay on Mueller-Hinton agar with commercial antimicrobial susceptibility disks according to the recommendations of the committee on standardization of susceptibility testing. (MOARD, 2008); (MOARD, 2011).

The antibiotics tested and their corresponding disk concentrations were as follows: Trimethoprim 1,25µg + Sulfamethoxazole (SXT) 23,75µg, Chloramphenicol (C 30) 30µg,

Toxicity Assay:

The toxicity test was conducted according to the “predetermined dose” method of guideline 420 (OECD, 2001) which consists of testing the essential oil at a dose of 2000 mg/kg. The test was carried out on 5 females albinos Wistar rats, after an overnight fast (removal of food but not water), the extract is administered in a single dose using a gastric tube for appropriate intubation. Their behavior was observed over a period of 14 days after force-feeding

(Mogue *et al*., 2020) and (Sawadogo *et al.*, 2018).

Anti-Inflammatory Assay:

Induction of Edema by Carrageenan:

The inflammation induced by the injection of a 1% carrageenan solution in albino mice was according to the method of (Winter *et al.*, 1962) and adapted by Antonisamy (2011). The mice used for this study come from the breeding Animal of Pasteur Institute Algiers. The experiments were carried out in adult mice. They were fasted overnight (16 hours) before the experiment. Mice received distilled water (control), essential oil of *R. officinalis* (200 /400 and 600mg/kg), or the essential oil of *R. officinalis*. The rats constituting the reference group received Diclofenac at a dose of 10mg/kg. One hour after treatment, 0.05 ml of 1% carrageenan solution, the evolution of the edema of the hind leg was measured every hour for 6 hours after injection of carrageenan.

The inhibition percentage of inflammation is measured according to the following formula:

% Inhibition = [(A – B)/ A] *100

A; an average of the increase in the control paw.

B; an average of the increase in the paw of the treated group.

Statical Analysis:

The results are compared by the analysis of variance and comparison of the results of the extracts two by two. The differences are considered statistically very highly significant at a P value of less than 5% (P <0.05). These statistical tests are carried out by STATISTICA software.

RESULTS AND DISCUSSION

Composition of the Essential Oil:

The result of the identified chemical compounds is represented in Table 1. Thirty-two compounds in the essential oil of *R. officinalis* were identified. The major constituent in the essential oil were found to be Eucalyptol (70,90%), Borneol (16.63%), α- Pinene (10,52%) β- Pinene (5,77%), Camphor (2,15 %), and α-terpineol (1,45%), respectively. Furthermore, in eastern Morocco, the 1,8-cineole content in the essential oil of collected rosemary leaves was (53.05%) followed by comphor (11.56%) (Imelouane *et al*., 2010), The essential oil generated from Tunisian Rosemary consisted of 35.2% of 1.8.cineole (Smeti *et al*., 2013) Another survey in turkey carried out (81.47%) of 1,8-cineole, α-pinene (8.90%), camphor (3.3%), camphene (2.64), cymene (1.95%) (Türkmena *et al., 2014)*, a study in Italy indicated that essential oil of Rosemary leaves contained 18.6% of 1.8.cineole (Canale *et al*., 2013).

This variation has been attributed to the composition of this volatile oil being variable according to the environment (climatic and edaphic) or genetic factors (Sagnard *et al*., 2002; Adams et *al*., 2006). The chemical composition can also be modified by the time of the collection and the method of extraction (Laggoune *et al*., 2013).

Table 1: Composition of the essential oil

N°	RT	KI	Compound	%
1	9,33	920	Tricyclene	0.0335
2	9,63	925	α- Thujene	0,1107
3	10,12	933	α- Pinene	10,5239
4	10,89	946	Camphene	2.1729
5	11,2	951	Thuja-2,4(10) -diene	0.0738
6	12,68	976	β- Pinene	5.7716
7	13,29	986	(3E) -OCTEN-2-ol	0.0378
8	13,63	991	Myrcene	0.8152
9	14,44	1004	α-Phellandrene	0.1178
10	14,8	1009	δ -3-Carene	0.1374
11	15,34	1017	α-Terpinene	0,3045
12	16,73	1036	Eucalyptol	70,9099
13	16,96	1040	(Z)-β-Ocimene	0,5055
14	17,61	1049	(E)- β -Ocimene	0,1039
15	18,28	1058	γ –Terpinene	0,5258
16	18,96	1068	Cis-Sabinene hydrate	0,0287
17	20,33	1087	Terpilonene	0,1247
18	21,55	1104	Linalool	0,2311
19	22,36	1116	Endo Fenchol	0,024
20	24,36	1143	Camphor	2,1582
21	25,46	1159	Trans-pinocamphone	0,0118
22	25,6	1161	Pinocarvone	0.0478
23	26,13	1168	Borneol	1,1899
24	26,87	1178	Terpinen-4-ol	0,2846
25	28,06	1195	α-terpineol	1,4536
26	29,03	1209	Verbenone	0,0459
27	31,56	1245	Carvone	0,2616
28	34,22	1284	Bornyl acetate	0,0823
29	42,89	1417	(E)-Caryophyllene	0,5806
30	45,02	1451	α-Caryophyllene	0,2099
31	52,81	1582	Caryophyllene oxide	0,0759
32	54,35	1608	Humulene epoxide. 2	0,0167
Total				99,9715

Antioxidant Activity:

The sample concentration providing 50% DPPH˙ scavenging effect was calculated from the graph of DPPH˙ scavenging effect percentage against sample concentration as a positive control. Table 2 summarizes the results. In comparison to the standard compound, the IC50 value was 18.04 ± 1.15 µg/ml. In a similar study Hussain *et al*., (2010) detected that their essential oil had adequate radical scavenging of 20.9 µg/ml. The essential oil of *R. officinalis* showed good antioxidant activity. On the same path Chen *et al*., (2008) found that the antioxidant activity is related to the presence of α-pinene, β-pinene, and 1,8-cineole. In another research, Ebrahimabadi *et al*., (2010) observed that the high antioxidant activity is due to the appearance of antioxidant substances, including 1,8-cineole, α-pinene, camphor, and borneol.

Table2. Antioxidant activity of essential oil of *R. Officinalis* in reducing power and DPPH assaysIC50) µg/ml.

Compound	DPPH (IC50) µg/ml
Quercetin	14.09 ± 1.3
Gallic acid	6.35 ± 0.41
Ascorbic acid	7.24 ± 0.97
Essential oil of Rosemary	18.04 ± 1.15

The Antibacterial Activity:

Table 3 details the sensitivity and resistance of the bacteria tested to the different antibiotics which are inside and indicates that the different antibiotics have more or less similar effects to that of essential oils tested after twenty-four hours. Rosemary essential oil against all tested microorganisms obtained by disk diffusion test is shown in Table 4. The current research results reveal that the essential oil exhibits a moderate antibacterial effect against the tested microorganisms. Most Gram-negative bacteria were susceptible to *Rosmarinus officinalis* essential oil as well as Gram-positive bacterias which are shown in Table 4. The inhibition zone diameter for *staphylococcus aureus* ATCC 25923 was from 18mm to 11 mm, *Escherichia coli* ATCC 25922 scored growth inhibition of 18mm to7 mm, for *Candida Albicans* ATCC 10231 18mm to 15mm, for *Bacillus* 19mm to 6mm ATCC 11778, and *Pseudomonas aeruginosa* ATCC 27853. The essential oil from *R. officinalis* tested by (Santoyo et *al*., 2005) reported Inhibition zones of *E. coli and aureus* fractions of about 17 mm, the study done by (Gachkar *et al*., 2007) showed an inhibition zone of 16 mm for the same stumps,16 mm to 0 mm.

The large amount of camphor, 1,8-cineole, and α-pinene. Camphor contained Rosemary essential oils exhibit a pronounced antibacterial effect (Stojanović-Radić et *al*., 2020), (Kadri *et al*., 2011). These compounds are associated to be the source of these antimicrobial properties of the essential oil of *R. officinalis L* (Bekirs *et al*., 2012).

Toxicity Assay:

Oral administration of a single dose of 2000 mg/kg of the oil did not cause any death or signs of toxicity in rats. It is therefore non-toxic for a dose less than or equal to 2000mg/kg. And the 50% lethal dose (the LD50) orally is greater than 2000mg/kg.

Table3. Sensitivity of pathogen tested on antibiotics

Pathogen	Zone of 1nhibition antibiotics (mm)			
	CMN	SXT 25	C 30	PEN
Candida Albicans	29 mm	30mm	22mm	35 mm
Staphylococcus aureus	29 mm	21mm	22 mm	43 mm
Escherichia coli	30 mm	27 mm	25 mm	35 mm
Bacillus	19 mm	30 mm	20 mm	23 mm
Pseudomonas aeruginosa	25 mm	33 mm	28 mm	44 mm

Table4. Sensitivity tests of bacterial pathogens to mint essential oils.

Pathogen	Zone of inhibition (mm)			
Candida Albicans	18 ±089 mm	17 ±035mm	15 ±047mm	15 ±023mm
Staphylococcus aureus	18 ±105mm	17 ± 047mm	11 ± 083mm	11 ± 013mm
Escherichia coli	18 ±012mm	17 ± 057 mm	15 ± 044 mm	7 ±014 mm
Bacillus	19 ± 067 mm	17 ± 055 mm	14 ± 035 mm	6 ±019 mm
Pseudomonas aeruginosa	16 ±042 mm	14 ± 047 mm	10 ±075mm	-

Anti-Inflammatory Assay:

The anti-inflammatory effects of *Rosmarinus officinalis were* analysed by administration of essential oil in mice using carrageenan-induced mouse paw edema, the results obtained are expressed in Table 5.

The inhibitory activity explained in Figure 1. shown by the essential oil of *Rosmarinus officinalis* (200 mg/kg) over a period of 6hours in carrageenan-induced paw inflammation was lower than diclofenac, however, we detected the augmentation of inhibition of REO dosed at 400mg/kg after 5 hours since carrageenan inducing, we

observed that REO concentrations of 600mg/kg had the higher inhibition percentage during the study.

Percentage inhibition of the different concentrations of REO and diclofenac, after the 6 hours of experimentation were quite similar ($P < 0.05$).

Bozin *et al.*, (2007) realised that *Rosmarinus officinalis* has anti-oxidative effects as well as anti-inflammatory, this essential oil contains a rich mixture of terpenes; the most represented compound was 1.8-cineole, which is known to have anti-inflammatory effects (Santos and Rao 2000; Juergens *et al.*, 2003), in particular, the highest concentration of REO (containing eucalyptol, α-pinene and borneol) inhibited the acetylcholinesterase (Savelev *et al.*, 2003).

Table 5. Effect of treatments on the diameter evolution (mm) of mince's plantar edema.

Treatment	1h	2h	3h	4h	5h	6h
Control	0,960 ± 0,099	0,914 ± 0,059	0,880 ± 0,069	0,932 ± 0,046	0,926 ± 0,035	0,934 ± 0,068
Diclofenac	0,341 ± 0,034**	0,385 ± 0,022**	0,314 ± 0.074**	0,306 ± 0,038**	0,364 ± 0,072**	0,433 ± 0,045**
REO 200mg/kg	0,746±0.157*	0,624±0.066**	0,586±0.056**	0,394±0.073**	0,456±0.044**	0,427±0.046**
REO 400mg/kg	0,382±0.048**	0,409±0.079**	0,342±0.041**	0,288±0.048**	0,312±0.066**	0,431±0.069**
REO 600mg/kg	0,240±0.042**	0,158±0.061**	0,161±0.074**	0,189±0.067**	0,181±0.089**	0,441±0.094**

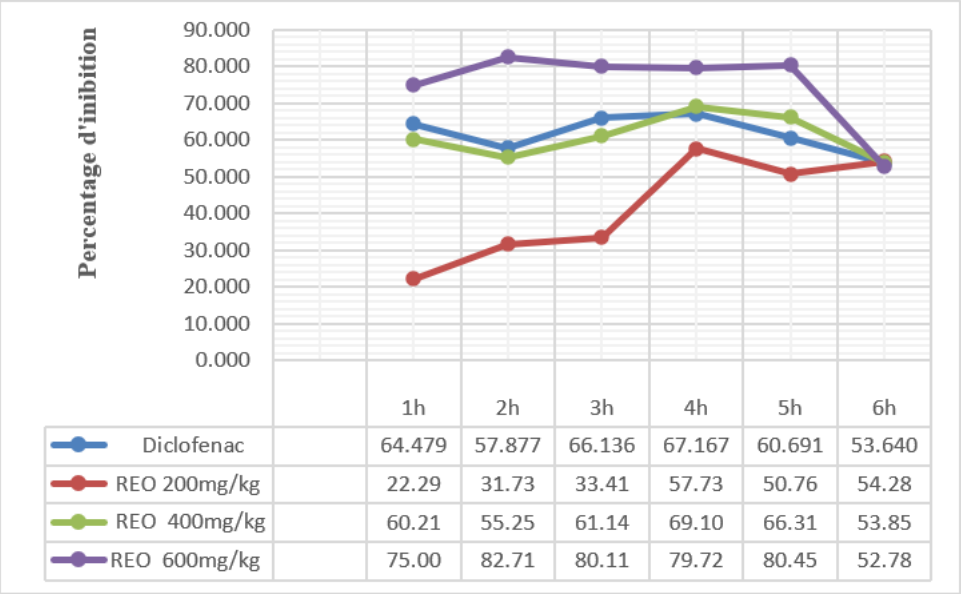

Fig.1 Percentage inhibition of inflammation.

CONCLUSION

The present study worked on the valorization of hydro-distilled essential oil of *Rosmarinus officinalis*, cultivated in the region of El Bayadh (south-west of Algeria), its composition was determined by GC and GC/MS. 32 components representing 99.97% of the essential oil were identified with the main components were eucalyptol and borneol which is well corroborated with the results obtained in other regions over the world. Over well, the differences in chemical composition could be explained by several factors, including pedoclimatic and seasonal variations. Its main components, essential oil have several antioxidants, and antimicrobial and anti-inflammatory activities, however, the anti-inflammatory effects of rosemary essential oil should be interpreted with caution. The experiment showed no sign of toxicity at a dose lower than or equal to 2000mg/kg, which makes it possible to promote it in the pharmaceutical and parapharmaceutical industry.

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