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Study of Antimicrobial, Anti-Inflammatory and Anticorrosive Effects of Rosmarinus Officinalis Essential Oil Cultivated in Morocco

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ABSTRACT

The present study aimed to evaluate the antibacterial, antifungal, anti-inflammatory and anticorrosive effects of *R. officinalis* essential oil. The antimicrobial activity was assessed using micro dilution method against two gram positive, one gram negative bacterial strains and two fungal strains. The anti-inflammatory activity of *R. officinalis* was determined using carrageenan-induced paw edema in rats and the inhibitive effect essential oil on the corrosion behavior of mild steel in 1 M HCl was studied using scanning electron microscope. The Minimal Inhibitory Concentration (MIC) showed that *R. officinalis* essential oil exhibited strong inhibitory effect, against all tested microorganisms with minimum inhibitory concentrations ranging from 0.031% to 1% (v/v).Also this oil induces a very important decrease in inflammation compared to control group rats with inhibition of 66%.SEM study demonstrated that the addition of *R. officinalis* in 1 M HCl medium causes decreased of the roughness of mild steel surface and consequently possesses very good corrosion inhibition ability. These findings showed that the studied essential oil has antimicrobial, anti-inflammatory and anti-corrosive potential that can be applied to the food, drug and steel industries.

Keywords: *R. officinalis*, Antibacterial, Antifungal, Anti-inflammatory, Anticorrosive effect

INTRODUCTION

Medicinal aromatic plants have a considerable advantage thanks to the discovery of their essential oils applications in health care as well as their uses in other areas of economic interest [1,2]. Their numerous uses make them aware of a growing demand on world markets. So, essential oils investigation stills a hot topic despite its seniority and exponential developments in vegetable biotechnology. Essential oils

present a very large diversity of compounds both in quantity and in variety. Their screening would make possible to discover new antiseptics and drugs, which could constitute an alternative to use of conventional antimicrobial that have become ineffective and therefore to solve the serious problem of antibiotic-resistance. Furthermore, stopping or delaying the maximum attack of a metal in an aggressive solution has become increasingly needed in order to reduce corrosion, which constitutes a constant and continuous problem, often difficult to eliminate completely. It represents with material degradation a major economic problem with considerable financial losses [3]. Face to this problem using inhibitors to prevent the process of dissolving metals remains an inevitable and widespread application. Due to the toxicity of synthetic corrosion inhibitors, there has been increasing search for green corrosion inhibitors which are environment friendly and are obtained from natural products such as oil plant and extracts [4]. Literature showed that *R. officinalis* essential oil received considerable attention in various domains. This plant was highly recommended for antioxidant, antibacterial, bio preservative, insecticidal, anticancer, anti-inflammatory effects [5-9]. In the investigation reported in this paper, we aimed to evaluate *R. officinalis* essential oil as antibacterial, antifungal, anti-inflammatory and a non-toxic natural anti-corrosive.

MATERIALS AND METHODS

Plant material

Fresh aerial part of *R. officinalis* was harvested from Er-Rich. The botanical identification was performed and then voucher specimen was deposited at the Herbarium of National Institute of Medicinal and Aromatic Plants, Morocco.

Essential oil extraction

Leaves and stems of *R. officinalis* were hydro distilled for 3 h using a Clevenger-type apparatus. The Essential Oil (EO) was kept in dark at 4°C until further use.

Gas chromatography-mass spectrometry analysis conditions

The essential oils were analyzed using GC-MS (Polaris Q ion trap MS) according to the protocol previously described [10]. Hence, analyses were performed on a Hewlett-Packard (HP 6890) gas chromatograph (flame ionization detector), equipped with a 5% phenyl methyl silicone HP-5 capillary column (30 m × 0.25 mm × film thickness 0.25 µ m). The temperature was programmed from 50°C after 5 min initial hold to 200°C at 4°C/min. N_2 was used as chromatography carrier at 1.8 mL/min, split mode was used with a flow of 72.1 mL/min and a ratio of 1/50, temperature of injector and detector was 250°C, and final hold time was 48 min. A computer system type "HP Chem Station" was used to led the machine and manages its functioning allowing the evolution of chromatographic analyses. Diluted samples (1/20 in methanol) of 1 µ L were injected manually.

Target strains

Tested bacteria include three isolates of Escherichia coli DH_5 α, Staphylococcus epidermis and *Acinetobacter baumannii*. Before use, strains were revivified by subcultures in Luria-Bertani (LB) plates at 37°C for 24 h. As regards the tested fungi, include *Aspergillus niger* and *Penicillium expansum*. Revivification of molds was made by subcultures in malt extract-agar plates (MEP) at 25°C for 7 days. After incubation, their spores were harvested by scraping the culture surface in sterile Tween 20 (1%) solution. These microorganisms spore suspension was concentrated by centrifugation at 10000 g for 15 min at 4°C until use. Then the spore suspension concentration was adjusted to 106 spores /mL (counted with a hemocytometer). These strains belong to Laboratory of Microbial Biotechnology, Faculty of Science and Technology of Fez (Morocco).

Determination of minimum inhibitory concentration against bacteria

The Minimum Inhibitory Concentration (MIC) was determined in 96 well-micro plate using the micro dilution assay according to the

protocol previously described [11]. Bacteriological agar at 0.15 % (w/v) was used as an emulsifier of the essential oil in the culture medium. For bacteria, the EO was serially diluted in Muller Hinton broth supplemented with agar to obtain final concentrations ranging between 4% and 0.0039% (v/v). The 12th well was considered as growth control (free-essential oil control). Then, 50 µ L of bacterial inoculum, previously prepared and adjusted to 0.5 McFarland, were added to each well to reach final concentration of 106 CFU/mL. After incubation at 37°C for 24 h, 10 µ L of resazurin were added to each well as a bacterial growth indicator. After further incubation at 37°C for 2 h, the bacterial growth was revealed by coloration change from purple to pink. Experiments were carried out in triplicate.

Determination of minimum inhibitory concentration against fungal strains

To investigate the antifungal activity of the studied essential oil against *Aspergillus niger* and *Penicillium expansum* a modified micro dilution technique was used [12]. Firstly, 50 µ l of malt extract broth were added from the second to the 12th well. The EO was diluted in Tween 20 (1% v/v) at a final concentration of 40% (v/v), then 100 µL of this solution were deposed in the first well. Afterwards, scalar dilution was made by transferring 50 µL from the 1st to the 11th well. The 12th well was considered as growth control. Thereafter 50 µ L of the fungal spore suspension were added to each well to reach final concentration of 106 spores/mL. The micro plate was sealed and incubated for 72 h at 30°C. The lowest essential oil concentration that prevents visible fungal growth was defined as the MIC.

In vivo anti-inflammatory activity carrageenan-induced rat paw edema

Essential oil anti-inflammatory activity was evaluated using carrageenan-induced paw oedemain rats technique [13]. Male wistar rats were divided into four groups of five animals each (1) Control group (10 ml/kg of 0.9% NaCl solution) (2) and (3) groups received reference drugs (10 mg/kg of indomethacin and voltaren) (4) groups were orally administered *R. officinalis* EO in 1 ml/kg. Animals were pre-treated with drug and essential oil before injection of carrageenan. Inflammation of the hind paw was induced by injecting 0.1 ml of 0.5% carrageenan suspension into the sub-plantar surface of the right hind paw. Measures of the paw circumference were determined at 3, 4, 5 and 6 h (after edematogenic agent injection) intervals later (St). The difference between St (3, 4, 5 and 6 h) and S0 was taken as the edema size. The inhibition percentage of the inflammatory reaction was determined for each animal by comparison with controls and calculated by the following equation.

$$\text{Inhibition}(\%) = \frac{(\text{St} - \text{S0})control - (\text{St} - \text{S0})\ treated}{(\text{St} - \text{S0})control} \times 100$$

Mild steel and HCl solution

The chemical composition of the mild steel used in this experiment is 0.230 % Si, 0.370 % C, 0.680 % Mn, 0.077 % Cr, 0.016 % S, 0.059 % Ni, 0.011 % Ti, 0.009 % Co, 0.160 % Cu and balance Fe. The carbon steel sheets were polished successively with a series of SiC papers (120, 400, 600 and 1200) rinsed with double-distilled water degreased in acetone in an ultrasonic bath immersion (for a period of 5 min), washed once again with bidistilled water and then dried at room temperature before use.

For the corrosive solutions used, 1 M (HCl) was prepared by dilution of an analytical reagent grade 37% HCl with double distilled water, while inhibitor-containing corrosive media were prepared in 1 M HCl with the concentration of 1 g/l with 6 h of immersion.

Scanning electron microscope study

Surface morphology of mild steel specimens was evaluated by Scanning Electron Microscope (SEM) in the absence and the presence of *R. officinalis* essential oil. For this purpose, the test specimen that exhibited the highest efficiency of corrosion inhibition from the weight loss measurement was examined with blank (without inhibitor) the acceleration voltage employed was 0.5 to 30 kV with a resolution of 3.5 nm.

RESULTS AND DISCUSSION

Chemical composition

The studied essential oil was previously subjected to a gas chromatography-mass spectrometry analysis. This analysis revealed 25 different compounds accounting for 98.14% of the whole *R. officinalis* essential oil, where the major constituents were 1,8- cineole (33.88%), α -Pinene (12.76%), β -Pinene (7.17%) and Myrcene (4.54%) (Table 1).

Kovats index	Constituents	%
931	α -Thujene	0.48
939	α -Pinene	12.76
953	Camphene	2.47
976	β -Pinene	7.17
990	Myrcene	4.54
1033	1.8- Cineole	33.88
1143	Camphor	14.66
1162	β -Trans-terpineol	3.46
1185	α -Terpineol	0.63
1194	Myrtenol	1.23
1235	Myrtenyl acetate	0.22
1282	α -Terpineol	2.28
1351	α -Cubebene	0.15
1376	α -Copaene	0.1
1384	β -Bourbonene	0.16
1418	β -Caryophyllene	3.8
1473	γ -Gurjunene	0.2
1480	Germacrene-D	0.42
1493	Ledene	3.92
1499	α -Murolene	0.32
1513	γ -Cadinene	0.8
1581	Caryophylleneoxide	2.75
1584	copaen-4- α -ol	0.43
1611	Tetradecanal	0.21
1653	τ -Cadinol	0.19
Total		98.14

Table 1: Chemical composition of *R. officinalis* essential oil.

In agreement with results obtained by other authors, the chromatographic analysis of *R. officinalis* EO showed that 1,8-cineole was the component present in the greatest percentage [5,14]. Regarding other works, our results diverge from those published [15,16].which stipulates that α-pinene was the major compound of their essential oils from Iran and Morocco (Rabat) respectively. This variation in chemical composition is due to several factors such as pedoclimatic and seasonal factors, cultural practices, extraction process and plant maturity collection period [17].

Antimicrobial activity

The antibacterial activity of tested essential oil was determined by evaluating its minimum inhibitory concentrations using micro dilution method. *R. officinalis* EO exercised an important inhibitory activity against all studied strains especially S. epidermis, since it was inhibited by a very low concentration of 0.031% (v/v). Also, a concentration of 0.062 % (v/v) was sufficient to inhibit the growth of *S. aureus*. Against Gram-negative bacteria the tested EO was more active against *E. coli* (MIC=0.125%). In contrast, *A. baumannii* was the most resistant strain with MIC value of 1% (v/v). As regards molds, *A. niger* was more sensitive than *P. expansum* against this essential oil's action with MIC values of 0.062 and 0.125 % (v/v) respectively (Table 2).

Strains	**Concentrations %**										
	4	2	1	0.5	0.25	0.125	0.062	0.031	0.015	0.007	0.0039
S. epidermis	-	-	-	-	-	-	-	-	+	+	+
S. aureus	-	-	-	-	-	-	-	+	+	+	+
E. coli	-	-	-	-	-	-	+	+	+	+	+
A. baumannii	-	-	-	+	+	+	+	+	+	+	+

Table 2: The minimum inhibitory concentrations of *R. officinalis* essential oil against the tested bacterial strains.

Overall, the EO showed very promising antibacterial activity. It was more selective for the Gram-positive bacteria than Gram-negative ones which is in accordance with studies reporting that Gram-positive bacteria are generally more sensitive than Gram-negative bacteria to EOs [10,18] (Table 3).

Strains	**Concentrations %**										
	4	2	1	0.5	0.25	0.125	0.062	0.031	0.015	0.007	0.0039
A. niger	-	-	-	-	-	-	-	+	+	+	+
P. expansum	-	-	-	-	-	-	+	+	+	+	+

Table 3: The minimum inhibitory concentrations of *R.officinalis* essential oil against the tested fungal strains.

These results could be related to the membrane composition of gram-negative bacteria. Indeed, these latter have a membrane which exhibiting selective permeability, lipopolysaccharides surface containing negative charges, which prevent the diffusion of hydrophobic molecules, and porins that block the passage of molecules at high molecular weight, in contrast to the simple membrane structure of Gram-positive bacteria [19].

Similarly, it has been reported that antifungal activity of essential oils is due to a modification in cell membrane composition. These oils diffuse into the fungal membrane structures and damage them by increasing their permeability. They interfere also with cell wall enzymes or inhibit intercellular and extracellular enzymes [20,21]. The broad spectrum and the significant antimicrobial activity of the tested essential

oil may be attributed to its chemical composition and especially to its main components. In fact antimicrobial screening of some essential oils main components, conducted by research team [22] demonstrated that 1,8-Cineole and α -Pinene were active against *E.coli, B.subtilis, P.aeruginosa, S.aureus and A.niger*. Also,several investigators have reported that components of herbs EOs such as camphor have antimicrobial activities [23] and that high camphor percentage is accompanied with an important bacteriostatic power [24].

In vivo anti-inflammatory activity carrageenan-induced rat paw edema

Inflammation is characterized by classic symptoms such as heat, redness, swelling and pain. Edema measurement is therefore an excellent tool for quantification of cutaneous inflammation induced by phlogistic agents such as Carrageenan. In order to justify the presumed anti-inflammatory activity of *R. officinalis* plant in pharmacopoeia tests were carried out with its essential oil and reference drugs on "Rat paw edema induced by carrageenan". All tested products have an inhibitory effect on the inflammatory reaction the tests inhibition results are shown in Figure 1.

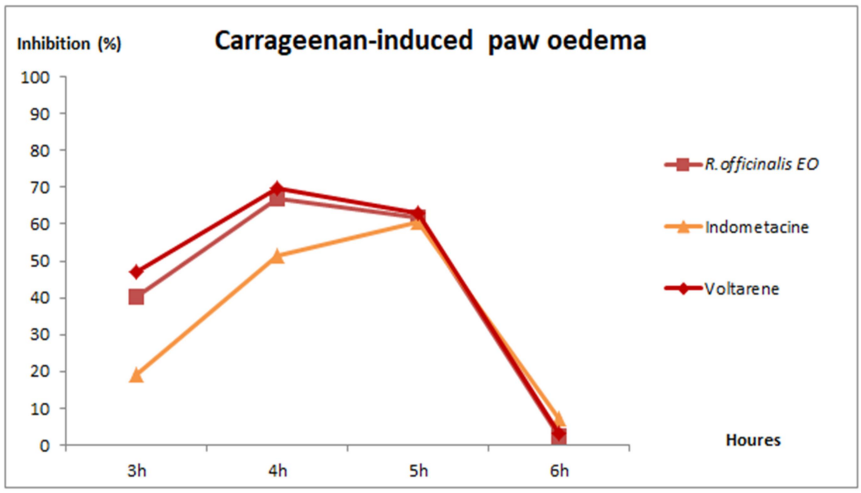

Figure 1: Effect of *R. officinalis* essential oil, indomethacin and voltarene on carrageenan-induced paw edema at various time intervals.

Note: —■— *R. officinalis,* —▲— Indomethacin, —♦— Voltarene.

After carrageenan treatment, control group rats have developed edema in their paws. While the group locally treated with indomethacin and voltarene have showed a very significant reduction in the legs thickness compared to that of the mice of the control group, with an inflammation inhibition of 51.42 and 69.57% respectively. The rats' treatment with *R. officinalis* essential oil induces a very important decrease in inflammation compared to control group rats with inhibition percentage of 66%, which is higher than that obtained with indomethacin. These results could be explained by the components contained in the tested essential oil. Indeed, sesquiterpenes which represent 13.74% of this oil are known for their high anti-inflammatory activity [25,26]. Moreover, being the major compound with 33.88 %, the anti-inflammatory activity of 1.8 cineole has been proven previously. So, it can be concluded that the anti-inflammatory activity found in rosmary E.O could be linked to its major compounds especially 1,8 cineole and sesquiterpenes.

SEM study

The studied essential oil effect on corrosion is presented in Figure 2a representing micrographs of the mild steel surface after being polished Figure 2a immersed 6 h in 1 M HCl Figure 2b and immersed in 1 M HCl with 1 g/L of *R. officinalis* essential oil. The electron micrographs reveal a smooth surface in absence of any treatment with parallel features on the polished steel surface which are associated with abrading

scratches Figure 2a. After an immersion in HCl medium without inhibitor addition, the high resolution SEM micrograph Figure 2b shows that the steel surface was strongly damaged due to corrosion attack of protons H^+ present in the acidic medium. However, a smooth morphology can be observed in the presence of *R. officinalis* EO Figure 2c, this may be due to the important inhibitory

effect of the essential oil on protection of the surface of carbon steel, and this is attributed to the formation of protective film of *R. officinalis* essential oil on the metallic surface. Corrosion inhibition of mild steel in 1 M HCl medium can be explained also by adsorption of *Romarinus officinalis* essential oil on the metallic surface [27].Consequently, this essential oil extracted from *R. officinalis* possesses very good corrosion ability in 1.0 M HCl solution for mild steel (Figures 2a-2c).

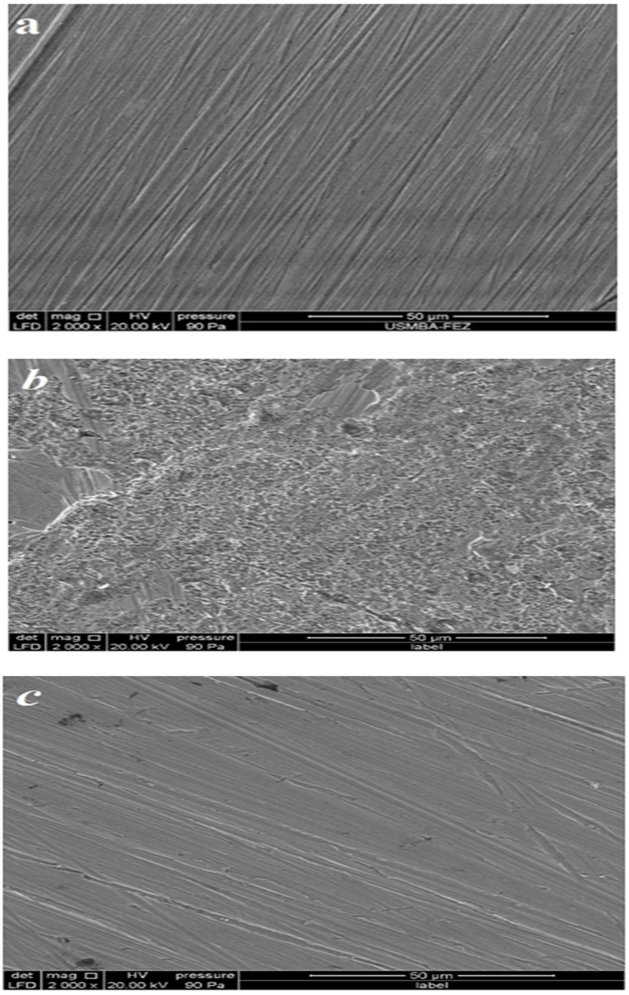

Figure 2: SEM micrographs of the mild steel surface (a) Metallic surface after being polished, (b) metallic surface after 6 h immersion in 1 M HCl and (c) metallic surface after 6 h immersion in 1 M HCl with 1 g/L of *Romarinus officinalis*.

CONCLUSION

This work aims to evaluate the antimicrobial, anti-inflammatory and anticorrosive effects of Moroccan cultivated rosemary essential oil. These preliminary results showed that *R. officinalis* oil could act as an effective and natural drug in terms of growth inhibition of a wide spectrum of microbial strains, known for their implications in human or animal infections on one hand, and on the other hand that it could help in forming an effective and inexpensive anti-inflammatory and anticorrosive alternative for low socio-economic communities. Hence,

the studied essential oil can be used as antimicrobial complement in developing countries to develop new therapeutic agents, or used as anti-corrosive agent thanks to its high potential which allows its use as a preservative to increase the performance and life span of the stainless steel material or as anti-inflammatory agent. So, it is clearly evident from this study that the Moroccan medicinal studied plant has great potential to be used which open new perspectives linked with the valorization of Moroccan plant's EOs and their use as candidates for the development of new ecofriendly agents especially in developing countries.

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