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Galenic Formulation of an Antibacterial Ointment Based on Derivatives of *Coriaria myrtifolia* and *Pistacia lentiscus*

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ABSTRACT

Faced to therapeutic limits of chemical drugs, research development programs on medicinal plants are oriented toward the obtaining of phytomedicines. In this context, this work aims to develop a dermal ointment intended for external use. In order to achieve this objective, the derivatives of two studied plants: *Pistacia lentiscus* (100 µl of Essential oil) and *Coriaria myrtifolia* (1 g of the ethyl acetate extract) are incorporated in 100 g of excipient (40 g of shea butter and 60 g of laboratorys white petrolatum "vaseline"). The quality control of this ointment has focused on the evaluation of organoleptic properties (color, odor), homogeneity, consistency or spread ability, pH and antibacterial activity. The observation has indicated that the ointment is of dark green color, homogeneous, and ensures a good adhesion to the skin. The assessment of its antibacterial power by the disk diffusion method has indicated that this ointment has an important antibacterial effect against *Staphylococcus aureus* and *Bacillus sp.* This activity is translated by inhibition zone diameters of 18.3 and 15.6 mm respectively, hence it can be concluded that the derivatives of the plants studied in galenic form retain their biological effect.

Keywords: *Pistacia lentiscus*, Antibacterial activity, *Coriaria myrtifolia*, Dermal ointment, Formulation.

INTRODUCTION

Morocco offers a great ecological and floristic diversity, because of its biogeographical position [1]. As everywhere in Africa, a strong ethnomedicinal tradition is still alive throughout all the country [2], particularly in the north of Morocco. Since these popular practices are not totally devoid of scientificity [3], it is therefore necessary to rehabilitate the medicine and the traditional toxicology in Morocco, this work falls within this context.

Pistacia lentiscus is known for its medicinal properties since the Antiquity. The aerial part is traditionally used in the treatment of arterial hypertension thanks to its diuretic properties [4,5]. The leaves are provided with anti-inflammatory, antibacterial, antifungal, antipyretic, astringent, hepatoprotective, expectorant and stimulating activities [6-11]. They are also used in the treatment of eczema, oral infections, diarrhea, nephrolithiasis, jaundice, headache, asthma and respiratory problems [7,12,13]. Finally, the lentisque's essential oil is known for its therapeutic virtues with regard to lymphatic and circulatory problems [14].

The results of preliminary studies have shown that *Coriaria myrtifolia* is rich in bioactive compounds (flavonoids, tannins), conferring an important antibacterial activity [15-17].

The galenic formulation complete and valorize the promising results of the previous studies [11,15,16], by developing a dermal ointment, having an antibacterial effect, intended for external use.

MATERIAL AND METHODS

Plant material

P. lentiscus was collected from Taounate (Altitude: 475 m, north: 34° 35.203', West: 004°38.533'; and *C. myrtifolia* from Bab Berred (altitude: 1290 M, N 35° 00' 979", W 004° 58' 092"); in the north of Morocco. The identification and conservation of both plants were carried out at the National Institute of aromatic and medicinal plants of Taouante (Morocco).

Preparation of derivatives of the two plants

The essential oil of *P. lentiscus* was obtained by hydrodistillation on a Clevenger type device [18] (100 g of fresh leaves were treated with

hydrodistillation during three hours), then the essential oil (E. O.) was stored in optimal conditions (4°C in the dark in the presence of anhydrous sodium sulfate) [11].

After drying the *C. myrtifolia* sample in open air, the leaves were separated from the stem and crushed until obtaining a degree of particle size adapted to an optimal dissolution. The extract preparation was carried out by Soxhlet: 45 g of powder first undergoes a delipidation by 200 ml of hexane; then extraction was carried out by the addition of 200 ml of ethyl acetate to the delipidated and dried powder. The Extract was recovered after vacuum evaporation of the solvent by using a rotary evaporator (90 rpm /min, 40°C) [15].

Galenic formulation

The ointment preparation

In order to prepare an ointment at 1%, 1 g of the ethyl acetate extract of *C. myrtifolia*, 100 µl of the foliar EO of *P. lentiscus* and 100 g of the excipient (40 g of the shea butter and 60 g of laboratory white petrolatum "vaseline") were used. Firstly, the extract, measured by a precision balance, was placed in a previously cleaned and dried porcelain mortar. The EO and the excipient were then gradually added while grinding until obtaining a homogeneous mixture [19]. The ointment thus obtained was conditioned thanks to a spatula in a glass pot, which was then hermetically closed and labeled [19].

Quality control of the ointment

The control has focused on the evaluation of organoleptic properties (color, odor), homogeneity, consistency or spreadability, pH and antibacterial activity of the formulated ointment.

Organoleptic property

The ointment color and odor were assessed in relation to those of the pure excipient [20].

Homogeneity

The homogeneity was verified by observing the excipient's dispersal; hence, after spreading a thin layer on a rigid flat surface using a spatula, regular or non-regular distribution of the extract was noted [19].

pH

The pH measurement of a tenfold dilution of the ointment in hot distilled water was carried out by using a pH meter [19].

Antibacterial activity

The disk diffusion method was used to evaluate the antibacterial activity of the formulated ointment [21]. The objective of this analysis was to ensure that derivatives of the studied plants put under galenic form retained their activity. Several concentrations were tested (1 g/ml, 500 mg/ml, 250 mg/ml and 125 mg/ml) against two bacterial strains responsible of dermal infections, *Staphylococcus aureus* and *Bacillus sp.* (Laboratory of Microbial Biotechnology, Faculty of Science and Technology, Fez, Morocco).

Statistical analysis

The results were expressed as the average and standard deviation. The comparison of the averages was performed by Student test and was considered significant at a value of $p < 0.05$.

RESULTS AND DISCUSSION

The results of our previous investigations on the chemical composition and the antibacterial effects of *C. myrtifolia* and *P. lentiscus* [11,15,16], a long with those of the scientific literature, have encouraged us to the exploitation and valorization of these plants by the formulation of a dermal ointment intended for external use. The quality control of the so-called ointment consists on the evaluation of the macroscopic characters (color, odor), homogeneity, pH and antibacterial activity.

Organoleptic property

The prepared ointment is of dark green color, homogeneous and soft. Characterized by a smell of lentisque and a pH of 6.5, it is stable to the usual conservation conditions and ensures a good adhesion to the skin.

Antibacterial activity

The results of Table 1 indicate that the ointment has an important antibacterial effect against the tested strains, which results in inhibition diameters of 18.3 and 15.6 mm respectively against *S. aureus* and *Bacillus sp.* (1000 mg/ml).

Table 1: The inhibition zones diameters of different concentrations of the formulated ointment

	T	1000 mg/ml	500 mg/ml	250 mg/ml	125 mg/ml
<i>Staphylococcus aureus</i>	7 ± 0	18.3 ± 0.4	14 ± 1.3	11 ± 1.1	8.3 ± 0.4
<i>Bacillus sp.</i>	6.6 ± 0.4	15.6 ± 0.4	14 ± 0.2	10.6 ± 0.8	6.3 ± 0.4

T: Blank (ethyl acetate and excipient)

The inhibition zones diameter is expressed in (mm), and represents the average of three repetitions.

This antibacterial activity can be explained by the presence of *P. lentiscus* essential oil and *Coriaria myrtifolia*'s ethyl acetate extract in this ointment. In fact, the antibacterial activity of these two plants has been attributed to their richness in biologically active secondary metabolites. Hence, Phytochemical screening indicated that several active compounds are present in the ethyl acetate extract of *C. myrtifolia* which in addition to polyphenols (480 ± 4.80) and flavonoids (17 ± 0.54) [15], contains tannins, steroidal heterosides and heterozoic triterpenes (Table 2) [22].

It is worth noting that polyphenols are recognized as secondary metabolites synthesized by higher plants in response to external aggressions, and have several roles in plant physiology and have potential healthy properties on human organism [23]. This, widely distributed, group of natural products have been shown to exert antioxidant, antiinflammatory, anticancer and antibacterial effects. Indeed, hundreds of publications reporting the antimicrobial activity of polyphenols have been recently published [24].

Moreover, polyphenols are known to be highly susceptible to auto-oxidation which induces formation of high molecular weight polymers. Indeed, polyphenols molecular weight is an important factor that governs their antimicrobial activity [25]. Because monomers are too small to establish enough hydrogen bridges while high molecular weight polymers are too large to cross the bacterial wall, so the ideal molecular weight would be that of oligomers [25]. It has been demonstrated that mechanisms of toxicity against microorganisms include either the deprivation of metal ions such as iron and non-specific interactions such as the establishment of hydrogen bonds with cell wall proteins (Adhesins) or enzymes [26].

While, the chromatographic analysis of *P. lentiscus* E. O. revealed that the major components were: myrcene (25.3%), limonene (15.7%), terpinene-4-ol (9.2%), β -gurjunene (2.6%), D-germacrene (2.3%) and α -pinene (1.6%), accounting for approximately 56.7% of its chemical composition (Table 3) [11]; beside other components such as 1,8-cineole, α -terpineol, linalool which also have a significant antibacterial activity.

Table 2: Chemical families' revelation results of the ethyl acetate extract of *C. myrtifolia* from Bab Berred [22]

Chemical families	Test Results
Polyphenols	+++
Flavonoids	+
Tannins	+
Steroidal glycosides	+
Triterpeneshétérosoides	+
Cardiac glycoside	-
Saponosides	-
Mucilage	-

+ presence/- absence

Table 3: Volatile components of the essential oil from leaves of *Pistacia lentiscus* [11]

RI	Constituents	Leaves (%)
939	α -pinene	1.6
953	Camphene	0.1
980	β -pinene	2.8
991	Myrcene	25.3
1005	α -phellandrene	3.2
1018	α -terpinene	1.7
1026	<i>P</i> -cymene	1.5
1031	Limonene	15.7
1033	1,8-cineole	1.3
1050	E- β -Ocimene	3.4
1088	α -Terpinolene	2.3
1098	Linalool	1.2
1143	Camphor	0.7
1177	Terpen-4-ol	9.2
1183	<i>P</i> -cymene-8-ol	0.9
1189	α -terpineol	3.5
1291	2-undecanone	0.2
1352	α -Cubebene	0.1
1376	α -Copaene	0.3
1391	β -Bourbonene	0.2
1395	β -Elemene	0.5
1403	Methyl eugenol	0.1
1418	Z-Caryophyllene	0.3
1432	β -gurjunene	2.6

1438	Epi-Bicyclic squiphellandrene	0.4
1454	α -Humulene	0.6
1461	Allo-aromadendrene	0.2
1477	d-murolene	0.1
1480	D-Germacrene	2.3
1524	δ -cadinene	2.7
1538	α -cadinene	0.5
1581	Caryophylleneoxide	0.2
1664	Humulene Epoxyde	0.2
1664	T-cadinol	0.6
1713	z-z-farnesol	0.3
	Total	86.80%

RI: Retention index, t : Traces (< 0.1%.)

Hence, 1,8-cineole is very active against some bacterial strains (*Salmonella sp.*, *Staphylococcus sp.*, *Bacillus sp.*) [27,28]. Moreover, the linalool-which is a terpene alcohol-is known for its inhibitory activity against *Enterococcus sp.* and *Escherichia coli* [29]. Furthermore, being among the major compounds of several medicinal plants such as *Cymbopogon citratus* [30], myrcene have been reported as antibacterial terpene [31]. Several other works have also demonstrated an antibacterial power for limonene [32,33], α -terpineol [34,35] and β -Ocimene [34] against several bacterial strains.

It should be noted that even if the ointment is diluted, the antibacterial effect is maintained (the inhibition diameter of the concentration 250 mg/ml against *S. aureus* is 11 mm). So, it can be concluded that the derivatives of the studied plants put under galenic form retain their effect.

CONCLUSION

Based on our previous studies results, exploitation of derivatives of *P. lentiscus* and *C. myrtifolia* was necessary. Hence the formulation of dermal ointment (based on these derivatives) intended for external use and having an important antibacterial effect was investigated. The galenic preparation and its quality control allowed obtaining of a dark green, homogeneous ointment, providing good adhesion to the skin and having an important antibacterial effect. In fact, inhibition zone diameters of 18.3 and 15.6 mm were obtained respectively against *S. aureus* and *Bacillus sp.* Hence, the objective of this work is therefore reached, and the valorization of the studied plants is performed.

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