Bioconversion of an Algerian Ovine Proteins to an Antimicrobial Agent for Skin Infection

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ABSTRACT

The aim of this study was the conversion of the ovine dermal proteins as a natural product that has an antimicrobial power used for tissue repair and skin lesion. After dehairing, fleshings, deliming the animal tissues (ovine) and purification, we supplied and fixed the essential oils of *Lavandula officinalis*, *Eucalyptus globules* and *Eugenia caryophyllata*. The product obtained is a spongy material communicating with open voids between the fibers. With a transparent light yellow color, it has a remarkable antibacterial power and a very strong inhibitory activity on all the bacterial strains tested whose average diameter of the inhibition zones exceeds 15mm. It is also characterized by a smell which can be aromatic, pleasant and spicy or fresh and spicy depending on the essential oil used.

KEY WORDS: Conversion, Ovine dermal proteins, natural substance, medical purposes, essential oils.

1. INTRODUCTION

The extraction technique of proteins and their preparation as biodegradable products from animal skins are well known for many years in medicine for the treatment of wound and the pathological cavity in the bones [1-3]. However, these products raise always a problem of potential transmission of an infectious agent, known or unknown, despite the severity of manufacturing and control procedures[4].

Some studies have been carried out actually to eradicate this problem from different antibiotics known by their antibacterial effect [5, 6] as it is the case for the gentamicin compress [7]. At the end of the 1980s and in the 1990s this process began to be no longer regarded as a miracle as it seems to be 40 years earlier. Indeed, many bacteria have developed a resistance to most antibiotics and, thus it seems important to find another alternative.

Studies have shown that essential oils could be a particularly credible application. Antimicrobial activity of essential oils is known empirically since antiquity [8]; their effectiveness as anti infection was scientifically established in vitro and in vivo. They have a genuine spectrum anti infectious including isolated cases of resistant infection to antibiotics [9]. Thus, our present study is looking into the preparation of an antimicrobial product based on ovine dermic protein powered by essential oils. This preparation is followed by tests of antimicrobial activity on the finished product.

2. MATERIALS AND METHODS:

2.1. Dermic protein preparation [10]:

The dermic protein preparation and transformation procedure was carried out from 24 months-aged north algerian (Mascara region) mortal remains ovine, in the manner described below:

Cleaning: the four parts of the weight of the mortal remains skin, weighing 5kg, was soaked in 5 to 15% aqueous sodium chloride solution, containing approximately 0.2 to 1 weight parts of sodium azide as preservative agent for 1000 parts in weight of the given solution, and from 0.5 to 2% in weight of non-ionic fat, a dispersing and wetting agent (phenilic ether of nonyl polyoxyethylene), with a temperature ranging from 18° to 20° for 24 hours, in a fuller, in order to remove impurities and stains.

Removal of wool: hairs are removed with a pelain bath, containing 4 weight parts with regards to the lime mortal remain weight 0.1M, with a temperature from 18° to 20° for 48 hours. The PH of the solution must be ranging between 12 and 13.5 in a fuller.

Hypoderm elimination: the subcutaneous tissue is eliminated mechanically with a defleshing instrument. The blades of the machine cylinder plane the subcutaneous tissue, which constitute an alcaline and a lot hydrated waste, called 'carnasse'.

Decalcifying: the defleshed tissue, consisting of 2 parts of its new weight, is soaked in a bath of a mixture of 2g/l of ammonium sulfate and 0.5g/l of sodium metabisulfite. This procedure is required to eliminate the alcaline products combined to dermic proteins.

The obtained pulp after these operations has a whitish color. 1 kg of it has been processed according to the Piter and Ries method [11], consisting:

Crushing: frozen from -10°C to -20°C, and finely crushed by means of a high-speed homogenizing knife. The temperature of the crushed matter has been kept below 4°C, by adding ices.

Paste cleaning: put in suspension, and shaked up strongly, at the same time, in order to wash well the paste in the 5 parts of its volume, in acetic acid at 0.5 M, and centrifuged.

Digestion: the solid centrifugal has been put in suspension in 5-litre acetic acid at 3%, containing 1g of

Purification: Ovine dermic proteins were purified by dissolution in acetic acid at 0.5 M, and precipitation by slow adding of 3% of aqueous sodium chloride.

Dissolution: Purified Ovine dermic proteins have been dissolved in acetic acid at 0.5M and diluted in water. Residual sodium chloride present in the proteins has been eliminated by cleaning using an ultrafilter. Ultrafiltration was carried on till the chloride ion will be undetectable in the eluate, after adding silver nitrate. The concentration of proteins increases to almost 2%.

Freeze-drying: the ovine dermic protein solution was filtered and poured in glass boxes, and freeze-dried at 0.3 cm in thickness.

Thermal processing: the semi-finite product has been heated to 100°C for an hour and half in a electrically heated drying classical oven, in order to improve its absorption and resistance against dampness, and then sterilized by irradiation with a dose of 2.5 billion of γ rays.

Technic pepsine for 1000 weight parts tissue, used as starting matter in 100ml of HCl at 0.01 N, in order to eliminate noncollagenous- type proteins and telopeptids. The suspension pH has been adjusted at 2.9 by means of HCl. The repeatedly shaken suspension is digested for 48 hours. The visquous ovine dermic protein solution was filtered by a G1drawing up filter, in order to eliminate non degraded residues.

Precipitation: the Ovine dermic proteins visquous filtrate was precipitated from the suspension by adding an aqueous solution of sodium hydroxide at 30% and separated by centrifugation.

2.2. Dermic protein transformations:

Feeding: It was divided into four parts. Each part is fed with essential oil, with a repeated tender shake for a half an hour to allow seepage and fixation of essential oils, except the last part which remains as a witness (see table 1).

The used oils are: lavender, clove and eucalyptus (see table 2). The used amounts depend on the efficient threshold, where the minimal inhibitive concentration is defined as the lowest oil concentration able to inhibit any bacterial growth [12]. Finally, the dampened products were packed in thermo-sealed polyethylene bags.

Table 1: Supply Formula of dermal ovine proteins *(semi-finite product).*

batchs	**% essential oil** (m/m)	**% vaseline** (m/m)
1rst batch : *Eucalyptus globulus*	0.1	10
2nd batch : *Eugénia caryophyllata*	0.1	10
3rd batch : *Lavandula officinalis*	0.8	10
4rth batch : without essential oil	0	10

Table 2: Characteristics and ch emical constituents of essential oils

Essential oil	Color	Smell	Main chemical constituents %		Aspect
Eucalyptus globulus	Very pale yellow	fresh and spicy	Eucalyptol	70%	Liquid
Eugénia caryophyllata	Light yellow	pleasant and spicy	Eugénol	78%	Liquid
Lavandula officinalis	Light yellow	aromatic	Linalool Linalyl acetate	51% 19%	Liquid

2. 3.Biological materials:

Bacterial strains: to estimate the anti-microbial activity, we have used wild strains and reference strains widely met in many human pathologies. Tests are performed on five bacteria from Pasteur Institute (Algeria) (see table 3).

Table 3: bacterial strain

Strain name	Reference	Gram	Family
Staphylococcus aureus	ATCC6538	+	Micrococaceae
Escherichia colis	ATCC25922	-	Enterobacteriaceae
Pseudomonas Aeruginosa	ATCC10145	-	Pseudomonaceae
Klebseilla pneumoniae	Sauvage	-	Enterobacteriaceae
Salmonella heiderlberg	Sauvage	-	Enterobacteriaceae

2.4. Anti-bacterial activity assessment:

The anti-bacterial activity assessment of various groups have been measured by the aromatogram technique, similar to that of the antibiogram used to test antibiotics [13].

We have used ovine dermic protein discs (products) of various groups of 5mm in diameter, put on the surface of a Mueller-Hinton gelosed environment. It's a standardized environment according to WHO standard, i.e in the way that it allows the growth of many bacteria. The environment is aseptically sown by 1ml of suspension of each strain, by using STRIS method. Boxes are pre-dryied before used [13, 14]. After incubation of knead boxes in the steam room at 37°C for 24 hours. The effect of the product is seen by the formation of an inhibition halo around the disc.

The reading of the results is made by the measure of the inhibition diameter (in mm), by means of a caliper rule or a ruler. HE dilution on the ovine dermic protein discs always happens in a solvent such as glycol ethylene [15], ethanol at 95% [16]. The principle of this method is always the HE migration by the diffusion in the gelose. This technique inspired from that of the antibiograms, has been generalized to HE [17].

A product is said active if it has an inhibition diameter greater or equal to 15mm [18].

2.RESULTS

The transformation of the ovine dermic proteins allowed to obtain a product which has a biodegradable spongy structure, communicating with open spaces between fibers.

The product obtained after deliming has a little bit white color (Fig.N°1), on the other hand the semi-finished product has a very white color (Fig. N°2). After supply and fixation of various types of essential oils, the color of the product turns on transparent clear yellow as seen on fig. N°3 with a spicy, fresh aromatic or pleasant spiced smell depending on the nature of essential oil used.

The study of the antibacterial power of products (ovine dermic proteins) fed by Eucalyptus globulus, Eugénia caryophyllata, Lavandula officinalis was made by the method of spreading on gelose. The measure of the diameter of the inhibition zones including the disc of our product (5mm) allowed to determine the antimicrobial activity of our in vitro products. Table N°4 followed by the graph N°4 show the results of the average antimicrobial activity tests of the various batch on bacterial strains of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas Aeruginosa*, *Klebseilla pneumoniae*, *Salmonella heiderlberg*.

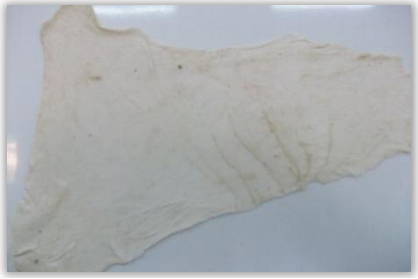

Fig. n°1: ovine skin after deliming

Fig. n°2: ovine protein semi finished

Fig. n°3: ovine protein - finished product

Table 4: Test of the protein ovine product's antimicrobial activity

Batchs	Means inhibition Zone (mm)									
	Staphylococcus aureus		*Escherichia coli*		*Pseudomonas Aeruginosa*		*Klebsiellia pneumoniae*		*Salmonella heiderlberg*	
1rst batch *Eucalyptus globulus*	19.5±0.1	+++	20±0.5	++	15.3±0.32	+	15.5±0.1	++	19±0.1	++
2nd batch *Eugénia caryophyllata*	22.5±0.5	+++	19.3±0.5		19±0.10	++	17.1±0.5	++	19±0.41	++
3rd batch *Lavandula officinalis*	22 ±0.2	++	16.1±0.1		17±0.26	++	16±0.50	++	17±0.21	++
4rth batch without essential oil	R	-	R		R	-	R	-	R	-

[18] R: resistant, insensitive (-), sensitive (+), very sensitive(++), extremely sensitive(+++)

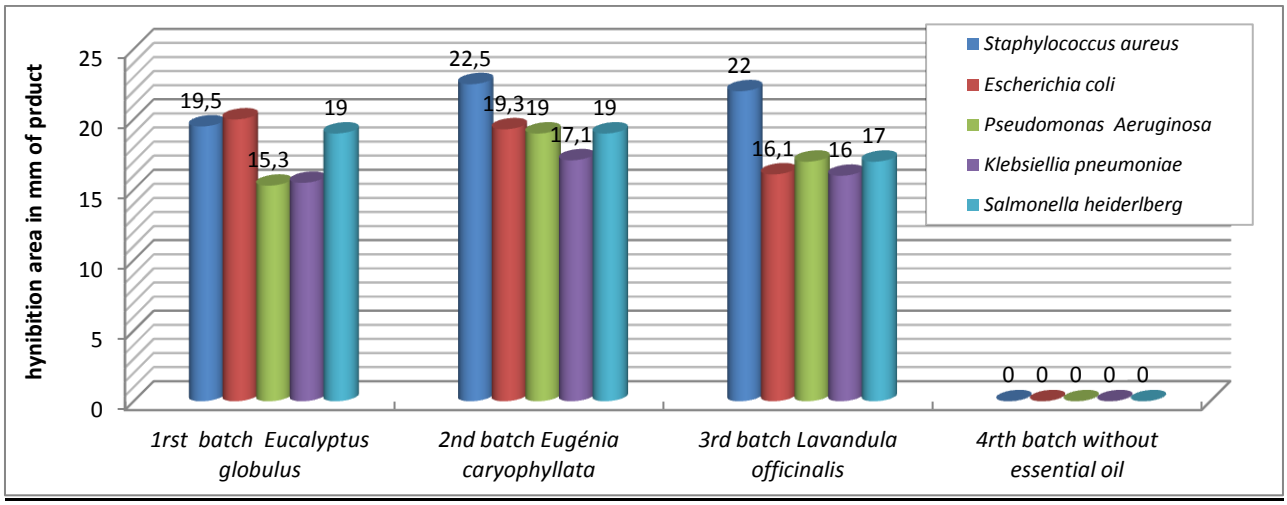

Fig. n°4: Test of the protein ovine product's antimicrobial activity.

3. DISCUSSION

We note that the essential oils were easily absorbed into our product. According to (Dorman and Deons, on 2000) [19]. active essential oils contain secondary metabolism components that are small enough to pass through the protein.

We think that the penetration and the fixation of essential oils in the product based on ovine dermic protein is caused by NH_3^+ of proteins which favor the fixation of the essential oil after the transfer of its proton (Fig.N°5),.

The product had a very strong inhibitive activity on all the tested bacterial strains with average values of the diameter of the inhibition zones exceeding 15 mm for the 1rd lot (*eucalyptus globulus)*. The Strains remained extremely sensitive for Staphylococcus aureus towards the 2nd lot *Eugénia caryophyllata* and the 3st lot *Lavandula officinalis*. According to Kalemba and Kunicka, the sensibility of a microorganism to essential oil depends on the properties of the essential oil and on the microorganism itself. It is well known that bacteria Gram (+) are more sensitive to essential oils, and confirm this phenomenon (Pool. [20] ; Brut. [21]; Beckechi. [22]).

The first three batches gave a broad-spectrum antibacterial activity acting as well on bacteria Gram(+) as Gram(-). The antibacterial properties of the main active components of essential oil (Eugenol, 1,8-cineole(Eucalyptol), linalool, Acetata of linalyl) are in part related to their lipophilic character leading to the accumulation in the bacterial cell walls [22-24]. This engenders a disturbance on the functioning and the permeability of the cellular membranes, a degradation of the cellular wall [25-26], damage to the cytoplasmic membrane and the cell content leaks [27-29].

On the other hand, the 4th batch remained resistant.

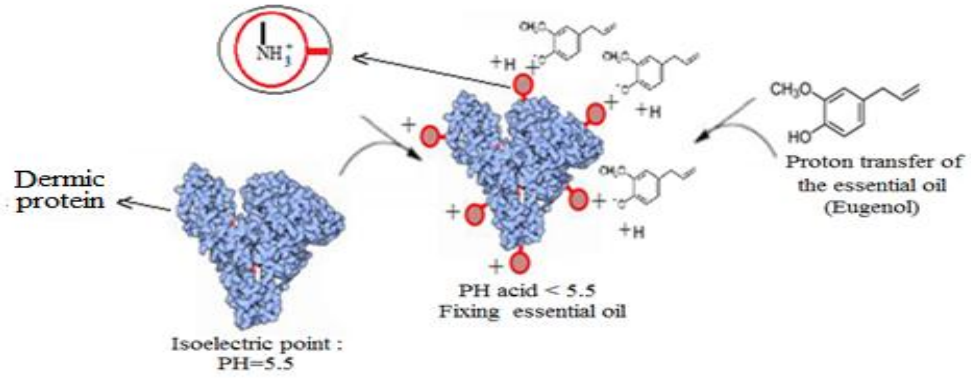

Fig. n°5: Insights hypothetical schematic of the proton transfer mechanism of the essential oil.

4. CONCLUSION

In this study we have shown how dermal proteins can be obtained from a ovine skin. These proteins are transformed into an antimicrobial product which has biodegradable spongy structure with a transparent light yellow color and an aromatic, or pleasant and spicy or

fresh and spicy smell depending on the nature of the essential oil used, in particular eucalyptus (*Eucalyptus globulus*) the clove (*Eugénia caryophyllata*) and lavender (*Lavandula officinalis*). The product has a very strong inhibitory activity on all tested bacterial strains of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebseilla pneumoniae*, *Salmonella heiderlberg* whose average values of the diameter of the inhibition zones is greater than 15 mm. This is particularly true for product supplied by (*Eucalyptus globulus*). The results are very promising, the strains remained extremely sensitive for *Staphylococcus aureus* towards the product supplied by *Lavandula officinalis* or Eugenia caryophyllata. The product gives a broad-spectrum antibacterial activity acting as well on bacteria Gram(+) as Gram(-). It is easy to see that the product obtained can be used for medical purposes.

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