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Biochemical Profile and Antimicrobial Activity of an Herbal-Based Formula and Its Potential Application in Cosmetic Industry

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Abstract: Microbial infections, and especially microbial resistance, are critical and actual problems that require targeted and efficient therapeutic intervention. Natural-based solutions are a viable alternative, at least for complementary therapy, due to few or no side effects and high safety and efficacy levels. The aim of this study was to demonstrate the potential use of a patented formula based on *Achillea millefolium*, *Origanum vulgare*, and *Lychnis coronaria* species as an antibacterial ingredient, mainly for skin and mucosal infections, in order to support its pharmaco-cosmetic application. The chemical composition of the formula was analyzed by HPLC and spectrophotometric methods. Furthermore, antioxidant and antimicrobial activity were evaluated. To determine the formula’s safety for topical application, it was used on a reconstructed human epidermal model. The formula showed inhibitory activity on both Gram-positive and Gram-negative bacteria, respectively, moderate inhibition on *B. cereus*, *Kocuria kristinae*, *P. aeruginosa*, *S. enterica* Typhimurium, methicillin-resistant and methicillin-sensible *S. aureus*, as well as high inhibition on *S. epidermidis*, *Serratia marescens*, and *Streptococcus pyogenes*. The developed product was biochemically characterized for its content in polyphenols, triterpenes, and polyphenol carboxylic acids. The formula was proven to have a nonirritant effect on the human epidermis and important antioxidant activity.

Keywords: *Achillea millefolium*; *Origanum vulgare*; *Lychnis coronaria*

1. Introduction

With the increasing prevalence of infectious diseases and the ability of microbials to develop resistance, the development of innovative routes to fight infections has become imperative.

Before the discovery of antibiotics, microbial infections were treated with herbal medicines or cauterization [1]. Natural compounds or extracts can be a valid choice to support the efficiency of allopathic medication, either as a monotherapy or as an adjuvant [2]. These phytochemicals can use several strategies; they can enhance antimicrobial drug activity, they can inhibit bacterial enzymes, or they can act as efflux-pump inhibitors. Many phytochemicals in combination with existing drugs were found to act as resistance modifying agents, and proper combinations may rescue the efficacy of important, lifesaving

antimicrobial agents [3]. Moreover, many antimicrobial herbal medicines possess simultaneous antibacterial, antifungal, antiprotozoal, and/or antiviral properties, but they also have immunostimulatory properties, which are particularly valuable in immunocompromised patients [4].

Herbal-based antimicrobial formulations are a proven alternative to synthetic compounds, with high applicability in various areas: food [5]; dermatology—for skin and soft tissue infections [6]; dentistry [7,8]; cosmetics [9]; and medicine [3]; Kolodziej, [10].

The *Achillea* genus comprises more than 100 species, many of them used in traditional medicine for their antispastic, anti-inflammatory, and cicatrizing effects [11]. The most widespread species, *Achillea millefolium* L. (yarrow or milfoil), has been listed among the most commonly used plant species in both folk and conventional medicine for over 3,000 years [12]. Its therapeutic effects are correlated to the rich content of various secondary metabolites, such as flavonoids, phenolic acids, terpenes, guaianolides, phytosterols, fatty acids, and organic acids [13]. As regards skin infections, it was found that *A. millefolium* extracts are the most potent antimicrobial ingredients, able to inhibit microorganisms, such as *S. aureus* and *P. aeruginosa*, which are usually resistant to plant extracts [14].

The genus *Lychnis* (syn. *Silene*), belonging to the tribe *Sileneae* of the family Caryophyllaceae, comprises 25–30 species growing in Northern and Eastern Europe and also in Asia, and includes weeds, ornamental plants, and species of medicinal value. Since ancient times, this plant was used to treat diarrhea, lung and liver ailments, leprosy, and beriberi [15]. Modern studies highlighted its antimicrobial effect; methanol and ethanol extracts are capable of inhibiting the growth of both Gram-negative and Gram-positive bacteria, such as *Klebsiella oxytoca*, *Escherichia coli*, *Proteus rettgeri*, *Citrobacter freundii*, *Pseudomonas aeruginosa*, *Alcaligenes faecalis*, *Enterobacter hormaechei*, *Pantoea agglomerans*, *Bacillus cereus*, *Staphylococcus epidermidis*, *Micrococcus luteus*, and *K. aerogenes*. The chloroform extract has a wider range of bactericidal activity, also inhibiting the growth of *S. aureus*, *Acinetobacter* sp., and *S. saprophyticus* strains, probably due to the presence of phytoecdysteroids (Mamadalieva 2012).

Other studies have confirmed that the ethanol, methanol, ethyl acetate, and propanol extracts of *S. coronaria* leaves and branches exhibit antibacterial activity against *S. aureus*, while the propanol extract is also active against *A. hydrophila* [16].

Different types of *Origanum* extracts are active against *E. coli* and *B. subtilis* [17], as well as *Salmonella* serotypes *Salmonella gallinarum*, *Salmonella enteritidis*, and *S. typhimurium* [18].

Although the literature data confirmed the benefits of using each one of these three herbal species in the treatment of various microbial diseases, this study aimed to demonstrate the potential use of a patented formula based on a mixture of them as an antibacterial ingredient, mainly for skin and mucosal infections, in order to support its pharmacocosmetic application. The research conducted on the formula containing *Achillea millefolium*, *Origanum vulgare*, and *Lychnis coronaria* extracts included an analytical characterization of the main compounds and a screening of the antimicrobial and antioxidant effects. In addition, to support its safety for topical application, the developed formula was tested for any irritant potential on a reconstructed human epidermal model.

2. Materials and Methods

2.1. Preparation of the Bioactive Solution

The solution was prepared according to patent no. RO118996/2004 with a slight modification.

Achillea millefolium, *Origanum vulgare*, and *Lychnis coronaria* L. were provided by S.C. Dacia Plant S.R.L., Bod, Romania which cultivates these species under ecological conditions. The raw material was dried and ground as a fine powder. Voucher specimens (AM22/2020, OV16/2020, and LC6/2020, respectively) were deposited at the manufacturer.

Briefly, a mixture of *Lychnis coronaria*, *Origanum vulgare*, and *Achillea millefolium* (5:3:2 w/w/w) raw materials was homogenized with 30% ethyl alcohol (in a ratio of vegetable raw material/solvent of 1:5 m/v) and left to soak at room temperature for 7 days with occasional stirring. After filtration, a reddish-brown solution was obtained and further concentrated at reduced pressure and 60 °C, up to 5:1 v/v compared to the initial solution. About 20 mL of concentrated ethyl alcohol was added to this solution and left in a refrigerator for 4 days, then filtered *in vacuo* to obtain a final, clear, reddish-brown solution. To this was added glycerin (70% of the total volume) to stabilize the solution.

2.2. Biochemical Profile

2.2.1. Spectrophotometric Analysis of Phenolic Compounds

Quantification of the total phenolic compounds expressed in gallic acid was performed by a Folin–Ciocalteu colorimetric method, which is based on the formation of colored complexes with maximum absorption in VIS at $\lambda = 750$ nm.

2.2.2. Spectrophotometric Analysis of Triterpenic Acids

The dosing of triterpene compounds was performed by a UV-absorption spectrophotometric method. The principle of the method consists in the dehydration of triterpenic acids in the presence of concentrated sulfuric acid at a temperature of 70 °C for 2 h, time necessary to complete the reaction when 2 absorption peaks appear in the product spectrum at $\lambda = 302$ – 320 nm and at $\lambda = 405$ – 410 nm.

2.2.3. Quantitative Analysis by High-Performance Liquid Chromatography (HPLC)

The optimal conditions for analysis were determined using specific standards for compounds of interest. Quantitative HPLC analysis of the main polyphenolic components was performed on a HPLC SHIMADZU, with a DAD detector; a Kromasil C18 column (100 mm × 4.6 mm) for polyphenol carboxylic acids at 25 °C, using a gradient elution. Separation of polyphenols was performed using water acidified with formic acid 1% and methanol acidified with formic acid 1%, respectively, as mobile phase at an initial flow rate of 0.1 mL/min, with an injection of 15 μ L. All reference substances (chlorogenic, rosmarinic, caffeic, and gallic acids were purchased from Sigma-Aldrich (Darmstadt, Germany).

2.2.4. Antioxidant Activity

The free radical scavenging activities of the product were determined according to a method adapted by Brand-Williams et al. [19], using the stable free radical, diphenyl-picryl-hydrazyl (DPPH). Briefly, 2 mL of 100 μ M ethanolic solution of DPPH was mixed with 1 mL plant formula, shaken vigorously, and further incubated for 30 min at room temperature in a dark place. The change in color from deep violet to light yellow was measured at 515 nm (A_{sample}), and the percentage of the radical scavenging activity (RSA) was calculated by using the following equation: $\% \text{ RSA} = (1 - [A_{\text{sample}}/A_{\text{control } t=0}])/100$. As a negative control, a DPPH 100 μ M solution in 80% ethanol was used, while as a positive control, a 0.02% L-ascorbic acid was used. The results were expressed as means for three measurements.

2.3. Pharmacologic Activity

2.3.1. Irritant Potential

To assess the skin irritation potential of the formula, a reconstructed human epidermal model, EpiDerm EPI-200 (MatTek Corporation, Ashland, MA, USA) consisting of normal, multi-layered, human-derived epidermal keratinocytes was used. The procedure was followed in accordance with the manufacturer's instructions. Cell viability was measured by the MTT reduction assay. As a positive control, 5% sodium dodecyl sulfate (SDS) was used, while as a negative control, Dulbecco's phosphate-buffered saline (DPBS) was

used. Relative cell viability was calculated for each tissue as a percentage of the mean of the negative control tissues. In accordance with UN GHS, the test substance was considered to be a nonirritant to skin if the tissue viability after exposure and post-treatment incubation were greater than 50%.

2.3.2. Antimicrobial effect

Tested microorganisms

For the antagonistic test, we took into account both Gram-positive and Gram-negative pathogenic bacteria, as well as some fungi showing pathogenic potential in humans and animals, such as *Candida* spp. and *Aspergillus* spp., as seen in Table 1.

Table 1. Pathogenic microorganisms used in the antagonistic tests.

No.	Microorganisms	Origin
Bacterii		
1.	<i>Bacillus cereus</i> CP1	UASMVB collection
2.	<i>Kocuria kristinae</i>	UASMVB collection
3.	<i>Escherichia coli</i> ATCC *8739	ATCC
4.	<i>Listeria ivanovii</i> ATCC 19119	ATCC
5.	<i>Listeria monocytogens</i> ATCC 7644	ATCC
6.	<i>Proteus vulgaris</i> ATCC 13315	ATCC
7.	<i>Pseudomonas aeruginosa</i> ATCC 9027	ATCC
8.	<i>Salmonella enterica</i> Typhimurium ATCC 14028	ATCC
9.	<i>Staphylococcus aureus</i> ATCC6538	ATCC
10.	<i>S. aureus</i> ATCC 33592 MRSA **	ATCC
11.	<i>S. epidermidis</i> ATCC12228	ATCC
12.	<i>S. epidermidis</i> ATCC 51625 MRSE **	ATCC
13.	<i>Serratia marescens</i> ATCC 14756	ATCC
14.	<i>Streptococcus pyogens</i> ATCC 19615	ATCC
15.	<i>Enterococcus faecalis</i> ATCC 29212	ATCC
Fungi		
16.	<i>Candida albicans</i> ATCC 10231	ATCC
17.	<i>C. parapsilosis</i> ATCC20019	ATCC
18.	<i>C. glabrata</i> ATCC 2001	ATCC
19.	<i>C. tropicalis</i> ATCC 44508	ATCC
20.	<i>C. guilliermondii</i> MI 40	UASMVB collection
21.	<i>C. krusei</i> 2016 MI 41	UASMVB collection
22.	<i>Aspergillus carbonarius</i>	UASMVB collection
23.	<i>Aspergillus niger</i>	UASMVB collection
24.	<i>Aspergillus flavus</i>	UASMVB collection

Legend: * ATCC—American Type Culture Collection; ** MRSA and MRSE—methicillin-resistant.

Testing the Inhibitory Activity on Pathogenic Microorganisms

For assessing the antagonistic activity of the product against pathogenic microorganisms, a slightly modified method of the disk-diffusion assay (Kirby–Bauer test) was used. The medium used for growing pathogenic bacteria was nutrient-agar, while for the yeast and molds, PDA (potato–dextrose–agar) (VWR, Leicestershire, UK). A double-layer media was prepared; the bottom layer contained 2% agar, while the upper layer 1% agar. The pathogenic microorganisms were incorporated in the second layer of the media, while a quantity of 100 µL of tincture was poured in small holes made in the first layer of the medium. After 48 h of incubation at 37 °C for bacteria and 28 °C for fungi, the inhibition

zones were measured. All experiments were performed in triplicate. A solution of 30% methanol (*v/v*) was used as a control.

The results were expressed as the mean $\pm$ standard deviation (SD), and all investigations were done in triplicate. The differences were analyzed by one-way analysis of variance (ANOVA). The significance level was set as follows: significant, $p \leq 0.05$; very significant, $p \leq 0.01$; and highly significant, $p \leq 0.001$.

3. Results

A patented formula made of *Achillea millefolium*, *Origanum vulgare*, and *Lychnis coronaria* (Patent RO118996/2004), was tested for its pharmaco-cosmetic potential. In this respect, the developed product was characterized for its biochemical composition and tested for its irritant activity and inhibitory activity against different human- and animal-pathogenic microorganisms.

3.1. Biochemical Profile

3.1.1. Total Polyphenols and Triterpenes

Phenolic compounds are a large and representative class for the *Origanum* and *Achillea* genera, and triterpenes are mainly found in *Lychnis*. The chemical content of the formula is presented in comparison to the raw material (see Table 2). It can be noted that *Origanum* has the highest polyphenol content.

Table 2. Spectrophotometric analysis of phenolic and triterpene compounds.

Sample	Total Polyphenols Expressed as Gallic Acid (%)	Total Triterpenes Expressed as Ursolic Acid (%)
Raw material <i>Origanum vulgare</i>	6.070	1.053
Raw material <i>Lychnis coronaria</i>	0.540	2.792
Raw material <i>Achillea millefolium</i>	1.098	1.051
Herbal formula	0.916	0.478

3.1.2. Polyphenol Carboxylic Acids

The main detected polyphenol carboxylic acids were chlorogenic acid, caffeic acid, rosmarinic acid, and gallic acid; their content in the formula is shown in Table 3.

The three genera used in this formula are rich in phenolic compounds, and some studies have linked this content to antimicrobial efficiency. *O. vulgare* is an important source of polyphenolic compounds as was suggested by Oniga et al., [20] who found that the representative phenolic compound is rosmarinic acid (12.83 mg/g), followed by chlorogenic acid (2.10 mg/g), while gentisic and *p*-coumaric acids are present in trace amounts [20].

Table 3. Quantitative analysis of the formula by high-performance liquid chromatography (HPLC).

Polyphenol Carboxylic Acid Content (HPLC)	mg/100 mL:
-Chlorogenic acid:	10.58
-Caffeic acid:	11.66
-Rosmarinic acid:	19.33
-Gallic acid:	0.68
Total:	42.26

In another *Achillea* species, *A. moschata*, it was shown that only the most polar fraction displayed antimicrobial activity, probably due to phenolics such as apigenin, centaureidin, and nevadensin [21]. The *A. schurii* extract also revealed a remarkable inhibitory activity against *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella typhimurium* which correlated with high phenolic content [22]. Several phenolic acids were also identified in *L. flos-cuculi* extracts: 4-hydroxybenzoic acid, caffeic acid, ferulic acid, vanillic acid, *p*-coumaric acid, and protocatechuic acid [23].

3.1.3. Antioxidant Activity

One of the methods for antioxidant potential assessment of a compound/preparation is DPPH assay. The DPPH radical is a stable, organic, free radical with an absorption maximum band around 515–528 nm, which can be reduced by antioxidant samples to a yellow-colored compound, diphenyl picrylhydrazine, the extent of the reaction depending on the hydrogen-donating ability of the antioxidants (Brand-Williams et al., 1995). L-ascorbic acid was used as positive control due to its capacity to reduce and decolorize 1,1-diphenyl-2-picrylhydrazine, reaching a maximum inhibition of 97.92 ± 0.62 %. In revanche, the plant extract formula reached a maximum inhibition activity of 83.24 ± 1.86 %, representing 85% of the inhibitory activity of the L-ascorbic acid. The antioxidant activity of *A. millefolium* was reported previously as different ethanolic, methanolic, or aqueous extracts; in ethanolic extract, the inhibitory activity at a concentration of 30 µg/mL extract was above 83.23% [24], which is close to the value obtained for our formula.

3.2. Pharmacologic Activity

3.2.1. Irritant Potential

A reconstructed human epidermal model is widely used to prove the safety of various products (chemicals, cosmetics, raw materials, etc.) in terms of skin corrosion, irritation, and penetration (Faller et al., 2002). According to the obtained data (Table 4), the formula was proven to be nonirritant for the human epidermis.

Table 4. Cell viability levels expressed as mean $\pm$ SEM, based on reconstructed human skin * $p < 0.05$ significantly different compared to untreated group.

Sample *	Average OD	Stdev	Relative viability	Conclusion
Positive control (DPBS)	1.1643 ± 0.125	-		
Negative control (SDS)	0.0875 ± 0.240	0.0016	8.86	
Formula	$1.0906^* \pm 0.135$	0.7890	110.88	Nonirritant (NI)

3.2.2. Antimicrobial Effect

The formula based on *Achillea millefolium*, *Origanum vulgare*, and *Lychnis coronaria* was tested in triplicate for their antagonistic effects against several pathogenic bacteria, as well as against *Candida* spp. and *Aspergillus* spp., which are frequent fungi in animal and human infections.

Generally, the formula exhibits moderate to high inhibitory activity against several pathogenic bacteria, as seen in Table 5. For instance, moderate inhibitory activity was registered in the case of Gram-positive bacteria, such as *B. cereus*, *K. kristinae*, and methicillin-resistant and methicillin-sensible *S. aureus*, as well as on Gram-negative bacteria, such as *P. aeruginosa* and *S. enterica typhimurium*. High inhibitory activity was noticed against Gram-positive species, such as *Staphylococcus epidermidis* and *Streptococcus pyogenes*, and on Gram-negative bacteria, such as *S. marescens*. Low inhibitory activity was limited to species like *E. coli*, *Listeria ivanovii*, and *Enterococcus faecalis*, while no inhibition was shown in the case of *Listeria monocytogenes* and *Proteus vulgaris*. Some relevant inhibitory activity can be observed in Figure 1.

Table 5. Antibacterial activity of the formula based on *A. millefolium*, *O. vulgare*, and *L. coronaria*.

Nr. crt.	Microorganism	Inhibition Diameter (mm)	Antibacterial activity
1.	<i>Bacillus cereus</i> CP1	17 ± 0.25	++
2.	<i>Kocuria kristinae</i>	18 ± 0.15	++
3.	<i>Escherichia coli</i> ATCC 8739	7 ± 1.2	+
4.	<i>Listeria ivanovii</i> ATCC 19119	4 ± 1.4	+
5.	<i>Listeria monocytogenes</i> ATCC 7644	-	-
6.	<i>Proteus vulgaris</i> ATCC 13315	-	-
7.	<i>Pseudomonas aeruginosa</i> ATCC 9027	12 ± 0.25	++
8.	<i>Salmonella enterica</i> Typhimurium ATCC 14028	16 ± 0.34	++
9.	<i>Staphylococcus aureus</i> ATCC6538	18 ± 0.25	++
10.	<i>S. aureus</i> ATCC33592 MRSA*	19.7 ± 0.14	++
11.	<i>S. epidermidis</i> ATCC 51625 MRSE	21 ± 0.15	+++
12.	<i>S. epidermidis</i> ATCC12228	20 ± 0.17	++
13.	<i>Serratia marescens</i> ATCC 14756	28 ± 0.25	+++
14.	<i>Streptococcus pyogenes</i> ATCC 19615	31 ± 0.35	+++
15.	<i>Enterococcus faecalis</i> ATCC 29212	5 ± 0.10	+

Legend: - = no inhibitory activity; + = 1–10 mm; ++ = 11–20 mm; +++ = >20 mm.

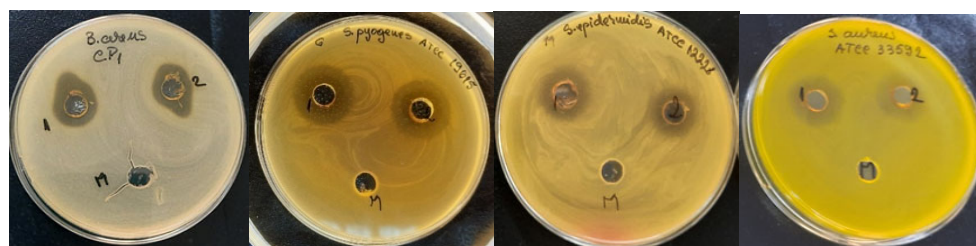


Figure 1. Visual aspects of inhibitory activity of the formula based on *A. millefolium*, *O. vulgare*, and *L. coronaria* on different pathogenic bacteria. From left to right: *B. cereus*, *S. pyogenes*, *S. epidermidis*, and *S. aureus*. 1 and 2 represent the duplicate of the developed formula.

Regarding antifungal activity, no inhibition was observed against any of the tested *Candida* and *Aspergillus* species.

The results indicate that the formula could be a candidate as an active ingredient of cosmetic products protecting the skin from the harmful impact of environmental factors. It is widely recognized that, in this area, plant extracts can act in two ways: by reducing skin infections and also by inhibiting the growth of microorganisms inside cosmetic products [25].

Among microorganisms causing skin infections, *Staphylococcus aureus* is one of the most widespread, and it is also partially responsible for atopic dermatitis (AD) symptoms

[26]; superficial, but also deep, ulcerative skin infections; infection of hair follicles; abscesses; wounds; and ulcers [27]. Moreover, *P. aeruginosa*, *S. aureus*, and *C. albicans* are considered the main potential pathogens in cosmetic products.

The inhibitory potential of our formula should be approached as a combined, cumulative activity of the antimicrobial active substances of all three plants. Different authors have reported microbial inhibition for two out of the three used plants (*A. millefolium* and *O. vulgare*). Among the three plant species included in the formula, *A. millefolium* was reported to be one of the most common medicinal plants used in the Balkan area for its antimicrobial activity [28]. Some studies are coming in support. Ethanolic extract of *Achillea* species showed a broad spectrum of strong antibacterial activities against *K. pneumoniae*, *E. cloacae*, *S. typhimurium*, *S. epidermis*, *E. coli*, *E. aerogenes*, *S. aureus*, *Klebsiella oxytoca*, *S. pyogenes*, *P. aeruginosa*, and *C. albicans* [29].

Romer et al [30], evidenced the fact that tincture of *A. millefolium* has significant inhibitory activity on *Staphylococcus aureus*, but not on *E. coli* or *P. aeruginosa*. A higher inhibition spectrum was exhibited by *A. millefolium* and its hybrid *Achillea collina*, Becker essential oils against *E. coli*, *Shigella flexneri*, *Klebsiella pneumoniae*, *Salmonella typhimurium* and *Staphylococcus aureus*, but no activity was noted against *Clostridium perfringens* or *Streptococcus pyogenes* [31,32]. It was suggested that the inhibitory effects could be attributed to the major constituents of the essential oils, respectively, caryophyllene, chamazulene, and β -pinene, and also of the minor components with strong antimicrobial activity, such as 1,8-cineole, limonene, or α -pinene [32]. Meanwhile, ethanolic extracts of the aerial parts of *O. vulgare* were proven to have some antibacterial activity on *Listeria monocytogenes* and *Staphylococcus aureus*, as well as antifungal activity on *Candida albicans* [33]. Our formula did not show any anti-*Candida* activity. *O. vulgare* essential oil (EO) contains high amounts of thymol and carvacrol, compounds known for their excellent antimicrobial activity alone or in combination with other antibiotics [9]. This may be a reason why different extracts of *O. vulgare* exhibit inhibitory activity against *E. coli*, *S. aureus*, *C. albicans*, and *P. aeruginosa* [9]. The species *L. coronaria* was not so often reported for its antimicrobial activity. However, it was proven that methanol and ethanol extracts of *Lychnis flos-cuculi*, from the same genus, inhibit the growth of microorganisms—both Gram-negative and Gram-positive bacteria [34]. It is known from the literature that slightly polar acylated ecdysteroids from *Lychnis* possess antimicrobial activity [35].

4. Conclusions

A formula made of *Achillea millefolium*, *Origanum vulgare*, and *Lychnis coronaria* was biochemically characterized and tested for its antimicrobial and nonirritant activity. Important content levels of polyphenols, triterpenes, and polyphenol carboxylic acids were found in the mixture, as a cumulative result of each plant compound. The formula proved to have high antioxidant potential. Regarding antimicrobial activity, the product showed inhibitory activity on both Gram-positive and Gram-negative bacteria, respectively, moderate inhibition on *B. cereus*, *Kocuria kristinae*, *P. aeruginosa*, *S. enterica* Typhimurium, and methicillin-resistant and methicillin-sensible *S. aureus*, as well as high inhibition on *S. epidermidis*, *Serratia marescens*, and *Streptococcus pyogenes*. No inhibitory activity was noticed in the case of *Candida* spp. and *Aspergillus* spp. The proven nonirritant action of the product supports its potential use in pharmaco-cosmetic products. Furthermore, we expect the development of a new cosmetic formula, based on this plant extract mixture, to be used for different skin infections associated with the presence of the staphylococci, such as acne.

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