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(54) **Title:** COSMETIC PREPARATIONS AGAINST DANDRUFF

(57) **Abstract:** Cosmetic preparations for the treatment of dandruff contain Ethyl Lauroyl Arginate HCl (LAE) in combination with a further active ingredient which is effective against dandruff. Such a further active ingredient may be at least one selected from the group consisting of zinc pyrithione, salicylic acid and piroctone olamine.



COSMETIC PREPARATIONS AGAINST DANDRUFF

Technical field

The present application relates to novel cosmetic preparations for the treatment of dandruff.

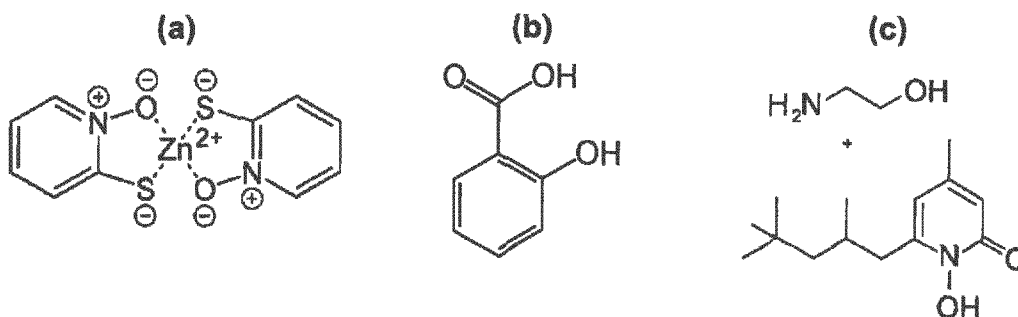
Background art

Dandruff, also called scurf, and historically termed *Pityriasis capitis*, is clinically defined as a light seborrheic dermatitis of the scalp, which affects almost half of the post-pubertal population of both genders of any ethnicity (Saint Léger, D. ; in: The science of hair care, 2nd edn (Bouillon, C., Wilkinson, J., eds), 2005, 609–631). This disorder consists of an exfoliation process of the scalp epidermis caused by several parameters, which leads to an unusual increase of a yeast-like fungus of the genus *Malassezia* around the corneocyte (McGinley, K.J. et al.; *J. Invest. Dermatol.* 1975; 64: 401–405). Corneocytes respond to this increase of the *Malassezia* population releasing a set of pro-inflammatory mediators responsible of the micro-inflammation frequently associated to dandruff. Local deposits of immunoglobulins appear and lymphokines are released that are responsible for the recruitment and local activation of leukocytes, which in turn lead to the eventual amplification of the inflammatory reaction (Piérard-Franchimont, C. et al.; *International Journal of Cosmetic Science* 2006; 28: 311–318). Several stimuli are known to cause this excess of sebum production on the scalp: stress, lack of adequate hygiene, allergies, vitamin B deficiency, hormonal factors and environmental factors, such as ultraviolet radiation and direct application on the scalp of some irritant chemical products. Dandruff represents an annoying aesthetical disturbance, because of the visible scalp flaking and the itching often associated.

The *Malassezia* genus needs for its growth to dispose of an exogenous source of C₁₂-C₁₆ fatty acids, since it is not possible for this micro-organism to synthesize these. *Malassezia* yeasts can usually be found on the skin of humans, however, when a major amount of sebum is produced on the skin because of the

aforementioned stimuli, its population increases contributing to the development of dandruff. The species of the genus *Malassezia*, which has mainly been related to dandruff is *Malassezia furfur* (also known as *Pityrosporum ovale*), but other species such as *M. globosa*, *M. restricta* and *M. sympodialis* have also been described (Piérard-Franchimont, C., et al.; *Dermatology* 2000; 200: 93–98; Saint-Léger, D., et al.; *J. Soc. Cosmet. Chem.* 1988; 40: 109–117).

Nowadays, the most common strategies used in cosmetics to fight against dandruff are aimed to remove scales, to reduce the adherence of *Malassezia* to corneocytes and principally to inhibit its growth. Some representative examples of anti-dandruff agents are: zinc pyrithione (see below a), salicylic acid (see below b) and piroctone olamine (see below c).



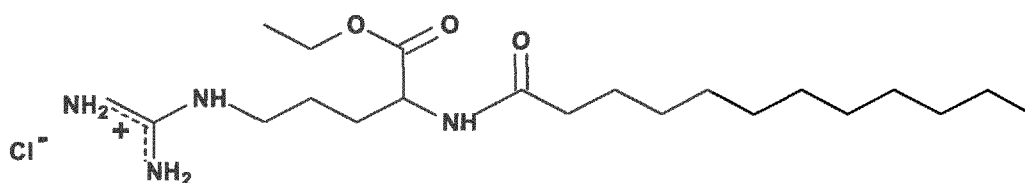
Zinc pyrithione is a powerful anti-fungal with some keratolytic properties. However, this compound has the disadvantage of being quite water-insoluble. Therefore, it has to be incorporated as a suspension to anti-dandruff shampoos or lotions. Besides, it is a compound with a certain degree of toxicity and it has been described that it can damage the DNA of keratinocytes (Lamore S. D. et al.; *Cell Stress and Chaperones* 2010; 15: 309–322).

Salicylic acid is a mild anti-fungal and anti-bacterial active with exfoliating properties on the basis of its capacity to soften the keratin filaments of the cornified layer of the epidermis. It is used in anti-acne and anti-dandruff treatments thanks to its keratolytic and comedolytic properties (Ranganathan S. & Mukhopadhyay T.; *Indian J. Dermatol.* 2010; 55(2): 130-4; Bunse T. & Mahrle G.; *Acta Derm. Venereol.* 1992; 72(1): 72-3). The acid form of salicylic acid is poorly soluble in water and even

though it is the most active form, usually the pH of cosmetic formulations needs to be in the range where mostly the basic form is present (pK_a salicylic acid = 2,97).

Piroctone olamine is the ethanolamine salt of the hydroxamic acid derivative piroctone. It is primarily used as anti-dandruff active agent due to its anti-bacterial and anti-fungal properties. It is considered to be safer and less irritating than zinc pyrithione (Dubini F. et al.; Azneim.-Forschung 2005; 55 (8): 478–83) being a good alternative to the latter for skin-sensitive individuals.

Ethyl Lauroyl Arginate HCl (LAE[®], trademark registered for Laboratorios Miret S.A.) is a cationic surfactant with antimicrobial properties derived from natural building blocks: Lauric acid and the amino acid *L*-arginine (see formula below). This compound inhibits the proliferation of several micro-organisms including bacteria, fungi and yeasts by means of disturbance of the membrane potential, altering cell permeability and therefore inducing the loss of cell viability (Infante R. et al.; International J. Cosm. Science 1984; 6: 275–282).



The MIC (Minimum Inhibitory Concentration) values of LAE for all micro-organisms are extremely low, being 12 ppm of active substance the corresponding MIC value for *Malassezia furfur* (Woods, G.L. & Washington, J.A.; in: Antibacterial susceptibility test: Dilution and disk diffusion methods en Manual of Clinical Microbiology eds. Murray, P.R. et al. ASM Press. Washington, D.C. 1995, 1327-1341).

LAE has been described as an active ingredient in the treatment of dandruff (see Commission Directive 2010/3/EU of February 1, 2010).

Since LAE is a cationic surfactant, with a very mild eco-toxicological profile and suitable for Ecocert formulations, it shows many advantages in terms of being aligned with the current market trends compared to the traditional anti-dandruff active agents.

The use of LAE in shampoos has been described before. For instance such a shampoo is described in EP 0 749 960 B1; but in that case the technological need to apply LAE was to preserve the shampoo and this use is different from the anti-dandruff use.

Among the cosmetic preparations which are used to treat dandruff, shampoos are the preferred ones, due to their convenience. Other sorts of cosmetic preparations which can deliver anti-dandruff active agents are lotions, rinse-off conditioners and leave-on treatments.

Disclosure of the invention

Objects to be solved by the invention

It is the object of the present invention to provide novel cosmetic preparations for the effective treatment of dandruff.

Means for solving the problems.

It was found by the present inventors that the combination of LAE with conventional anti-dandruff agents leads to a surprising activity. The activity which is observed was regularly higher than the sum of the activities of each of the components alone and is considered to represent a synergetic activity in such cases.

Shampoos containing LAE have been described before. One such shampoo is described in EP 0 749 960 B1 which contains 30 % by weight of sodium lauryl sulfate and 5 % by weight of lauric diethanolamide. LAE is present in amount of 0.04 to 0.05 % by weight. It is emphasized in the document that LAE is present to execute its function as a preservative of the shampoo. There is no description of a contribution of LAE to the effect of the shampoo.

Further shampoos containing LAE are described in EP 1 414 394 A1 and EP 1 414 395 A1. Also in these documents the presence of LAE was merely intended for the preservation of the cosmetic preparations. Apart from this preservation effect in

the shampoo by LAE, no other effects to humans were described in the documents, for example the anti-dandruff effect.

It has now been confirmed in experiments, that the presence of LAE is effective for the treatment of dandruff. This effect of LAE can be observed when the product 0.01 to 1.5 % by weight relative to the total weight of the preparation. This is within the normal range of the concentration of LAE which is normally adjusted to achieve a preservative effect. It is preferred to maintain the concentration of LAE within the range of 0.05 to 1.0 % by weight and more preferred to maintain this concentration within the range of 0.1 to 0.8 % by weight.

The treatment with LAE may be conducted on the basis of a conventional preparation. This conventional preparation may be an aqueous solution containing further ingredients which are usual in the art. It is also possible to provide the preparation as a shampoo, whereby LAE as the active ingredient is added to any type of shampoo preparation which has been described in the state of the art.

It is the surprising observation of the present invention that the addition of LAE to compositions prepared for the treatment of dandruff leads to a substantial improvement of the effect of these compositions. This means that a combination of LAE with another active ingredient with an effect on dandruff leads to an improved effect. The effect of the combination which is observed is better than the effect of each of the components alone.

This effect is observed in cosmetic preparations containing besides LAE at least one further active ingredient which is effective against dandruff.

The at least one further active ingredient may be selected from the group consisting of Aminopropyl Menthyl Phosphate, *Artemisia annua* Leaf Extract, *Asarum heterotropoides* Extract, *Betula ermanii* Stem Extract, *Boesenbergia pandurata* Rhizome Extract, *Brassica campestris* (Rapeseed) Flower Extract, *Capsicum annuum* Fruit Extract, *Cardiospermum halicacabum* Seed Extract, *Carpinus tschonoskii* Leaf Extract, Citronellic Acid, * Coal Tar, *Coptis japonica* Extract, *Fragaria ananassa* (Strawberry) Seed Oil, *Hedera helix* (Ivy) Stem Extract,

Hexamidine Diisethionate, Hydrolyzed *Saccharomyces*/Sodium Selenate Ferment Extract, Hydroxypropyl Bisstearamide MEA, *Juniperus communis* Sprout Extract, Kiwi Fruit Extract Beta-Glucan, Ketoconazole, Lactobacillus/Rice Bran/*Saccharomyces*/*Camellia sinensis* Leaf Extract Ferment, *Lactococcus*/Bean Seed Extract Ferment Filtrate, *Larix sibirica* Wood Extract, *Leuconostoc*/Radish Root Ferment Filtrate, Macelignan, Magnesium/Aluminum/Zinc/Hydroxide/Carbonate, *Mallotus japonicus* Leaf Extract, MEA-Hydroxyoctyloxypyridinone, *Metasequoia glyptostroboides* Extract, *Morus bombycis* Extract, Octadecenedioic Acid, sh-Octapeptide-4, Ovalicin, Oxidized Beta-Glucan, Panduratin A, *Peumus boldus* Leaf Oil, *Physocarpus amurensis* Stem Extract, Phytosphingosine Acetamide, *Pinus densiflora* Leaf/Sugar Ferment Filtrate, *Pinus pinaster* Bark/Bud Extract, *Pogostemon cablin* Leaf/Stem Extract, Pomegranate Fruit Peel Extract Octenylsuccinate, Pumpkin Fruit Extract Beta-Glucan, * Salicylic Acid, Saponinyl Acetosteardimonium Chloride, *Sciadopitys Verticillata* Root Extract, * Selenium Sulfide, Shikimic Acid, Silver Bicarbonate, *Smilax china* Extract, Sodium Citronellate, *Sorbus aucuparia* Seed Oil, * Sulfur, Undecylenamidopropyl PEG-2 Dimonium Undecylenate, *Vitis vinifera* (Grape) Seed Extract, Zinc Glycinate Salicylate, * Zinc Pyrithione (Pyrithione Zinc), Zinc Thiosalicylate, Urea, Lactic Acid, Piroctone Olamine, Climbazole, Resorcinol, Undecylenic Acid, Undecylenate Imidazole, Sodium Shale Oil Sulfonate.

The products in the above list characterized with an asterisk * are approved as drugs in the USA at the filing date of the application.

It is particularly preferred that the cosmetic preparations according to the invention contain at least one active ingredient selected from zinc pyrithione, salicylic acid and piroctone olamine besides LAE.

The cosmetic preparation may be any preparation which is known in the state of the art for the treatment of dandruff.

In view of the improvement of the effect the concentration of LAE and the at least one other active ingredient may be lower in comparison with the concentration in compositions containing LAE and the at least one other ingredient alone. Accordingly the concentration of LAE may be between 0.01 and 1.5 % by weight,

preferably between 0.05 and 1 % weight and more preferably between 0.1 to 0.8 % by weight relative to the total composition.

The ability of LAE to disturb the membrane of micro-organisms can help the other anti-dandruff active ingredient to penetrate more easily and therefore, lower concentrations of them might be necessary. This is particularly important when those active ingredients have an irritating effect, such as zinc pyrithione. Selenium sulphide presents a certain toxicity as well. The incorporation of LAE into the anti-dandruff cosmetic preparations may allow a considerable reduction in the amount of the ingredients necessary to reach an effect in the elimination of *Malassezia furfur*, and as a consequence, in the improvement of the problem in patients.

The concentration of the at least one further active ingredient may be regularly adjusted in the range of 0.01 to 3 % by weight, depending on the activity of the active ingredient which is selected for the combined preparation.

When zinc pyrithione is selected as the at least one further active ingredient the concentration of this compound in the total composition will be between 0.01 and 3 % by weight, preferably between 0.3 and 2 % by weight and more preferably between 0.5 and 1 % by weight relative to the total composition.

When salicylic acid is selected as the at least one further active ingredient the concentration of this compound in the total composition will be between 0.01 and 3 % by weight, preferably between 0.3 and 2 % by weight and more preferably between 0.5 and 1 % by weight relative to the total composition.

When piroctone olamine is selected as the at least one further active ingredient the concentration of this compound in the total composition will be between 0.01 and 3 % by weight, preferably between 0.3 and 2 % by weight and more preferably between 0.5 and 1 % by weight relative to the total composition.

The combinations of LAE with other anti-dandruff agents show an unexpected increase in the efficiency against *Malassezia furfur*.

EXAMPLES

The anti-dandruff activity of LAE and the other active ingredients alone or in combination is compared by means of *in vitro* tests, such as inhibition halo determination or suspension test for the specific micro-organism (in this case, *Malassezia furfur*). *In vivo* studies were carried out to compare the anti-dandruff activity of LAE with other shampoos that contain different anti-dandruff agents.

METHODOLOGY

Screening of anti-fungal activity – Inhibition halo

A diffusion plate procedure was used for the screening of anti-fungal activity of the different anti-dandruff active agents. *Malassezia furfur* (DSM 6170 / ATCC 14521) was grown in Potato Dextrose Broth up to 10^8 CFU (Colony Forming Units) per mL. Afterwards, 200 mL of liquid Potato Dextrose Agar were inoculated with 2 mL of the yeast suspension (approximately 10^6 spores), mixed homogeneously and poured onto Petri dishes (20 mL/plate). After the agar solidified at room temperature, two holes (8 mm diameter) were prepared at opposite sides of each Petri dish and filled with 0.1 mL of shampoo with a pipette (method as described in ES 2 234 757 T3). The incubation time was 48 hours at a temperature of 30 ± 2 °C. At the end of the incubation period, the inhibition halo diameters were measured using callipers and expressed in millimetres. When the diameter of the inhibition halo observed was equal to or higher than 7 mm, it was considered positive for anti-fungal activity.

***In vitro* Test – Suspension Test**

(Based on quantitative suspension test according to DIN EN 12054 (dilution-neutralization method).

Suspension test was performed to assess the anti-dandruff reduction ability of different active agents against *Malassezia furfur* in water solution and shampoo formulations. *Malassezia furfur* (DSM 6170 / ATCC 14521) was grown in Potato Dextrose Agar (PDA) up to 10^8 CFU (Colony Forming Units) per mL. Each test

sample was inoculated with this culture at 1% (time zero) and an aliquot of 1 mL of each solution was removed at different times (0, 10 minutes, 30 minutes and 2 hours). 0.1 mL of the appropriate dilution was spread onto Potato Dextrose Agar (PDA) plates for enumeration using a spiral automatic spreader (Eddy Jet, IUL Instruments). PDA plates were incubated at $30 \pm 2^{\circ}\text{C}$ for at least 48 hours, and afterwards they were read with an automatic colony counter (Counter Flash 4.2, IUL Instruments). The results were expressed as CFU/mL of test solution.

REFERENCE EXAMPLE 1.

A clinical assessment of the anti-dandruff effect of the following preparations in the same type of shampoo was conducted in volunteers:

- Preparation A: placebo
- Preparation B: LAE 0.8%.
- Preparation C: Piroctone olamine 0.8%.

The volunteers were not aware of the composition of the preparation with which they were treated.

The dandruff condition was assessed on the basis of the following scorage scale:

- 0 – absent dandruff condition
- 2 – light dandruff condition
- 4 – medium dandruff condition
- 6 – moderate dandruff condition
- 8 – important dandruff condition
- 10 – very important dandruff condition.

The volunteers were treated with the preparations A, B or C for a period of 4 weeks. At the beginning and at the end of the treatment period the evaluation of the dandruff condition was conducted by a dermatologist.

The following values were determined, whereby these values are provided as mean values:

Table 1.

	Week 0	end of week 4
Preparation A (n=11)	4.1 ± 1.3	3.6 ± 2.2
Preparation B (n=9)	3.9 ± 1.5	1.3 ± 1.0 *
Preparation C (n=11)	4.8 ± 1.9	2.5 ± 1.8 **

*: statistically significant ($p < 0.03\%$). This significant result is observed after treatment with the shampoo according to preparation B for 4 weeks.

**: statistically significant ($p < 0.06\%$). This significant result is observed after treatment with the shampoo according to preparation C for 4 weeks.

These effects were confirmed in the self-assessment by the volunteers, which had observed an improvement after the treatment with the preparations B and C.

EXAMPLE 1:

Shampoo formulas

An anti-dandruff shampoo formula was prepared in order to compare the effectiveness of several anti-dandruff agents.

a) Combinations of LAE and salicylic acid were evaluated.

Table 2.

INCI name	Composition (%)								
	1	1a	1b	2a	2b	3a	3b	4a	4b
Decyl Glucoside	8	8	8	8	8	8	8	8	8
Cocamidopropyl Betaine	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Ethyl Lauroyl Arginate HCl		0.2	0.4			0.1	0.1	0.2	0.2
Salicylic Acid				0.2	0.4	0.2	0.4	0.2	0.4
Citric Acid	e.q. to pH = 5								
Water	up to 100								

After inoculation with *Malassezia furfur* (DSM 6170/ATCC 14521) 6 – 7 log CFU/mL; the content of the micro-organisms was analysed after different periods of time, as the table below shows.

Table 3.

Time (min)	1	1a	1b	2a	2b	3a	3b	4a	4b
0	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9
1	6.7	5.8	5.1	6.0	6.0	4.5	3.4	< 2.0	< 2.0
5	6.8	4.0	3.5	5.8	5.8	2.5	< 2.0	< 2.0	< 2.0
60	6.5	< 2.0	< 2.0	5.7	5.5	< 2.0	< 2.0	< 2.0	< 2.0

Results expressed as log CFU/g. Limit of detection: 2 log CFU/g

A synergetic effect between LAE and salicylic acid was observed.

b) Combinations of LAE and zinc pyrithione were evaluated.

Table 4.

INCI name	Composition (%)								
	5	5a	5b	6a	6b	7a	7b	8a	8b
Decyl Glucoside	8	8	8	8	8	8	8	8	8
Cocamidopropyl Betaine	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Ethyl Lauroyl Arginate HCl		0.2	0.4			0.1	0.1	0.2	0.2
Zinc Pyrithione				0.2	0.4	0.2	0.4	0.2	0.4
Citric Acid	e.q. to pH = 5								
Water	Up to 100								

After inoculation with *Malassezia furfur* (DSM 6170/ATCC 14521) 6 – 7 log CFU/mL; the content of the micro-organism was analysed after different periods of time. The results of the corresponding suspension test are shown below.

Table 5.

Time (min)	5	5a	5b	6a	6b	7a	7b	8a	8b
0	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9

1	6.7	5.8	5.1	6.0	5.2	< 2.0	< 2.0	< 2.0	< 2.0
5	6.8	4.0	3.5	3.5	5.0	< 2.0	< 2.0	< 2.0	< 2.0
60	6.5	< 2.0	< 2.0	3.5	3.1	< 2.0	< 2.0	< 2.0	< 2.0

Results expressed as log CFU/g. Limit of Detection: 2 log CFU/g

It is clear from this example the surprising synergic effect of LAE with zinc pyrithione against *Malassezia furfur*, compared to LAE or zinc pyrithione alone.

c) Combinations of LAE and Piroctone olamine were evaluated.

Table 6.

INCI name	Composition (%)								
	9	9a	9b	10a	10b	11a	11b	12a	12b
Decyl Glucoside	8	8	8	8	8	8	8	8	8
Cocamidopropyl Betaine	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Ethyl Lauroyl Arginate HCl		0.2	0.4			0.1	0.1	0.2	0.2
Piroctone Olamine				0.2	0.4	0.2	0.4	0.2	0.4
Citric Acid	e.q. to pH = 5								
Water	up to 100								

The results of the corresponding suspension test after inoculation with *Malassezia furfur* (DSM 6170 / ATCC 14521) 6 – 7 log CFU/mL are shown below.

Table 7.

Time (min)	9	9a	9b	10a	10b	11a	11b	12a	12b
0	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9
1	6.7	5.8	5.1	6.4	6.2	3.1	2.5	< 2.0	< 2.0
5	6.8	4.0	3.5	5.7	5.5	< 2.0	< 2.0	< 2.0	< 2.0
60	6.5	< 2.0	< 2.0	5.0	4.8	< 2.0	< 2.0	< 2.0	< 2.0

Results expressed as log CFU/g. Limit of detection: 2 log CFU/g

Synergy between the active ingredients is demonstrated compared to the effects of LAE and piroctone olamine if present alone.

EXAMPLE 2:**Hair-conditioner formulas****a) Combination with salicylic acid**

An anti-dandruff hair-conditioner formula was prepared in order to evaluate salicylic acid alone or in combination with LAE.

Table 8.

INCI name	Composition (%)							
	1	1a	1b	2a	2b	3a	3b	3c
Cetrimonium chloride	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Cetearyl alcohol	3	3	3	3	3	3	3	3
Isopropyl myristate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Ethyl Lauroyl Arginate HCl		0.1	0.2	0.1	0.2			
Salicylic Acid				0.2	0.2	0.2	0.4	1.0
Citric acid	e.q. to pH = 5							
Water	up to 100							

The results of the corresponding suspension test after inoculation with *Malassezia furfur* (DSM 6170 / ATCC 14521) 6 – 7 log CFU/mL are shown below.

Table 9.

Time (min)	1	1a	1b	2a	2b	3a	3b	3c
0	6.2	6.2	6.2	6.2	6.2	6.2	6.2	6.2
1	6.1	5.5	5.0	3.6	3.1	6.0	6.0	5.8
5	5.8	4.0	3.2	3.1	2.2	5.9	5.8	5.5
60	5.0	< 2.0	< 2.0	< 2.0	< 2.0	6.0	5.5	5.0

Results expressed as log CFU/g. Limit of detection: 2 log CFU/g

Combinations of LAE with salicylic acid against *Malassezia furfur* show a surprising synergetic effect.

b) Combinations with zinc pyrithione

An anti-dandruff hair-conditioner formula was prepared in order to evaluate zinc pyrithione alone or in combination with LAE.

Table 10.

INCI name	Composition (%)						
	1	1a	1b	4a	4b	5a	5b
Cetrimonium chloride	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Cetearyl alcohol	3	3	3	3	3	3	3
Isopropyl myristate	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Ethyl lauroyl arginate HCl		0.1	0.2	0.1	0.2	-	-
Zinc pyrithione				0.2	0.2	0.2	0.4
Citric acid	e.q. to pH = 5						
Water	Up to 100						

The results of the corresponding suspension test after inoculation with *Malassezia furfur* (DSM 6170 / ATCC 14521) 6 – 7 log CFU/mL are shown below.

Table 11.

Time (min)	1	1a	1b	4a	4b	5a	5b
0	6.2	6.2	6.2	6.2	6.2	6.2	6.2
1	6.1	5.5	5.0	2.8	2.5	5.5	3.5
5	5.8	4.0	3.2	< 2.0	< 2.0	5.1	3.1
60	5.0	< 2.0	< 2.0	< 2.0	< 2.0	3.2	2.9

Results expressed as log CFU/g. Limit of detection: 2 log CFU/g

The combination of LAE with zinc pyrithione has a surprisingly improved effect against *Malassezia furfur* because only five minutes after treatment the log CFU/g of this microorganism is below the limit of detection.

c) Combinations with piroctone olamine

An anti-dandruff hair-conditioner formula was prepared in order to evaluate piroctone olamine alone or in combination with LAE.

Table 12.

INCI Name	Composition (%)						
	1	1a	1b	6a	6b	7a	7b
Cetrimonium chloride	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Cetearyl alcohol	3	3	3	3	3	3	3
Isopropyl myristate	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Ethyl lauroyl arginate HCl		0.1	0.2	0.1	0.2		
Piroctone olamine				0.2	0.2	0.2	0.4
Citric acid	e.q. to pH = 5						
Water	up to 100						

The results of the corresponding suspension test after inoculation with *Malassezia furfur* (DSM 6170 / ATCC 14521) 6 – 7 log CFU/mL are shown below.

Table 13.

Time (min)	1	1a	1b	6a	6b	7a	7b
0	6.2	6.2	6.2	6.2	6.2	6.2	6.2
1	6.1	5.5	5.0	3.5	2.3	6.0	5.5
5	5.8	4.0	3.2	2.2	< 2.0	5.1	4.5
60	5.0	< 2.0	< 2.0	< 2.0	< 2.0	4.0	3.4

Results expressed as log CFU/g. Limit of detection: 2 log CFU/g.

The combination of LAE with piroctone olamine surprisingly improves the efficacy against *Malassezia furfur*.

In conclusion, combinations of LAE with other anti-dandruff actives against *Malassezia furfur* show a surprising synergetic effect.

EXAMPLE 3:

Hydro-alcoholic lotion

a) Combination with Salicylic Acid

Anti-dandruff hydro-alcoholic lotions were prepared in order to evaluate the activity of salicylic acid alone or in combination with LAE.

Table 14.

INCI name	Content (%)						
	1	1a	1b	2a	2b	3a	3b
Ginseng extract	3	3	3	3	3	3	3
Polyglyceryl-3 caprylate	1	1	1	1	1	1	1
Polysorbate 20	2	2	2	2	2	2	2
Ethyl lauroyl arginate HCl		0.1	0.2	0.1	0.2	-	-
Salicylic acid				0.2	0.2	0.2	1
Citric acid	e.q. to pH = 5						
Ethanol	10 %						
Water	up to 100						

The results of the corresponding suspension test after inoculation with *Malassezia furfur* (DSM 6170 / ATCC 14521) 6 – 7 log CFU/mL are shown below.

Table 15.

Time (min)	1	1a	1b	2a	2b	3a	3b
0	5.8	5.8	5.8	5.8	5.8	5.8	5.8
1	6.7	3.6	3.1	2.8	2.2	5.8	5.2

5	6.8	2.9	2.6	< 2.0	< 2.0	5.7	4.8
60	6.5	< 2.0	< 2.0	< 2.0	< 2.0	5.0	4.5

In a hydro-alcoholic lotion matrix, a synergetic effect has been observed in combinations of LAE with salicylic acid.

b) Combination with zinc pyrithione

Anti-dandruff hydro-alcoholic lotions were prepared in order to evaluate the activity of zinc pyrithione alone or in combination with LAE.

Table 16.

INCI name	Content (%)						
	1	1a	1b	4a	4b	5a	5b
Ginseng extract	3	3	3	3	3	3	3
Polyglyceryl-3 caprylate	1	1	1	1	1	1	1
Polysorbate 20	2	2	2	2	2	2	2
Ethyl lauroyl arginate HCl		0.1	0.2	0.1	0.2	-	-
Zinc pyrithione				0.2	0.2	0.2	0.4
Citric acid	e.q. to pH = 5						
Ethanol	10 %						
Water	up to 100						

The results of the corresponding suspension test after inoculation with *Malassezia furfur* (DSM 6170 / ATCC 14521) 6 – 7 log CFU/mL are shown below.

Table 17.

Time (min)	1	1a	1b	4a	4b	5a	5b
0	5.8	5.8	5.8	5.8	5.8	5.8	5.8
1	6.7	3.6	3.1	< 2.0	< 2.0	5.7	3.2
5	6.8	2.9	2.6	< 2.0	< 2.0	5.4	2.8
60	6.5	< 2.0	< 2.0	< 2.0	< 2.0	2.2	<2.0

In a hydro-alcoholic lotion matrix, synergetic effect has been observed in combinations of LAE with zinc pyrithione.

c) Combination with piroctone olamine

Anti-dandruff hydro-alcoholic lotions were prepared in order to evaluate the activity of piroctone olamine alone or in combination with LAE.

Table 18.

INCI name	Content (%)						
	1	1a	1b	6a	6b	7a	7b
Ginseng extract	3	3	3	3	3	3	3
Polyglyceryl-3 caprylate	1	1	1	1	1	1	1
Polysorbate 20	2	2	2	2	2	2	2
Ethyl lauroyl arginate HCl		0.1	0.2	0.1	0.2		
Piroctone olamine				0.2	0.2	0.2	0.4
Citric acid	e.q. to pH = 5						
Ethanol	10 %						
Water	up to 100						

The results of the corresponding suspension test after inoculation with *Malassezia furfur* (DSM 6170 / ATCC 14521) 6 – 7 log CFU/mL are shown below.

Table 19.

Time (min)	1	1a	1b	6a	6b	7a	7b
0	5.8	5.8	5.8	5.8	5.8	5.8	5.8
1	6.7	3.6	3.1	< 2.0	< 2.0	5.7	4.8
5	6.8	2.9	2.6	< 2.0	< 2.0	5.4	4.2
60	6.5	< 2.0	< 2.0	< 2.0	< 2.0	3.9	3.6

In a hydro-alcoholic lotion matrix, synergetic effect has been observed in combinations of LAE with piroctone olamine.

Comparing the efficacy results of the three combinations of LAE with the other antidandruff agents applied in a hydro alcoholic lotion, a surprising synergetic affect is observed as the result of these combinations.

EXAMPLE 4:
Aqueous solutions.

Anti-dandruff actives are evaluated in water solution by mean of inhibition halo. The results are the mean value of three replicates. When the diameter of the inhibition halo observed was higher than 7 mm, it was considered positive for anti-fungal activity.

Table 20.

ACTIVE AGENT	STRAIN	<i>Malassezia furfur</i> (DSM 6170 / ATCC 14521)
No active		7 ± 0.5
0.1 % LAE		11 ± 1
0.1 % Salicylic acid		8 ± 0.5
0.1 % Zinc pyrithione		12 ± 1
0.1 % Piroctone olamine		10 ± 2
0.1 % LAE + 0.1 % Salicylic acid		14 ± 1
0.1 % LAE+ 0.1 % Zinc pyrithione		18 ± 0.7
0.1 % LAE + 0.1 % Piroctone olamine		16 ± 0.5

As is observed in the results, LAE is more effective than salicylic acid and similar to zinc pyrithione and piroctone olamine. Nevertheless, the combination of any of the active ingredients with LAE increases significantly the effectiveness compared to the active ingredients alone.

CLAIMS

1. Cosmetic preparation for the treatment of dandruff containing Ethyl Lauroyl Arginate HCl (LAE) in combination with a further active ingredient which is effective against dandruff.
2. The cosmetic preparation of claim 1, wherein the further active ingredient is at least one selected from the group consisting of zinc pyrithione, salicylic acid and piroctone olamine.
3. The cosmetic preparation of claim 1 or 2, wherein the concentration of Ethyl Lauroyl Arginate HCl is in the range of between 0.01 and 1.5 % by weight, preferred range from 0.05 to 1% by weight and most preferred range 0.1 to 0.8% by weight.
4. The cosmetic preparation of any of claims 1 to 3, containing Ethyl Lauroyl Arginate HCl in combination with zinc pyrithione whereby zinc pyrithione is present at a concentration of between 0.01 to 3%, preferred range from 0.5 to 1% and most preferred 0.05 and 0.4 % by weight.
5. The cosmetic preparation of any of claims 1 to 3, containing ethyl lauroyl arginate HCl in combination with salicylic acid whereby salicylic acid is present at a concentration of between 0.01 to 3%, preferred range from 0.5 to 1% and most preferred 0.05 and 0.4 % by weight.
6. The cosmetic preparation of any of claims 1 to 3, containing ethyl lauroyl arginate HCl in combination with piroctone olamine whereby piroctone olamine is present at a concentration of between 0.01 to 3%, preferred range from 0.5 to 1% and most preferred 0.05 and 0.4 % by weight.
7. The cosmetic preparation of any of claims 1 to 6 to be applied for the hair treatment in the form of shampoo, leave-on hair conditioner, rinse-off conditioner or hydro-alcoholic lotions.

8. Use of the cosmetic preparation of any of claims 1 to 7 for the treatment of dandruff.