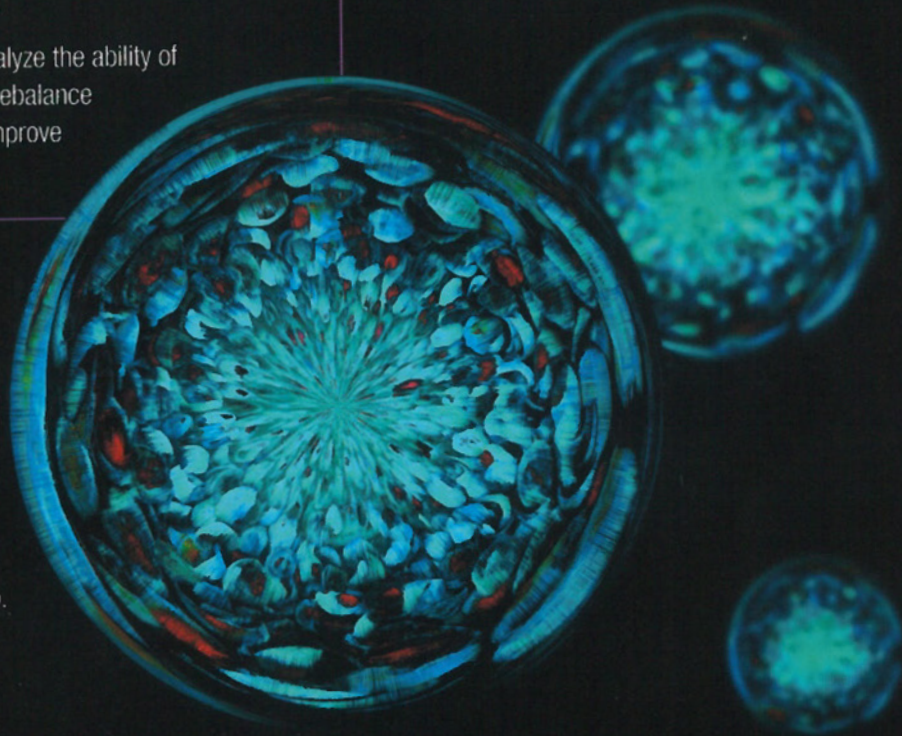




KEY POINTS

- An imbalanced microflora can cause skin problems such as acne, atopic eczema and fungal infections.
- Here, authors analyze the ability of bioflavonoids to rebalance microflora and improve skin health.



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Mastering Microflora

Bioflavonoids for Better Skin Appearance



The microflora is an important part of the skin. Sebum and corneocytes as well as sweat create the ideal feeding ground for the tolerated microbes. The

present article describes a new active containing bioflavonoids from *Maclura cochinchinensis*, which was tested for its capability to rebalance the disturbed microflora of oily skin and, in turn, reduce acne blemishes as well as axillary odor.

The Microbial Settlement on the Skin

The skin is our largest organ, with a surface area of roughly 1.7 square meters. It protects us from the environment, is important for thermoregulation and is definitely key for our appearance. However, we are not alone in our skin—millions of small companions are settling on our skin's surface: microbes. In total, 100 billion bacteria



The microflora is not the root cause of skin problems, but rather a reaction to dysregulated skin functions.

and fungi use our skin as their habitat.¹ This incredibly large number corresponds to the entirety of our skin cells.²

Recent advances in microbiome analysis techniques revealed a much higher bacterial diversity on skin than previously detected by culture-based methods. For example, studies by the authors' company on acne prone skin have revealed bacteria from six phyla, more than 80 families and no less than 500 different species. One can imagine this is only a fraction of what can be found in normal skin. Furthermore, skin of different ethnicities and of people who live on different continents may harbor even greater microbe diversity.

The skin's microflora is an important guard against potentially harmful intruders. By occupying all skin niches (see **Figure 1**), the settlement of germs is strongly reduced. Sweat also plays an important role to provide the best conditions for this beneficial microflora in that it creates a slightly acidic pH on the skin surface.³

Microflora produce a human being's typical scent. In moist areas, in particular, apocrine sweat glands secrete a special sebum composition in which *Staphylococci*, *Corynebacteria* and *Bacilli* degrade sebum, dead cell material and components of the skin surface. These produce body odor by releasing their metabolites.

Skin Problems Due to Imbalanced Microflora

An imbalanced microflora condition—referred to as *dysbiosis*—can cause skin problems such as acne, atopic eczema and fungal infections. Despite this, the microflora is not the root cause of skin problems, but rather a reaction to dysregulated skin functions.

Microorganisms typically respond to the "food supply" of the skin. In the case of greasy skin, a lot of sebum is available, which supports the growth of *Staphylococci* and *Propionibacteria*. The latter, being trapped in anaerobic zones of clogged skin pores due to hyperkeratinization, are the main drivers of acne vulgaris and the formation of inflammatory spots.

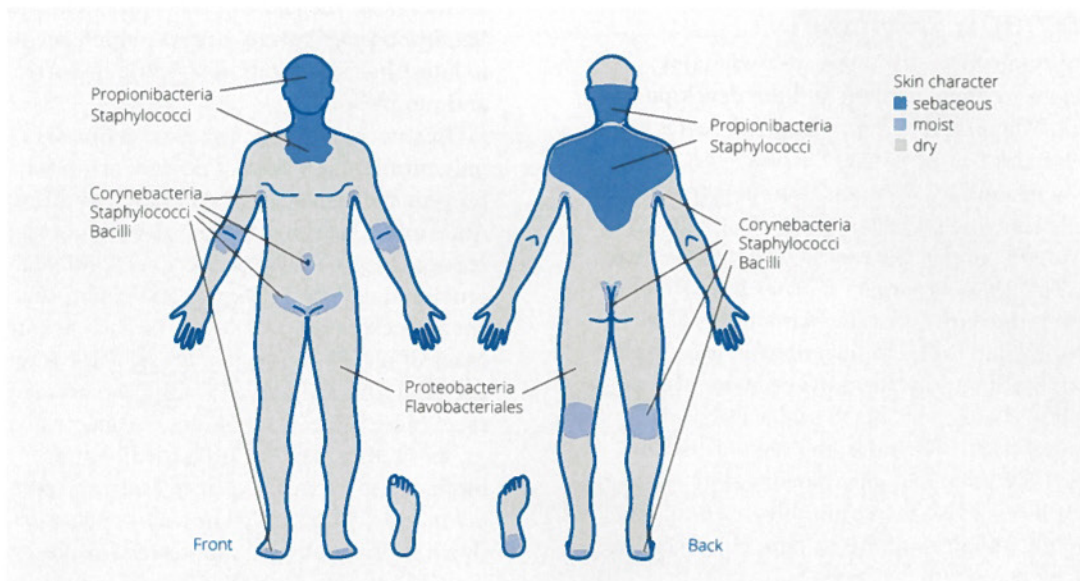
Due to sebum overproduction, the sebaceous duct is filled with excess sebum and dead keratinocytes. Oxidation turns the greasy texture of sebum into a wax-like consistency, while melanin of dead keratinocytes oxidizes to become black—this is recognized as blackhead comedones. The following sebum will be trapped in an anaerobic tube. This is the best condition for the colonization of *Propionibacterium acnes* (*P. acnes*)—an anaerobic Gram-positive bacterium—settling on the sebum-rich parts of the body.

With an estimated density of 100–1,000,000 per square centimeter on the skin, *P. acnes* accounts for approximately half of the total skin microflora⁵ and may represent more than 90% of the microflora in acne conditions. It feeds on lipids and secretes metabolites such as porphyrins and lipopolysaccharides; all while attracting immune cells, which aim to fight the bacterial infection.⁶ In the late stage of an acne pustule, the epidermis around the hair shaft ruptures to create scarring in the skin (see **Figure 2**).

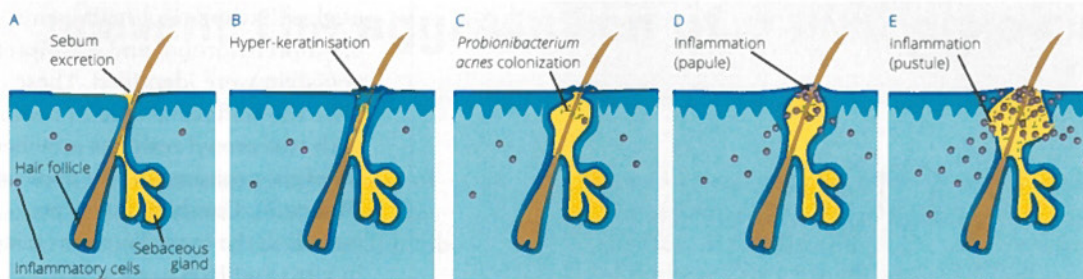
The topical acne market is expected to grow at a 4.6% CAGR from 2017–2024; *Data Bridge Market Research* notes this growth will be due to new clinical results and product launches.



Source: Global Cosmetic Industry
(www.GCI Magazine.com)



● **Figure 1.** Location of the most abundant bacterial phyla on the body surface. Depending on the production of sebum or the humidity, different areas of the human body are populated by different bacterial species.^{3,4}



● **Figure 2.** The development of acne is the escalation of several unfavorable events: a) due to an elevated inflammatory ground state or testosterone excess, the sebaceous gland is stimulated to increase sebum production, leading to oily skin; b) hyperkeratinization of keratinocytes leads to pore-clogging (blackheads); c) colonization of the entrapped sebum by *P. acnes* induces an immunological reaction of the skin; d) inflammatory cells are attracted and begin to fight the bacterial infection causing a papule to appear that develops into a pustule; and e) continued build-up of infection leads to the rupture of the sebaceous duct, spreading the inflammation into the dermis.



Therapies Against Acne and Oily Skin

Pharmacologically, there are two major strategies to fight oily skin and the development of acne. The first is to reprogram sebocytes to produce smaller amounts of sebum—i.e., the feeding ground for *P. acnes*. Second is to use strong cleansing products containing benzoyl peroxide to control the excess settlement of bacteria. This therapy targets *P. acnes* in particular; however, the entirety of the skin's microflora is affected due to the radical nature of benzoyl peroxide. Due to this, benzoyl peroxide therapies should only be utilized under medical supervision. Furthermore, the regular use of benzoyl peroxide may provoke dry skin.

Antibiotics also are commonly used to fight a severely imbalanced microflora. However, the

use of non-specific broad-spectrum antibiotics increases the risk of generating resistant *Staphylococcus aureus* strains, which according to latest investigations, also settle in pores and pimples.⁷

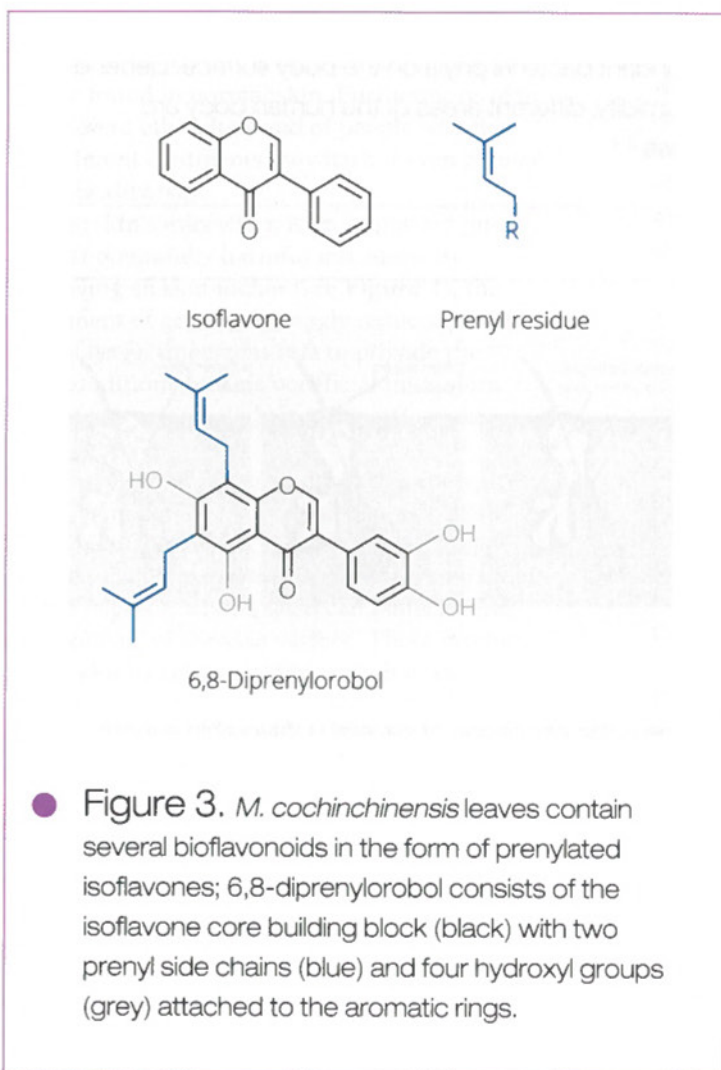
Cosmetic solutions for acne-prone skin can only mimic one's dermatological armory to prevent the aggravation of a mild condition. And since a healthy microflora is important to reduce the growth of deleterious species, it is crucial to choose intelligent molecules that target a specific sub-population of bacteria; in the cases of oily skin or moist areas, these targets are predominantly *P. acnes* or *Corynebacterium spec.* (which creates malodor), respectively.

As such, a blend of propanediol and bioflavonoids extracted from *Maclura cochinchinensis*, commonly known as cockspur thorn, was developed^a and tested for its anti-acne effects. Isoflavones, a subclass of bioflavonoids, are secondary plant metabolites that defend plants against pathogens. These were hypothesized to function as balancing ingredients in cosmetic formulations for the skin's microflora.


The *M. cochinchinensis* leaf extract was first characterized. As its main constituents, the prenylated isoflavones isolupalbigenin, 6,8-diprenylorobol and 6,8-diprenylgenistein were identified. These have the isoflavone core in common, with two prenyl residues attached at different positions on the core (see **Figure 3**). Combined with propanediol, the ingredient was tested in vitro and in vivo as described next to determine its efficacy on the skin's microflora and microbiome.

Material and Methods

MIC of skin microflora: The *M. cochinchinensis* ingredient was diluted in different concentrations in Müller-Hinton broth and the growth of selected bacterial strains was analyzed under aerobic or anaerobic conditions for *P. acnes*



^a Seboclear-MP (INCI: Propanediol (and) Bioflavonoids) is a product of Rahn AG.



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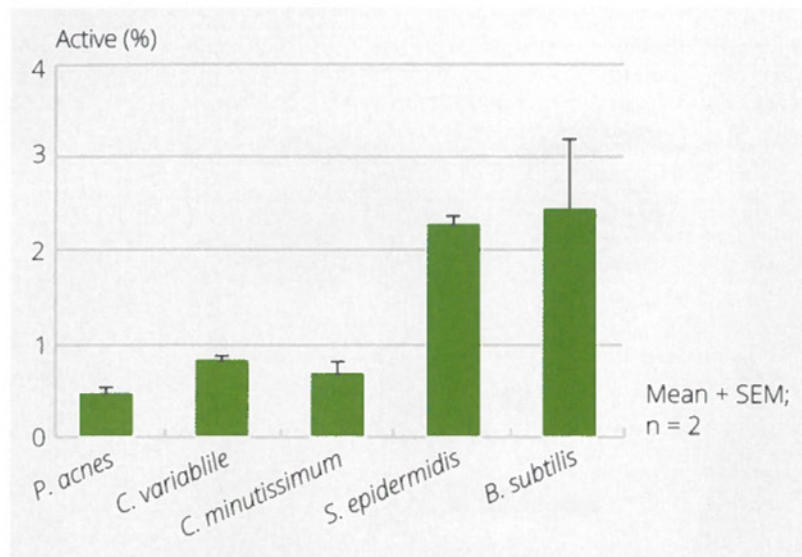
for 24–48 hr. The minimal concentration of the active at which no bacterial growth was observed corresponded to the minimal inhibitory concentration (MIC).

In vivo guidelines: In vivo studies were performed in accordance with the principles of good laboratory practice (GLP), good clinical practice (GCP), and in compliance with the quality assurance system requirements. The studies also were in accordance with the World Medical Association's Declaration of Helsinki. All study participants signed a written informed consent at the beginning of the studies.

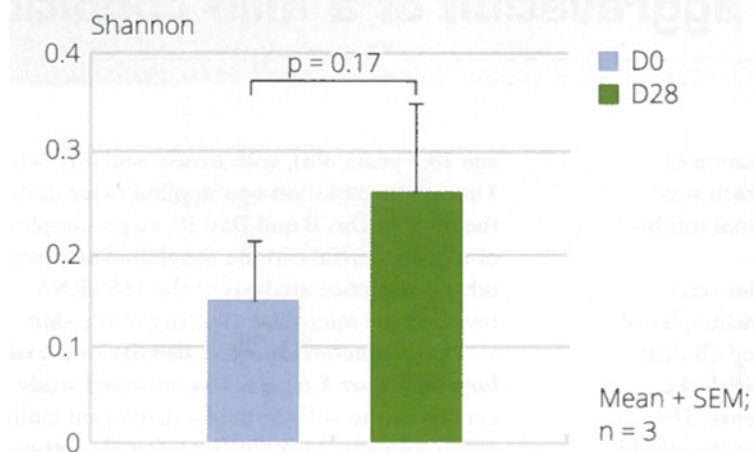
Acneic skin microbiome, before and after treatment: A small application study was carried out using a formulation containing 3% of the *M. cochinchinensis* ingredient for 28 days on three female subjects, 18–45 years old (aver-

age 28.7 years old), with acneic and oily skin. The test formulation was applied twice daily to the face. At Day 0 and Day 28, swab samples of the skin surface in the nasolabial area were taken. Sequence analysis of the 16S rRNA revealed the microbial diversity of the skin.

Corynebacterium spec. density and axillary malodor: A placebo-controlled study was carried out to substantiate a deodorant claim on 20 subjects, 11 male and 9 female, between 35–64 years old (average 52.6 years old) with pronounced axillary odor. After 10 days of applying a standardized cleansing product, subjects washed their armpits in a defined way at a test institute (pre-conditioning). Three evaluators judged malodor by sniffing subjects' armpits both 6 hr and 24 hr later. After 24 hr, the armpits were washed again and a simple



● **Figure 4.** The *M. cochinchinensis* ingredient controlled the growth of skin microflora—especially *P. acnes*, the main aggravating bacterium in the development of acne, and *Corynebacteria*, which is responsible for malodor in axillary areas.



● **Figure 5.** Shannon diversity index of the microbiome; after 28 days of *M. cochinchinensis* application, the index increase of 73% indicates diversification of the skin's microflora. This was significant per the Student's t-test.

spray, containing either 1% or 0% of the *M. cochinchinensis* ingredient, was applied. The development of malodor was then assessed in the same way as before. Swab samples were taken after 6 hr to investigate the colony-forming units (CFU) of bacteria and of *Corynebacteria* on Columbia blood agar.

Effect of active ingredient on acne-prone skin: In a double-blind, placebo-controlled, randomized study, 21 female subjects 16–24 years old (average 19.4) with healthy, oily Caucasian skin, applied an emulsion containing either 3% of the *M. cochinchinensis* ingredient or a placebo twice daily to half of their face. Inflammatory spots were assessed with the red channel of a VISIA photographic system while porphyrins were detected in the UV channel.

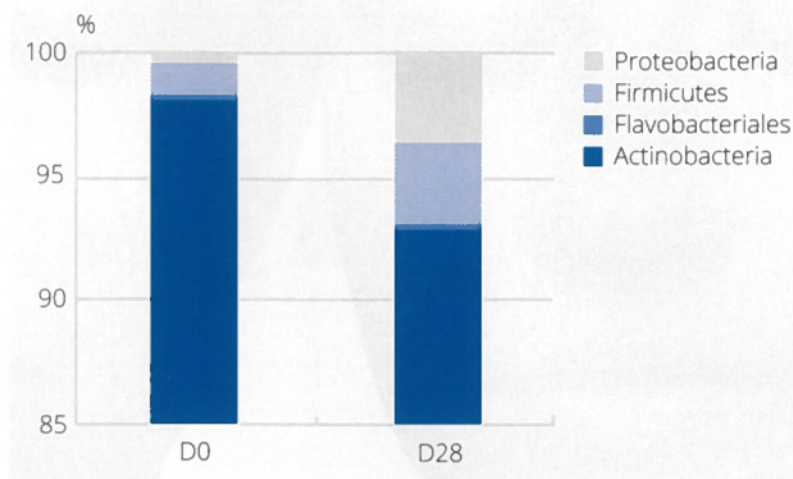
Results

Selective suppression of actinobacteria: The *M. cochinchinensis* ingredient differentially regulated the growth of the skin's microflora. This effect was attributed to the extract's prenylated isoflavones. Depending on the bacterial strain, they suppressed the growth of bacteria and were most active on both *P. acnes*, the main

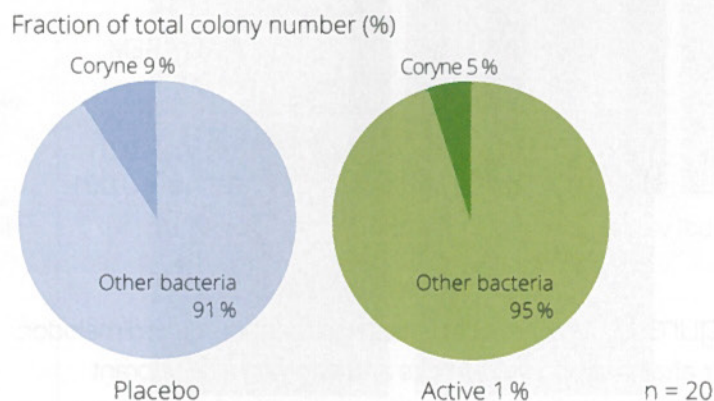
aggravating bacterium of acne vulgaris, and *Corynebacterium spec.*, responsible for malodor (see **Figure 4**). At 0.5–1.0%, the *M. cochinchinensis* ingredient only affected these unfavorable *Actinobacteria*. At higher concentrations, the growth of *Staphylococci* and *Bacillus subtilis* were regulated as well.

Microbiome diversity in vivo: Analysis of the skin microflora in subjects having acne lesions before application of the *M. cochinchinensis* ingredient proved dysbiosis in each study participant. The majority of skin bacteria was represented by *Propionibacteria* (87–98%), of which 99% were *P. acnes*. In all study participants, the abundance of *Actinobacteria*, to which *Propionibacteria* and *Corynebacteria* belong, was reduced by application of the active (see **Figure 6**). After 28 days, the amount of *P. acnes* decreased by 10% or 12% in two-thirds of study participants. In contrast, biodiversity in the phyla *Firmicutes* (e.g. *Staphylococcus sp.* and *B. subtilis*) and *Proteobacteria* increased.

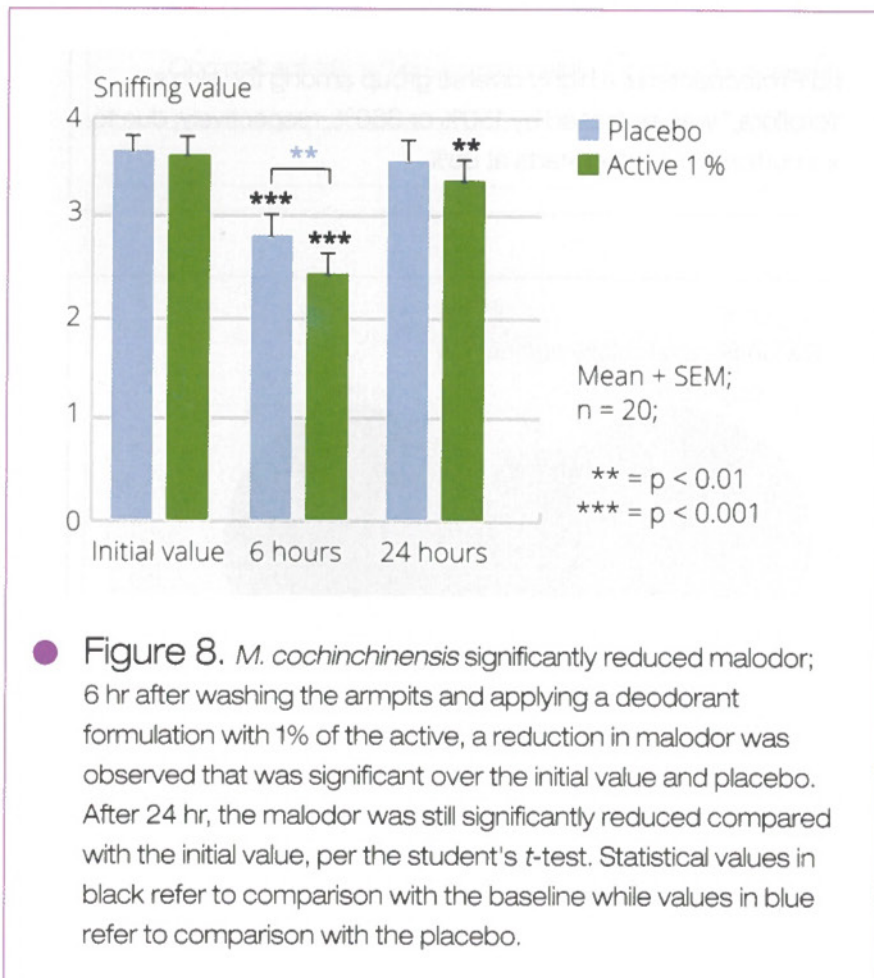
In relation, the median Shannon index was increased for all study participants, moving toward the more diversified microbiome of normal and balanced skin (see **Figure 5**). This result was in accordance with the antimicrobial specificity profile determined in **Figure 4** but due to high inter-



● **Figure 6.** Biodiversity of the skin microbiome of a 23-year-old study participant. After applying 3% *M. cochinchinensis* for 28 days, the predominance of actinobacteria (e.g., *P. acnes* and *Corynebacterium sp.*) was reduced. The diversity in the phyla *Firmicutes* (e.g., *Staphylococcus sp.* and *B. subtilis*) and *Proteobacteria*, a highly diverse group among the skin's microflora,⁸ was increased by 150% or 860%, respectively; due to exemplification, scaling starts at 85%.



● **Figure 7.** After 6 hr, *M. cochinchinensis* application reduced the fraction of odor-causing *Coryneform* bacteria among the axillary microflora without affecting the other bacteria, ultimately reducing odor.



● **Figure 8.** *M. cochinchinensis* significantly reduced malodor; 6 hr after washing the armpits and applying a deodorant formulation with 1% of the active, a reduction in malodor was observed that was significant over the initial value and placebo. After 24 hr, the malodor was still significantly reduced compared with the initial value, per the student's *t*-test. Statistical values in black refer to comparison with the baseline while values in blue refer to comparison with the placebo.

individual diversity and the small number of study participants, significance was not established.

Corynebacteria and axillary malodor: Analysis of swab samples after 6 hr of placebo or serum application in the armpits revealed a reduction of *Coryneform* bacteria by 44.4%, presumably due to the activity of the prenylated isoflavones in *M. cochinchinensis* (see **Figure 7**). With 1% *M. cochinchinensis*, the fraction of *Coryneform* bacteria among all colony-forming units was 5% while for the placebo, it was 9% after 6 hr. This aligns with the sniff test results of the deodorant study, since *Coryneform* bacteria are responsible for the creation of malodor.

Untreated armpits developed a pronounced malodor after 24 hr with

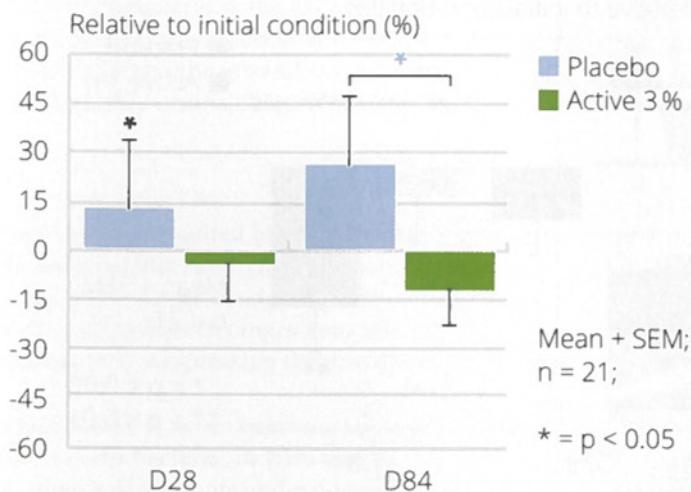
a sniffing value of roughly 3.5. After application of a deodorant spray, the sniffing value was reduced by 1.2 units for the test active and 0.83 units for placebo (see **Figure 8**). Thus, *M. cochinchenensis* significantly outperformed the placebo after 6 hr. In addition, after 24 hr, the malodor in the armpits treated with active was still significantly decreased by 0.25 units while the placebo returned back to the initial value.

P. acnes and signs of acne: While application of the test formulation on the hemiface significantly increased the porphyrin count, the same formulation with 3% *M. cochinchenensis* continuously decreased the porphyrins in sebaceous ducts, which are a measure for *P. acnes* colonization, significantly over the placebo (see **Figure 9**). This effect was easily visible by VISIA image in the UV channel (see **Figure 10**).

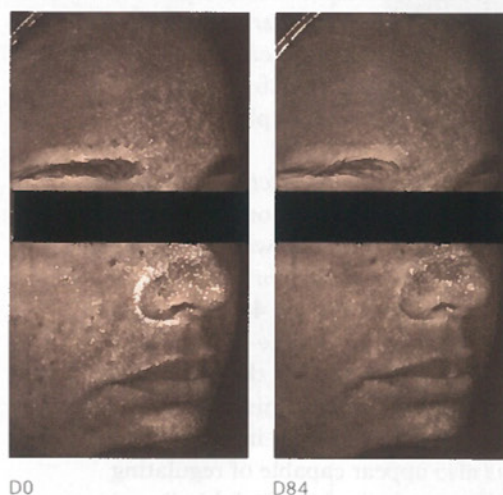
As noted, red inflammatory spots also were measured by VISIA photography. After 28 days of application, *M. cochinchenensis* decreased the number of red spots by 8% in acne-prone skin. After 84 days, spots were reduced significantly over the baseline and placebo by 22%, while the placebo did not significantly change (see **Figure 11**).

Discussion

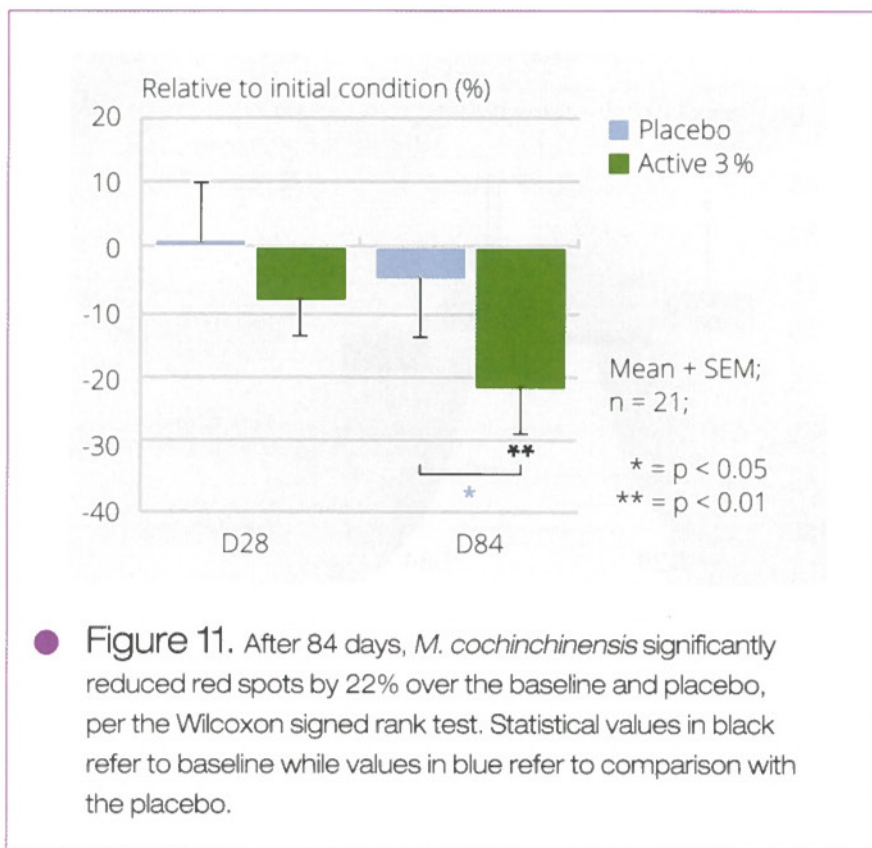
Normal skin harbors numerous different bacterial and fungal microorganisms on its surface. These strains cohabit to create the larger beneficial microflora. Under certain conditions, though, the balance of the microflora can shift unfavorably.



● **Figure 9.** *M. cochinchenensis* reduced porphyrine levels significantly after 84 days compared with the placebo, per the Wilcoxon signed rank test. Statistical values in black refer to baseline while values in blue refer to comparison with the placebo.



● **Figure 10.** VISIA image of porphyrines; after 84 days of treatment, the number of pores with porphyrins were significantly reduced



In the described study, the Shannon index of one subject was extremely low at 0.15. This shows that predominantly, *P. acnes* strains were collected in the swab samples due to the severe acneic condition. However, treatment with 3% *M. cochinchinensis* for 84 days led to a 73% increase of the Shannon index and a large diversification of bacterial phyla except the *Actinobacteria*, which were reduced. Although after treatment, the Shannon index showed the overall bacterial diversity was still very low, the trend toward a normalized skin microflora was

An excess of *P. acnes*, for example, can lead to acne vulgaris; and the overgrowth of *Staphylococci* may lead to skin inflammation and atopic dermatitis.³ *Corynebacteria*, which grows primarily in moist areas, also can cause pitted keratolysis on the soles of the feet, manifested clinically as hyperhidrosis, unpleasant odor and pain.⁹

Even *Staphylococci* and *Bacilli* are desirable for healthy skin flora, under normal conditions, and since *M. cochinchinensis* was shown to act selectively on *Propionibacterium spec.* and *Corynebacterium spec.* (see **Figure 4**), the potential to develop antibiotic resistance to *S. aureus* can be excluded. Additionally, the suppressive activity was the smallest for this strain.

The prenylated isoflavones in *M. cochinchinensis* also appear capable of regulating the microbiome of oily skin. High biodiversity reflects skin health but this diversity is reduced in oily, acne-prone skin due to an abundance of *Propionibacteria*. Said biodiversity is measured using the Shannon index, where normal skin has a value of about 6.8 and the lower the Shannon index, the lower the diversity.¹⁰

obvious. This result underlines the limitations of cosmetic active ingredients compared with drugs, which are only allowed under medical supervision.

To investigate the potential of *M. cochinchinensis* to inhibit the growth of *Corynebacteria*, a common sniff test was performed. Axillary malodor is a result of the decomposition of sweat and sebum by moist-loving *Corynebacteria*. Interestingly, the development of axillary malodor goes along with hormonal changes during puberty. As experience has shown, babies and children do not typically develop malodorous sweat until they enter adolescence.

In the present tests, *Corynebacteria* clearly were reduced after the use of the active ingredient, compared with the placebo. This reduction was manifested by a significant reduction in axillary malodor after 6 hr.

The suppression of *P. acnes* growth also was investigated in an 84-day study with a placebo vs. 3% active applied to the face. As a result, the porphyrins, indicative for growth of *P. acnes*, were reduced by 12% over the baseline of 38%. This translated to a visible reduction in red

spots, most likely because *P. acnes* is known to aggravate inflammatory processes in acne-prone skin and lead to spots and pimples.

Taken together, the reduction of *Corynebacteria* in the axillary study, the reduction in porphyrin count, as well as the reduction of *Actinobacteria* in the 28-day facial study demonstrate the growth suppression profile of *M. cochinchinensis*, supporting the results observed in vitro in the broth culture experiments.

Conclusions

It is important to reduce the number of unwanted bacteria in favor of a balanced microbiota to maintain a healthy skin. The overgrowth of *P. acnes* or *Corynebacteria* can aggravate acneic conditions and malodor. However, the described *M. cochinchinensis* ingredient acts as a microbiota-regulating agent by selectively suppressing the growth of these unwanted species.

The number of porphyrins, a measure of *P. acnes* colonization, was reduced as well as the number *Coryneform* bacteria, in vitro and in vivo. This leads to a reduction in inflammatory spots and a normalized skin appearance.

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