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Selected Alkaloids Used in the Cosmetics Industry

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Synopsis

Plants are a rich source of a wide variety of bioactive compounds that can be used for the preparation of cosmetics. Natural cosmetics with plant components such as vitamins, polyphenols, and alkaloids have become more and more popular. Alkaloids are important secondary metabolites in plants. They are known to possess therapeutic properties. Alkaloids can be used in the production of tonics, creams, lotions, face and hair masks, compresses for skin problems with numerous inflammations, and discoloration and antiaging products, as well as for reducing the formation of cellulitis. Alkaloids are also used in the production of ampoules for cosmetologists and aesthetic medicine doctors. However, at higher doses, they may exhibit toxic properties. Several studies have been carried out in evaluation of the activity of alkaloids from various plants for their use in cosmetics. This review describes alkaloids (caffeine, capsaicin, berberine, piperine, spilanthol, and anatabine) derived from various plants that are used in cosmetics, as well as their reported activities.

INTRODUCTION

Alkaloids are a group of secondary plant metabolites containing nitrogen atoms. According to Pelletier (1), alkaloid is "a cyclic organic compound containing nitrogen in a negative oxidation state which is of limited distribution among living organisms." Most of them stimulate physiological processes of living organisms. They are found in plants from the family of poppies (Papaveraceae), leguminous (Fabaceae), nightshade (Solanceae), papilionaceous (Papilionaceae), and buttercup (Ranunculaceae), and in fungi (*Boletus*, *Fusarium*, and *Psilocybe*), and club moss (Lycopodiaceae) and horsetails (Equisetaceae) included in the lower plants. Hegnauer (2) defines alkaloid plants as those species that contain more than 0.01% in weight of alkaloids. These compounds are most often stored in tubers, roots, seeds, fruits, leaves, flowers, and the bark. The content of alkaloids is influenced by plant development conditions, such as season and microclimate, as well as the degree of maturity of the plant raw material. A single plant may contain several alkaloids with similar or different effects (3).

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Among other properties, alkaloids used in medicine increase blood pressure (caffeine), are used as local anesthetics (morphine), relax smooth muscles (ephedrine and papaverine), have a diuretic effect (theobromine, theophylline, and caffeine), are immunostimulatory (anatabine and piperine), and have anticancer properties (berberine and codeine), as well as antirheumatic properties (capsaicin). However, prolonged use of some alkaloids, e.g., caffeine or codeine, can cause addiction (Table 1). The function of these compounds depends on the dose used, e.g., atropine in low doses is used in medicines, whereas in higher ones, it is a strong poison (4).

REPORTED COSMETIC USES OF ALKALOIDS

The cosmetics industry uses alkaloids with antimicrobial (berberine and spilanthol) and antioxidant (caffeine, berberine, spilanthol, and capsaicin), for lightening discoloration (berberine), reducing cellulite and wrinkles (caffeine, capsaicin, piperine, and spilanthol), soothing and anti-inflammatory (anatabine and piperine), and protecting against the harmful effects of ultraviolet ray properties (caffeine) (4). Most often, in the production of cosmetic products, alkaloids are extracted from plants in pure form or their aqueous or alcoholic extracts are used. Because of the aforementioned properties, these compounds can be used in the production of tonics, creams, lotions, face and hair masks, compresses for skin problems with numerous inflammations, and discoloration and antiaging products, as well as reducing the formation of cellulite. In addition to traditional external application on the skin, alkaloids are used in the production of ampoules for cosmetologists and aesthetic medicine doctors.

This article summarizes the experimental data relative to the cosmetic effects of some alkaloids.

CAFFEINE IN COSMETICS

Caffeine (1,3,7-trimethylxanthine) is one of the most popular purine alkaloids of plant origin (Figure 1). Depending on the botanical from which it is obtained, it takes different names. The most popular raw material from which caffeine can be obtained is coffee beans (*Coffea L.*). Caffeine is also found in tea (*Camellia*) and is called theine. Caffeine is also present in cola nuts (*Cola acuminate* and *Cola nitida*), in leaves of the Yerba mate (*Ilex paraguariensis*) and guarana (*Paullinia cupana*); hence, it is called guaranine. It is also found in small amounts in cocoa. Its content depends on the species of the plant from which the raw material comes, and the method of preparing the drink or preparing the seeds for consumption (5). Purified caffeine comes in the form of a white crystalline powder. It has no fragrance and bitter taste. This alkaloid is an active ingredient in some pharmaceutical preparations; it is used in cardiological, neurological, and respiratory medicinal products (6,7).

Caffeine has been used in different kind of cosmetics. Commercially available topical formulations and anti-cellulite cosmetics normally contain 3–7% of this compound (8). Lupi et al. (9) conducted a study on 99 women (20–39 years) with clinically apparent cellulite, who used a solution containing 7% caffeine for 30 d. Women applied 15 mL of the solution to one thigh, while the other was used as control. In 67.7% of women, a reduction in diameter of the hip was observed. The median circumference reduction in the

Table 1
Selected Alkaloids Used in Cosmetics

Name	Group	Occurrence	Action	Used in cosmetics	LD50	References
Caffeine	Purine alkaloids	Caffe (*Coffea L.*), tea (*Camellia*), cola (*Colae*), Yerba mate (*Ilex paraguariensis*), and guarane (*Paullinia cupana*)	Firming, elasticity blood vessels, antioxidant, stimulate hair high, and antiaging	Anti-cellulite balms, antiaging creams, highlight creams, and shampoo and conditioners on hair high	80–100 mg/L, 367 mg/kg BW	(7,21)
Capsaicin	Protoalkaloids	Chili peppers (*Capsicum chili*), jalapeño (*Capsicum annuum* '*Jalapeño*'), and cayenne pepper (*Capsicum annuum* "Cayenne")	Analgesic, antioxidant, weight loss, antibacterial, and thermoregulatory	Lipsticks, lip glosses, hair masks, wipes, shampoos, creams, and anti-cellulite balms	512 mg/kg BW	(4,30,36,78)
Berberine	Isoquinoline alkaloids	Barberry (*Berberis vulgaris*) and abuta (*Abuta grandifolia*)	Antibacterial, antifungal, antimicrobial, antioxidant, and highlight	Masks, creams, tonics, balms, and natural sun filter factors UVA i UVB	329 mg/kg BW	(4,53)
Anatabine	Pyridine alkaloids	Annual paprika (*Capsicum annuum L.*), tomatoes (*Solanum lycopersicum L.*), eggplant (*Solanum melongena L.*)	Inflammatory, soothing, and regenerative	Creams, pastes, tonics, balms, and gels	–	(75,76)
Piperine	Piperidine alkaloids	Black pepper (*Piper nigrum L.*)	Anti-inflammatory and anti-cellulite	Face creams, lotions, tonics, soaps, and anti–nail biting preparations	330 mg/kg BW	(61,62)
Spilanhtol (afinine)	Alkiloamids	*Acmella oleracea*	Anti-wrinkles, antiaging, antibacterial, and firming	Antiaging creams, anti-cellulite creams, gels and emulsion care, and aqua extract for cleaning skin	113 mg/kg BW	(63,65,67)

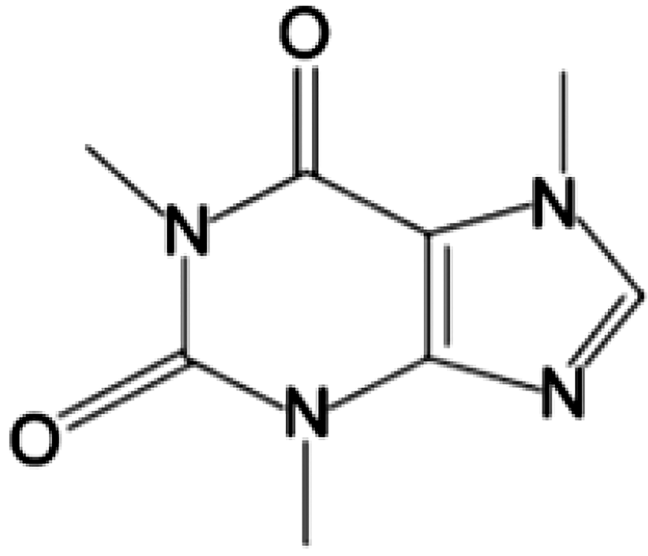

Figure 1. Chemical structure of caffeine (6).

thicker portions of treated thighs was 2.1 cm. When considering the lower portion (10 cm above the patella), the difference between the first and last evaluation was 1.7 cm, in treated thighs.

Caffeine is also an active ingredient in many anti-cellulite cosmetics. This alkaloid shows firming and elasticizing the skin. In vitro studies on human hepatoma HepG2 cells have shown that caffeine can slow down lipogenesis and stimulate lipolysis, which may translate into a decrease in the level of fat accumulated in the body (10). Significant decreases in the accumulation of hepatic lipids, such as triglyceride (TG), and cholesterol were observed when HepG2 cells were treated with caffeine in concentration of 6–24 mM. Caffeine decreased the mRNA level of lipogenesis-associated genes (SREBP1c, SREBP2, FAS, SCD1, HMGR, and LDLR). By contrast, the mRNA level of CD36, which is responsible for lipid uptake and catabolism, was increased. Phosphorylation of MP-activated protein kinase (AMPK) and acetyl-CoA carboxylase were evidently increased when the cells were treated with caffeine as indicated for 24 h. Caffeine effectively depleted TG and cholesterol levels by inhibition of lipogenesis and stimulation of lipolysis through modulating AMPK-SREBP signaling pathways (11).

This was also confirmed in studies in rats (eight rats per group) that were fed a high-fat diet supplemented with 0, 0.025%, 0.05%, or 0.1% caffeine for 21 d. Authors noted reducing the body fat mass and body fat percentage in a dose-dependent manner in rats fed with a high-fat diet, presumably because of increased lipolysis *via* catecholamines. Rats receiving 0.025%, 0.05%, and 0.1% caffeine had about 7, 10, and 11 g less body fat mass and 2.8, 3.1, and 4.5% less body fat percentage, respectively, than rats from the control group (12).

Caffeine has penetrating properties, unchanged by the thickness of the skin or the occlusive layer. The maximal absorption rates of caffeine through the human skin were found to be 2.24 ± 1.43 μg/cm^2/h (13). For determination of the maximal absorption rate of

caffeine, this alkaloid was applied at a concentration of 4.0 mg/mL ethanol/water (1:1, v/v) on the human skin membrane. The application volume was 25 μL/cm^2, which was considered the minimum volume necessary to produce a homogeneous distribution on the skin surface. This represented a finite dose (100 μg/cm^2), to mimic occupationally relevant situations. The exposure time was 24 h. Aliquots of the receptor fluid were collected at various time points (minimally at 1, 2, 4, 8, and 24 h post-dosing). A cumulative amount absorbed per unit skin area *versus* time course was constructed from the amount of test substance in the receptor fluid, and the maximum absorption rate was determined from the steepest, linear portion of the curve.

The penetration of caffeine through the skin depends on the ethnicity. Because of the greater number of sebaceous glands per square cm, caffeine is better absorbed by the skin of Asians in relation to the Caucasian. Mustafa et al. (14) studied the effect of different concentrations of caffeine (1, 3, and 5%) on *in vitro* release through synthetic membrane and on *ex vivo* permeation of this alkaloid through human skin. The lowest lag time (Tlag) and higher absorption rates were obtained with gel at 1% of caffeine applied at 1 mg/cm^2. The diffusion flux of caffeine permeation does not depend on the concentration but rather on the quantity of formulation applied (15).

In addition, there is a linear relationship between percutaneous caffeine penetration and transepidermal water loss (TEWL) (16). This was confirmed in the studies by Lotte et al. (17). The authors of the presented studies applied to volunteers 20 μL radiolabelled 10^{-3} gCi/nmol caffeine to an area of 1 cm^2. This alkaloid was applied on the forehead, postauricular area, arm (upper and outer), and abdomen. The highest TEWL values were obtained when caffeine was applied on the forehead (1.8), next when caffeine was applied on the postauricular area (1.2), on arm (1.0), and the lowest when caffeine was applied on the abdomen (0.9). The maximal absorption of caffeine is reached at 100 min after local application *in vivo* (18).

Amnuaikit et al. (19) performed experiment on the 34 volunteers divided into four groups, and the studies were taken for four successive days (about 8–9 volunteers/group/d). They were assigned to lie on the bed but did not sleep for one night. No face washing in the next morning was allowed. The length of each puffy eye was immediately measured using a thread starting from the corner of the eye near the nose to the end of the other side of the eye curving *via* the puffiness and then the thread length was determined using a ruler. The swollen area under each eye was marked, and then the samples, i.e., a selected caffeine gel and its gel base, were randomly applied on different eyelids. The observations were taken at 10, 15, 30, 90, 150, and 180 min after applying the gel formulations. Ahmadraji and Shatalebi (20) evaluated *in vivo* efficacy of an anti-wrinkle and dark circle eye pad consisting of 3% caffeine and 1% vitamin K. Then, research was carried out on 11 healthy women. In a single blind trial, the sample pad was applied under the right eye, and the placebo pad, consisting of water, was placed under the left eye simultaneously. The content of water in the surface layer of the skin, the content of pigmentation, and the percent of elasticity of the skin were measured using apparatus multi-skin center MC 900. After 4 weeks, the skin around the right eye of all the subjects experienced a reduction in the depth of wrinkles and dark circles, and from a subjective point of view, the appearance and elasticity of the skin were improved. Topical caffeine use reduces blood flow by narrowing the blood vessels; wherefore, this alkaloid reduces eye shadows and swelling, as well as signs of skin fatigue (21).

Caffeine has antioxidant properties, helping to fight free radicals, which play a role in accelerating the skin aging process. Combinations of caffeine and green tea polyphenols have been reported to have anti-wrinkle properties and the ability to brighten and firm up the skin in older individuals (22). The authors drew such conclusions on the basis of studies in which 126 women aged 30–70 years with mimic wrinkles (moderate to pronounced) took part. The women applied the selected product to half of their face twice a day (morning and evening) for 4 weeks. In the experiment, women from group 1 used daytime SPF 30 lotion, from group 2 used night cream, and from group 3 used eye cream containing caffeine. Authors did not present information on how much caffeine was in the topically applied product. The FOITS technique (Fast Optical In vivo Topometry of human Skin) was used to measure changes in crow's feet. In addition, changes in skin barrier function were determined using triplicate TEWL measurements. Four weeks of treatment with the caffeine preparation improved skin smoothness and reduced the depth of wrinkles (23).

It has been reported that caffeine helps to stimulate hair growth and inhibit their loss (22). Beneficial changes in the mechanical properties of individual scalp fibers were observed after the caffeine preparation. Fisher et al. (24) took hair follicles from the vertex areas from 14 male patients. Hair follicles were cultivated for 120–192 h *in vitro* with normal William's E medium (control) or William's E medium containing different concentrations of caffeine (0.001–0.15%). Hair shaft elongation was measured daily, and at the end of cultivation, cryosections of follicles were stained with Ki-67 to evaluate the degree and localization of keratinocyte proliferation. They showed that caffeine concentrations ranging from 0.001% to 0.005% led to *in vitro* stimulation of human hair follicle growth. The stimulating effects of caffeine on hair growth can also be explained due to the caffeine phosphodiesterase inhibition activity with consequent increase of cAMP intracellular concentration and stimulating cellular metabolism (24).

CAPSAICIN IN COSMETICS

Capsaicin, (6E)-N-[(4-hydroxy-3-methoxyphenyl) methyl]-8-methyl-6-nonenamide, is an active ingredient in capsicum plants (Figure 2). Capsaicin is obtained, among others, from fruits of chili peppers (*Capsicum chili*), jalapeño (*Capsicum annuum* '*Jalapeño*'), or cayenne pepper (*Capsicum annuum* '*Cayenne*') (25,26). It is known primarily for its characteristic sharp taste and irritating properties on nerve endings, which is used in the food, pharmaceutical, and cosmetics industries. Capsaicin has analgesic, antioxidant, weight loss, antibacterial, and thermoregulatory effects. Discussions are currently underway about the effect of capsaicin on cancer development. Test results of Hwang et al. (27) suggest that capsaicin may act as a cocarcinogen, and thus potentiate the carcinogenic effect of 2-O-tetradecanoylphorbol-13-acetate (TPA).

The properties of capsaicin are used primarily in the pharmaceutical industry as an additive to drugs that inhibit pain associated with rheumatoid arthritis and neuralgia. They have also been used in the cosmetic industry as active, flavoring, or preservative agents (28).

Applying a small amount of capsaicin to the skin causes a feeling of warmth or gentle burning. Because of the side effects of long doses of capsaicin, such as secondary hyperalgesia, the maximum use of capsaicin treatment in drugs is 0.075%. On the other hand,

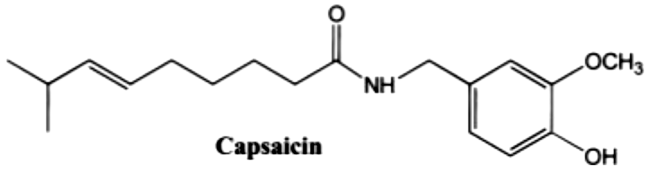

Figure 2. Chemical structure of capsaicin (77).

the amount of capsaicin in over-the-counter preparations, including cosmetics, is allowed at a concentration of 0.025% (10). Warm feeling is created as a result of the release of pro-inflammatory peptides (substance P and CGRP), which dilate blood vessels and increase the heat exchange surface with the environment, which increases heat loss (29). Capsaicin in lip products may be used in the cosmetic industry in all types of lip glosses or lipsticks designed to enlarge lips. Increased blood flow through the blood vessels, caused by capsaicin, has the effect of enlarging the lips, which is manifested in the form of slightly swollen, toned, and red lips (30). However, this is a short-term result. Boudreau et al. (31) studied the effect of capsaicin on human sensory and vascular changes in 13 people. Applications of 1% capsaicin or vehicle cream to the glabrous lips and tongue were randomized between two two-trial sessions. The capsaicin trial followed the vehicle trial for each session. Before and after 5, 15, and 30 min capsaicin or vehicle cream application, blood flow and temperature were measured by laser-Doppler imaging and thermography. Increases in blood flow and temperature occurred after 5 min of capsaicin application. Van der Schueren et al. (32) showed that 1,000 micrograms of capsaicin produced the maximum response between 30 and 45 min after application, and at 60 min time point, there was decrease in the blood flow.

By using hair and scalp care products (masks with capsaicin), there is increased microcirculation of the scalp blood. Because of local irritation caused by capsaicin and increased blood flow, platelets are delivered to the scalp, including growth factors and cytokines. These compounds act immediately, leading to the activation of cell renewal, which affects skin regeneration as well as hair growth. The effect of capsaicin on hair growth was studied by Lee et al. (33). After depilation of the back skin of mice, they divided mice into 4 groups, i.e., control, capsaicin, minoxidil, and co-applied group. They examined hair regrowth after depilation in terms of macroscopic examination, image analysis using phototrichograms, measurement of hair regrowth length, and microscopic examination. Hair growth of capsaicin group and minoxidil group began faster than that of co-applied group and control. Thereafter, hair growth of the capsaicin group was observed as the fastest, followed by the minoxidil group, co-applied group, and control. On microscopic examination, capsaicin was able to make the hair cycle faster and shorter than control. These results suggest that capsaicin can not only induce the anagen phase quickly but also sustain constant effect on hair growth.

Capsaicin shows the highest solubility in a lipophilic solvent (fat and oil) at pH = 8, but at pH = 5, the solubility of capsaicin is slightly lower. The pH of the skin is normally acidic, ranging in pH values of 4–6 (34); combining these two facts, it can be concluded that capsaicin may show a high affinity for lipophilic cell membranes of the scalp, penetrating deep into the tissues transdermally and so transcellularly (35). Another group of cosmetics with capsaicin are creams, slimming balms and products aimed at help in the fight against cellulite.

According to research conducted on Sprague–Dawley rats by Joo et al. (36), capsaicin prevents the transcription of protein genes that intensify the process of adipogenesis, in other words, accumulation of white adipose tissue. In this study, rats were divided into the normal control group, the high-fat group with capsaicin, and the high-fat group without capsaicin. This alkaloid was administered in dose 10 mg/kg BW by oral injections. During the proteomic analysis of white adipose tissue, 37 proteins were detected and identified whose expression was modulated in response to high-fat diet. Joo et al. (36) showed that under the influence of dietary capsaicin, the amount of mRNA of the key white adipose tissue transcription factors, PPARγ and C/EBPR, decreased significantly. The weight difference at the beginning and at the end of the study for control group was 240 g, for the high-fat group without capsaicin was 313 g, and for high-fat group with capsaicin was 285 g (36).

Capsaicin also contributes to the regulation of transcription of genes responsible for the production of enzymes that catalyze the hydrogenation of fats. In Lee et al. (37) studies conducted on the mouse 3T3-L1 cell line, fully differentiated adipocytes were treated with 0.1, 1, or 10 μmol/L capsaicin for 24 h. The intracellular lipid content was decreased in a dose-dependent manner in the presence of 0.1, 1, or 10 μmol/L capsaicin, by 3.2%, 6.9%, and 14%, respectively. The TG content of cells was also decreased by 15%, 21%, and 33%. Treatment with capsaicin resulted in a decrease in the intercellular lipid content and an increase in the amount of glycerol released into the medium, an indicator of the stimulation of lipolysis. Capsaicin is involved in the breakdown of fat in rodent adipocytes, and also increases the catabolism of intracellular fats (37). These processes are controlled by regulating the expression of genes encoding proteins involved in catabolic lipid processes. Research on laboratory animals applies to humans; therefore, it can be assumed that the mechanisms that occur in animals will be the same as in humans. Each of these processes leads to a decrease in the amount of body fat, which can be a method of fighting cellulite.

Capsaicin also has antioxidant properties that consist in reducing the intensity of lipid peroxidation processes. As an example, scientists cite delayed LDL lipoprotein oxidation (38). Serum lipid oxidation was examined by incubation of human serum with increasing concentrations (0.1, 0.5, 0.7, 1, 2, and 3 μM) of capsaicin, dihydrocapsaicin, and subjected to copper (100 μM)-induced oxidation. Copper-induced oxidation of serum was undertaken using the method described by Schnitzer et al. (39). At a concentration of 0.7 μM, the rate of oxidation was reduced by 42 and 45% for capsaicin and dihydrocapsaicin, respectively (40). Delaying the lipid oxidation process can be of great importance when extending the shelf life of cosmetics containing a high fat content.

Capsaicin has been shown to maintain the durability of various artificial materials, e.g., polyethylene—one of the most popular component cosmetic packaging. Capsaicin blocks the production of oxygen-free radicals and hydroxide anions, even in the presence of γ waves that activate their formation (41). The evident increase in the oxidation induction time (OIT) is the proof of the efficient antioxidant efficiency of capsaicin. The presence of capsaicin in low-density polyethylene (LDPE) brings about the delay in the OIT values by 1.6 times relative to neat polymer. The oxidation times of 150 and 260 min represent the values of 10 times longer for LDPE stabilized with capsaicin than for pure LDPE received irradiation dose of 30 kGy (41).

Published research shows that a plant extract with high capsaicin content has the effect of inhibiting the growth of some bacterial cultures. Both alcoholic and aqueous paprika

extracts (*Capsicum annuum L.*) in concentration 100 mg/L have antibacterial activity against strains of *Staphylococcus aureus*, *Salmonella typhimurium*, and *Vibrio cholerae*. The concentration of capsaicin in the extract and IC 50 was not reported in the quoted article (42). Therefore, capsaicin can be considered a natural preparation with antimicrobial activity against selected bacterial strains.

BERBERINE IN COSMETICS

Berberine is an isoquinoline alkaloid present in the bark, root, and other organs of the species barberries (*Berberis vulgaris*) and goldenseal (*Hydrastis canadensis*). Berberine is obtained in the cosmetic industry also from roots and aerial parts of the abuta plant (*Abuta grandifolia*) (Figure 3) (43,44). Berberine at concentrations of 50, 100, and 200 µg/mL displayed a significant antibacterial and antifungal activity against *Staphylococcus aureus* and different *Candida* spp. A decoction of the bark of this raw material is recommended in the treatment of mycosis of the skin (45). According to Cernáková and Kostálová (46), berberine was found to be moderately active against the tested bacteria, yeasts, and fungi. IC50 for *S. aureus* was 14.6 mg/L, for *B. subtilis* 143 mg/L, for *P. aeruginosa S* 39.8 mg/L, for *P. aeruginosa* 99.2 mg/L, for *E. coli S* 73.2 mg/L, for *E. coli* R 87.0 mg/L, and for *Z. ramigera* 145 mg/L. They tested different concentrations of berberine in the solid medium being 0 (control), 100, 250, 500, and 1,500 mg/L. Gram-positive and Gram-negative bacteria were grown in peptone water during static culture. The cultivation lasted 1 d at 37°C. The evaluation was performed by reading A630 after 1 d (for bacteria) and 2 d (for yeasts); the percent growth was calculated by comparing to A630 of the corresponding control. The research has shown that berberine chloride inhibits activity Gram-positive bacteria stronger than Gram-negative bacteria, whereas antifungal properties of this compound are based on the cell membrane damage (45,47).

Berberine in addition to antimicrobial properties also exhibits on action anodyne, anti-inflammatory, and antioxidative properties, and reduced pressure blood and control of cholesterol and sugar level in blood (44).

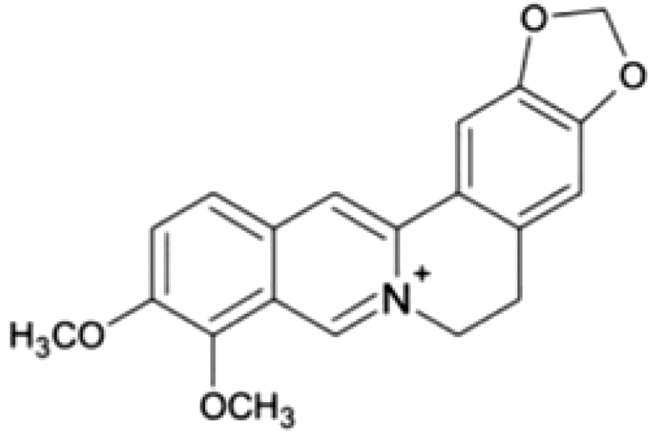

Figure 3. Chemical structure of berberine (42).

Berberine fruit masks are used to treat acne vulgaris as well as skin hyperpigmentation. Seki and Morohashi (48) noted that lipogenesis in the hamster sebaceous glands was suppressed 63% by 10^{-4} M berberine. They suggest that this alkaloid can be used for acne vulgaris because of inhibition of lipogenesis.

Anti-inflammatory properties are associated with the process of inhibiting the activity of pro-inflammatory lipoxygenase enzyme causing skin diseases (49,50). Kuo et al. (51) used cancer cell to evaluate anti-inflammatory properties of berberine. In cancer cell line OC2 and KB cells, a 12-h berberine treatment (1, 10, and 100 mM) reduced prostaglandin E2 production dose dependently with or without 12-O-tetradecanoylphorbol-13-acetate (TPA, 10 nM) induction. This berberine-induced effect occurred rapidly (3 h) as a result of reduced COX-2 protein. Further analysis showed that berberine inhibited activator protein 1 binding directly.

Despite the evidenced potentiality of berberine in the treatment of skin diseases, its topical application is limited because of its high hydrophilicity, the approximate log p value of –1.5 hinder its delivery across the skin layers (44). According to Torky (52), to increase its dermal bioavailability, berberine can be formulated with sodium oleate as a complexing agent. This complex displays about 250-fold higher saturation solubility in n-octanol, endorsing the improved lipid solubility of the complex compared with free alkaloid (52).

They are a rich source of antioxidants. Zovko Koncic et al. (53) studied the antioxidant activities of the ethanolic extracts of roots, twigs, and leaves of *Berberis vulgaris L.* and *Berberis croatica Horvat*. For preparation of extracts, powdered herbal material (10 g) was extracted with 96% ethanol (50 mL) in ultrasonication bath at 45°C for 45 min. They noted that all the extracts were found to possess some radical-scavenging and antioxidant activities, as determined by the scavenging effect on the 2,2-diphenyl-1-picrylhydrazyl free radical, reducing power and β-carotene–linoleic acid model system.

Toxicity of berberine depends on the route of administration and experimental animal species. The LD50 value of powdered root *Berberis vulgaris* is 2,600 mg/kg in mice on oral administration. On oral administration of root extract fraction of *B. vulgaris*, the LD50 values are 1,280 and 520 mg/kg in rat and mice, respectively. In mice, the LD50 values of pure berberine on intraperitoneal (IP) and oral administration are 23 and 329 mg/kg, respectively. Berberine sulfate isolated from *Berberis aristata* on IP administration in rats has LD50 value equal to 205 mg/kg (54).

PIPERINE IN COSMETICS

Piperine (1-[5-(1,3-benzodioxo-5-yl)-1-oxo-2,4-pentadienyl] piperidine) belongs to the group of piperidine alkaloids (Figure 4). Piperine can be obtained from black pepper (*Piper nigrum L.*). Black pepper contains approx. 2–3% of volatile oils (55) and about 5–9% of alkaloids such as piperine, piperidine, peperitin, and a whole range of other similar substances (56). The process of piperine isolation consists in the extraction of oleoresin from ground pepper with supercritical fluid and then crystallization with ethanol. This alkaloid can also be obtained by methods such as maceration, Soxhlet extraction, or hydrotropic solubilization (57).

Black pepper has a variety of physiological properties, ranging from stimulation of pancreatic digestive enzymes, through anti-inflammatory effects in many autoimmune

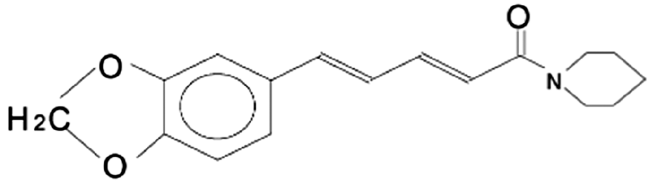

Figure 4. Chemical structure of piperine (50).

diseases, to improving the bioavailability of certain drugs (58). This plant, because of its content of piperine and its derivatives, has found itself in the interest of cosmetic manufacturers and cosmetologists. In studies conducted on 3T3-L1 cells, Park et al. (59) noted that, piperine, by affecting adipogenic transcription factors such as PPARγ, SREBP-1c, and C/EBPβ, influences the transcription of genes responsible for adipocyte differentiation. By inhibiting the activity of PPARγ genes, it is expected to slow down the fat formation process (59).

By doing so, the process of absorption of active ingredients through the skin increases. This property is used in face creams, lotions, or tonics. Piperine, like caffeine, has also been used as a weight loss supplement. By stimulating the nerve endings in the gastrointestinal tract, piperine activates the secretion of digestive enzymes so that the food components are more thoroughly digested and thus absorbed. Platel and Srinivasan (60) in research conducted on Wistar rats, which were fed diet with addition of 10 mg piperine, noted enhanced pancreatic lipase, amylase, trypsin, and chymotrypsin activity.

Piperine has also been used in various types of detergents, including soaps (61). This alkaloid has also been appreciated as an ingredient of preparations discouraging nail biting, which is quite a popular behavior among children. The receptors of the taste buds in contact with the preparation are irritated, and the feeling of delicate baking discourages from biting nails (onychophagy). Piperine is not classified as acute, irritating, or skin sensitizing (62).

SPILANTHOL IN COSMETICS

Spilanthol ((2E,6Z,8E)-N-isobutyl-2,6,8-decatrienamide), also known as affinin (Figure 5), belongs to a group of compounds called alkylamides. The substance is found in a wide range of plants, in particular Asteraceae and Piperaceae. The highest content of spilanthol is found in extracts of root, leaves, or buds of *Acmella oleracea*—plant of Asteraceae (63,64). Spilanthol is considered to be a natural, safe, and nontoxic botox alternative. Research was conducted on nerve–muscle coculture, a culture model used to recreate human striated

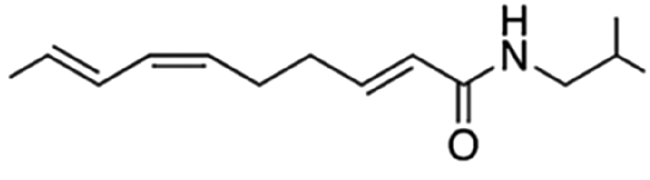

Figure 5. Chemical structure of spilanthol (59).

muscle cell innervation using spinal cord and spinal ganglion explants from rat embryos. The cells were treated with lyophilized extract of *Acmella oleracea* diluted to 50% in maltodextrin or pure spilanthol (40×10^{-5}% or 160×10^{-5}%). The frequency of contraction in nerve–muscle coculture with spilanthol was determined after 5 min, 1 h, and 6 h of incubation with the substance. Both extract and pure spilanthol, applied externally, reduced muscle tension, thus hopefully contributing to the reduction of facial expression wrinkles (65).

Spilanthol perfectly overcomes the epidermal barrier and migrates deep into the skin. Veryser et al. (66) analyzed the transdermal behavior of spilanthol using human skin in a Franz diffusion cell setup. The dose solutions (30% w/w spilanthol in ethanol or products with spilanthol) were applied on the 0.64 cm^2 skin surface with a micropipette. Authors noted that spilanthol penetrates through the human stratum corneum and the viable epidermis, thereby reaching the dermis and thus the systemic circulation, with permeability coefficient (*Kp*) values between 0.6 and 53.3 × 10–4 cm/h, depending on i.a. the used dose formulation.

Cosmetics based on spilanthol as an active ingredient are gaining popularity, especially anti-wrinkle products (mainly creams). They also occur in the form of water extracts from *Acmella oleracea*, used to wash the skin. Cosmetics containing spilanthol are also gels, care emulsions in types oil/water or water/oil, or nanoemulsions (67).

They are mainly similar to botulinum toxin activity. It is responsible for the reduction or complete elimination of unaesthetic skin creases in various areas of the face—from very fine to deep wrinkles. The effects of cosmetic products containing spilanthol are comparable to those after using botulinum toxin. Hence, its name is "herbal botox." Spilanthol is safe and nontoxic. No contraindications for its use have been shown. Spilanthol properties were studied on nerve–muscle coculture model, which is a model suited to studying the influence of a substance on muscle contraction frequency, as well as to studying the recuperation of contractile activity after blockage of muscle contractions by a substance. The frequency of contraction was determined after 5 min, 1 h, and 6 h of incubation with spilanthol. At 6 h, the substance was eliminated, and recuperation of contractile activity was studied 1 h and 24 h later. At the concentrations 40×10^{-5}% and 160×10^{-5}%, pure spilanthol blocked muscle contractions after 5 min of incubation. The blockage was maintained until 6 h and the fibers remained blocked for 24 h after elimination of the substance (65).

Spilanthol works in a variety of ways, including larvicidal (10–14 μg/mL), antimicrobial (25–300 μg/mL), and fungicidal and bacteriostatic (5–150 μg/mL). This compound also has anti-inflammatory properties (90–180 μM in the macrophage cell line), and antianxiety (3–30 mg/kg i.p. in mice) and diuretic (800 mg/kg *per os* in mice) effects (68,69).

To estimate the bacteriostatic activity of spilanthol, Molina Torres et al. (70) used liquid cultures, which were grown at 28 or 37°C, depending on the microorganism, in the PDB medium with vigorous agitation. The growth of *E. coli* (Gram –) and *B. subtilis* (Gram +) was 100% inhibited at spilanthol concentrations of 75 and 150 μg/mL, respectively. *E. carotovora* (Gram –) was not sensitive to the higher concentration of this amide. The minimum inhibitory concentration of spilanthol was estimated by testing different quantities of this compound incorporated into the PDA culture medium (potato dextrose agar). Spilanthol was incorporated into the medium at about 50°C, before it solidified in

the Petri dishes. The solidified plates at room temperature were inoculated with a 5-mm mycelium disk, and the mycelium dry weight was determined after 10 d of incubation. *S. rolfsii* and *S. cepivorum* were more sensitive to spilanthol, with a mycelial growth inhibition of 100 and 94%. A lower sensitivity was detected with *Verticillium* sp. and *Fusarium* sp. even at the higher concentration of spilanthol assayed (70).

ANATABINE IN COSMETICS

Anatabine (3-[(2S)-1,2,3,6-tetrahydropyridin-2-yl] pyridine) belongs to the group of pyridine alkaloids (Figure 6.). It occurs most frequently in the nightshade plants (Solanceae), as well as in annual paprika (*Capsicum annuum L.*), tomatoes (*Solanum lycopersicum L.*), eggplant (*Solanum melongena L.*), and tobacco (*Nicotiana tabacum L.*) (71). Because of the presence of other toxic alkaloids, e.g., nicotine, obtaining anatabine from plants is problematic. This compound is first extracted with ethanol, water, steam, or carbon dioxide. Then, the extract is purified by means of analytical techniques, e.g., liquid chromatography. Anatabine is alkaline in aqueous solutions, which is why it is used in the production of cosmetics to neutralize the pH of the cosmetic mass. In the cosmetic industry, products with anatabine are most often used for the production of emulsions, creams, gels, pastes, and lotions (72,73).

Anatabine has been shown to have anti-inflammatory effects in *in vivo* and *in vitro* studies. Studies conducted in mice, which received anatabine in dose 2 mg/kg BW, IP show that this substance reduces the level of pro-inflammatory amyloid β (from about 4,300 pg/mL in control group to about 3,000 pg/mL in group receiving 2 mg/kg anatabine) in blood plasma (74) and inhibits the production of pro-inflammatory cytokines such as TNFα, IL-1β, and IL-6 in tissues and blood (75). The quoted numbers are obtained by extrapolating from graphs in the quoted article. The anti-inflammatory and immunity-enhancing effects of anatabine can be used in the production of cosmetics designed to complement

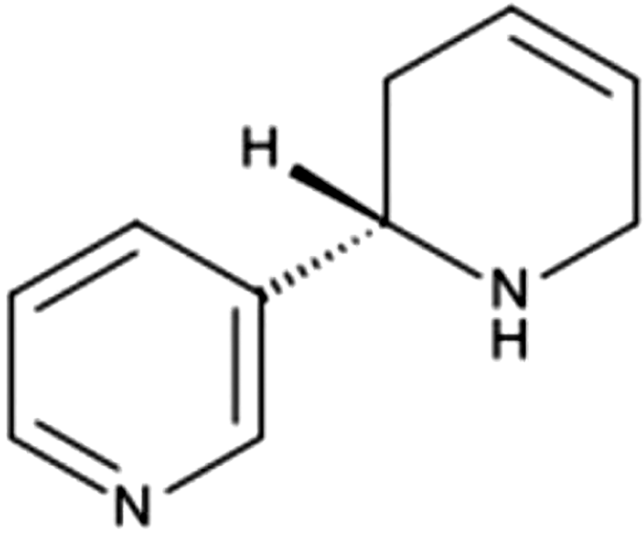

Figure 6. Chemical structure of anatabine (67).

the care of acne skin. Lanier et al. (76) conducted a study on 10 volunteers with mild and moderate rosacea who were applying a cream containing this alkaloid twice a day. After 30 d, half of the respondents noticed a marked improvement in the skin condition (reduction of erythema, inflammation, and flaking off the skin).

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