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Natural Products Supporting the Extracellular Matrix: Rice Ceramide and Other Plant Extracts for Skin Health

Hiroshi Shimoda, PhD

Oryza Oil & Fat Chemical Co., Ltd., Ichinomiya, Japan

Summary

Natural cosmeceutical products are attracting increasing attention, especially from the ecological and safety point of view. Collagen, hyaluronic acid,

Aaron Tabor and Robert M. Blair (eds.), *Nutritional Cosmetics: Beauty from Within*, 319–334, © 2009 William Andrew Inc.

elastin, and ceramides are components of the skin's extracellular matrix. Ceramides and glucosylceramides are contained in the stratum corneum of the epidermis and play roles in the barrier function and moisturization of the skin. Some of these components can be extracted and purified from plants. Rice ceramide extracted from rice germ and bran consists of at least six types of glucosylsphingolipids. This extract supports skin moisturizing and barrier function, suppresses melanin synthesis, promotes cell proliferation, and acts anti-inflammatorily and antiallergically. Therefore, application of rice ceramide in cosmeceutical products and as dietary supplements should be beneficial for maintaining a healthy skin extracellular matrix. Besides rice ceramide, we found that litchi seed extract, purple rice extract, and grape extract inhibit skin-tissue-degrading enzymes, for example collagenase, hyaluronidase, and elastase. Selective and combined application of these extracts is thus expected to help maintain skin health.

16.1 Introduction

In development of new skin care products, raw materials with novel physiological functions and proven safety play a key role. Choice of synthetic or natural materials for cosmetic composition depends on their cost, safety, and effectiveness. With a safe, novel, and ecological image, more and more consumers are favoring natural cosmetic components recently. In addition, consciousness of environmental aspects has led to the increased demand for organic cultivation, non-solvent extraction, and non-animal- and non-fish-derived materials. We have developed a number of extracts from various plants for application in foods and cosmetics. Ceramide, a skin component with moisturizing activity, can either be synthesized or extracted from plants such as rice, wheat, and corn. The demand for plant-derived ceramides, which can be used for both cosmetics and foods, is increasing. In this chapter, we introduce skin-healthy effects of rice-derived ceramide. We also describe beauty effects of litchi seed extract, purple rice extract, and grape extract.

16.2 Rice Ceramides

16.2.1 Ceramides in Skin

In 1884, ceramides were found in human brain tissues, and later in skin and mucosal tissue. Human skin consists of epidermis, corium, and tela

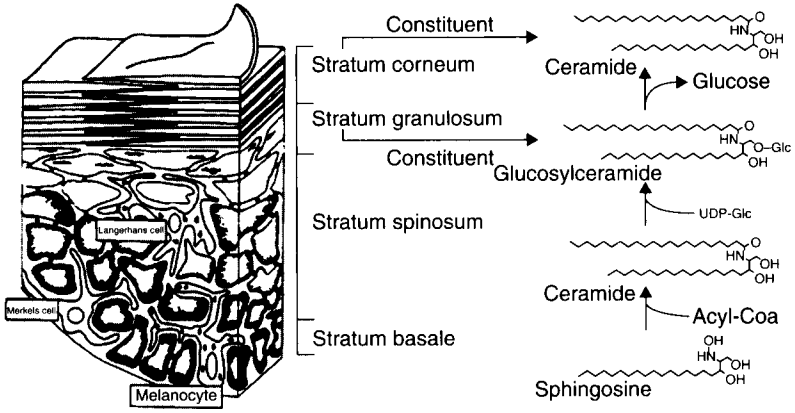


Figure 16.1 Sphingolipids and their biosynthesis scheme in human skin.

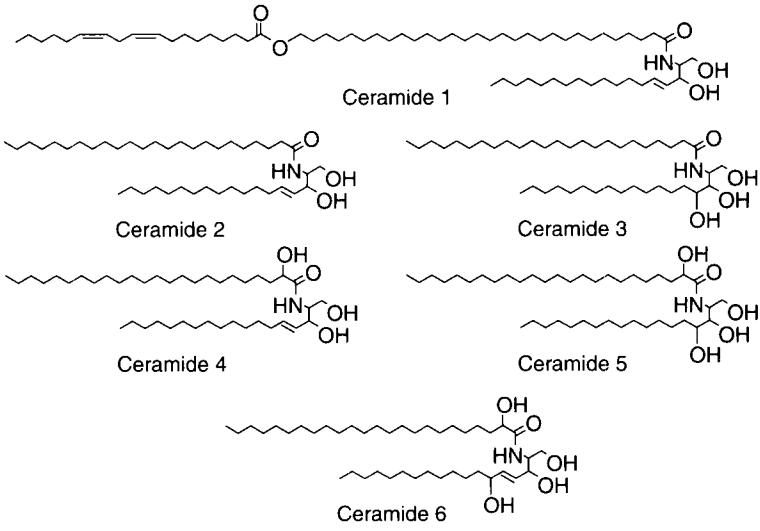


Figure 16.2 Ceramides in stratum corneum.

subcutanea. The epidermis is classified into four layers, namely the stratum corneum, stratum granulosum, stratum spinosum, and stratum basale (Fig. 16.1). More than six types of ceramides have been identified in human skin (Fig. 16.2) [1,2]. These ceramides are produced via several biosynthetic pathways in the epidermis and accumulated in the stratum corneum as the major constituent lipids (40–60% of the total lipids). In the epidermis,

ceramides play an important role in forming lamella phases and in maintaining barrier function [3].

Imokawa et al. [4] demonstrated that the content of skin ceramides declines with age (Fig. 16.3). Forearm skin of elderly persons (especially those older than 70 years) is often xerotic, suggesting an association between ceramide decrease and skin drying. Marked reduction of ceramides was found in both lesional and nonlesional forearm stratum corneum of patients with atopic dermatitis (Fig. 16.4). These findings suggest that ceramides are a key factor for moisture maintenance and barrier function of the stratum corneum. A decrease in ceramide content is also thought to be associated with wrinkle formation. Thus, a sufficient amount of ceramides is considered to be essential for maintaining healthy skin.

16.2.2 Rice Ceramide

Rice (*Oryza sativa* L.) has been widely grown in Southeast Asia, not only as a major crop but also as an integral part of traditional culture and lifestyle in many Asian countries. In recent years, attention has been focused on rice bran and rice germ for its unique bioactive compounds and non-GMO profile. We have developed a number of products extracted from rice bran and rice germ as functional ingredients in medicines and cosmetics, as dietary supplements, and as food additives. One such functional compound is rice ceramide, which supports barrier function and moisture of skin. Rice ceramide contains a large amount of glucosphingolipids, which have

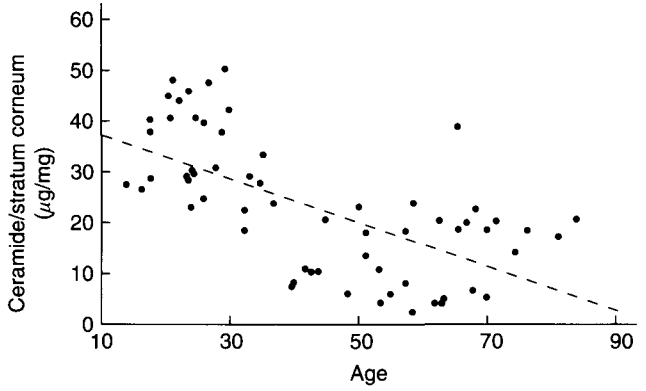


Figure 16.3 Ceramide content of the stratum corneum in healthy subjects of various ages [4].

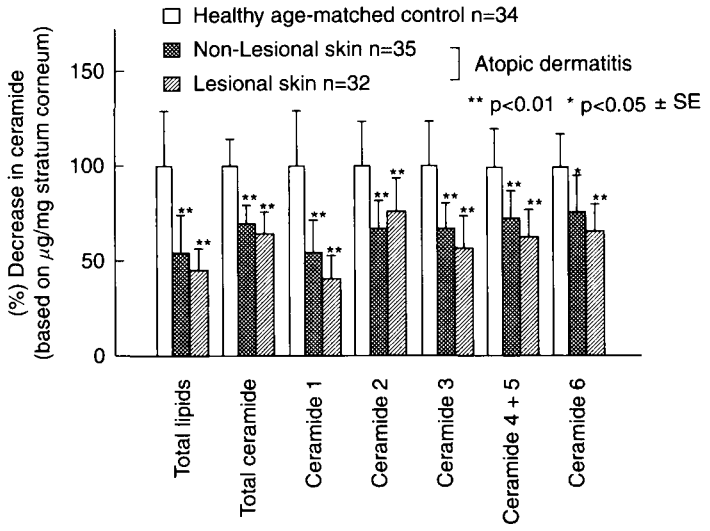
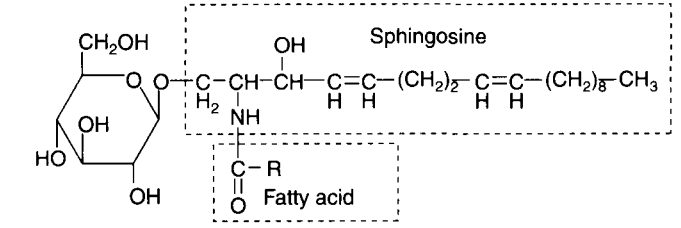


Figure 16.4 Ceramide contents in forearm skin of healthy subjects and atopic dermatitis patients [4].



R: 1. $-(CH_2)_7CH{:}CHCH_2CH{:}CH(CH_2)_4CH_3$ 2. $-(CH_2)_{14}CH_3$

3. $-(CH_2)_7CH{:}CH(CH_2)_7CH_3$ 4. $-(CH_2)_{16}CH_3$

Figure 16.5 Major glucosphingolipids in rice bran.

similar chemical structures to those from animals. Rice-derived glucosphingolipids consist of sphingoid bases conjugated with fatty acids by amide linkages. The terminal hydroxyl group is substituted to glucose. Glucosphingolipids are classified into different species depending on the structure of their sphingoid bases and fatty acids. Koga et al. identified more than 20 types of sphingolipids in rice bran [5]. We have identified four additional types of ceramides that are the major glucosphingolipids in rice bran (Fig. 16.5).

16.2.3 Digestion, Absorption, and Transport of Ceramides

Digestion, absorption, and metabolism of plant-derived sphingolipids have been studied by Schmelz et al. [6]. They examined the distribution and metabolism of sphingolipids in the intestine by tracing radio-labeled sphingomyelin in mice. Sphingomyelin appeared in all parts of the intestine, and most of it was catabolized to ceramides and their metabolites. Only 1% of undigested sphingomyelin was transferred from intestine to liver 30–60 minutes after administration. Transport of sphingomyelin and its metabolites from the intestinal canal to other tissues is thus not efficient. The absorption and metabolism of sphingolipids vary depending on the types of ceramides. Duan et al. [7] reported that sphingomyelin is digested by alkaline sphingomyelinase mainly in the middle and lower areas of the small intestine and that alkaline sphingomyelinase plays an important role in the first stage of digestion. Other researchers reported the effectiveness of oral application of ceramides for skin diseases. Kimata [8] gave ceramide (1.8 mg/day) to children with moderate atopic dermatitis for 2 weeks and confirmed that the treatment improved skin symptoms and reduced allergic responses. Asai and Miyachi [9] reported that topical application of rice ceramides for 3 weeks enhanced water contents in the stratum corneum. Thus, it is likely that at least a part of digested ceramides can be absorbed and can reach damaged skin, where they improve skin condition by retaining moisture.

16.2.4 Cosmeceutical Function of Rice Ceramide

In the past, mainly synthetic and animal-derived ceramides have been used for cosmetics. In recent years, risk of bovine spongiform encephalopathy has raised a safety concern for using cattle-derived ceramides. Consequently, more attention has focused on plant ceramides such as rice ceramide, which is considered suitable for both food and cosmetics. In this section, we review several cosmeceutical functions of rice ceramide. Most importantly, rice ceramide possesses an excellent moisturizing effect for skin, superior to that of ceramides from other plants, such as elephant foot and wheat, as demonstrated in our *in vitro* experiments (Fig. 16.6). Also, topical application of ceramide has been reported to improve skin moisturization [9,10]. Moreover, ceramide is effective in improving atopic dermatitis in animals and humans [11–13]. These observations suggest that ceramide is effective for supplementation of moisture for dry skin.

In addition, ceramide possesses an antipigmentation activity. We found that rice ceramide (100 μg/ml) inhibited melanin production in B16 melanoma

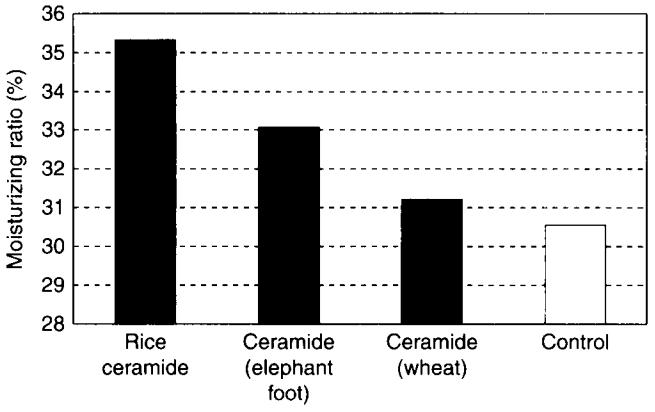


Figure 16.6 Moisturizing effect of plant-derived ceramides. Each ceramide was suspended in water at 3% solution in test tubes, and kept for 8 hours at 35°C and 4% humidity.

cells *in vitro* (Fig. 16.7[a]). At the same concentration, activity of rice ceramide was higher than that of ascorbic acid, arbutin, and ellagic acid. Similar to kojic acid, rice ceramide exhibited a similar concentration-dependent activity in inhibiting melanogenesis (Fig. 16.7[b]).

In collaboration with Professor Igarashi at Hokkaido University, we found that suppression of melanin formation by ceramides involves inhibition of tyrosinase, which is a key enzyme for melanin synthesis. As shown in Fig. 16.8, rice glucosphingolipids exhibited an inhibitory effect on tyrosinase and on melanin production in a concentration-dependent manner. In addition to the tyrosinase inhibitory activity, ceramide has been reported to affect other pathways leading to melanin production. Kim et al. [14] found that C(2)-ceramide suppressed proliferation of mouse melanocytes *in vitro* via inhibition of the Akt/protein kinase B (PKB) activation that produces phosphorylated Akt/PKB. Moreover, using human melanocytes, they found that C(2)-ceramide decreased the protein expression of microphthalmia-associated transcription factor, which is required for tyrosinase expression. They further reported that C(2)-ceramide induced delayed activation of extracellular signal–regulated protein kinase (ERK) and Akt/PKB, which may lead to suppression of cell growth and melanogenesis [15]. These findings suggest that ceramide is effective in preventing skin pigmentation.

Ceramides also enhance proliferation of dermal fibroblasts. As illustrated in Fig. 16.9, except wheat-derived ceramide, all ceramides enhanced cell

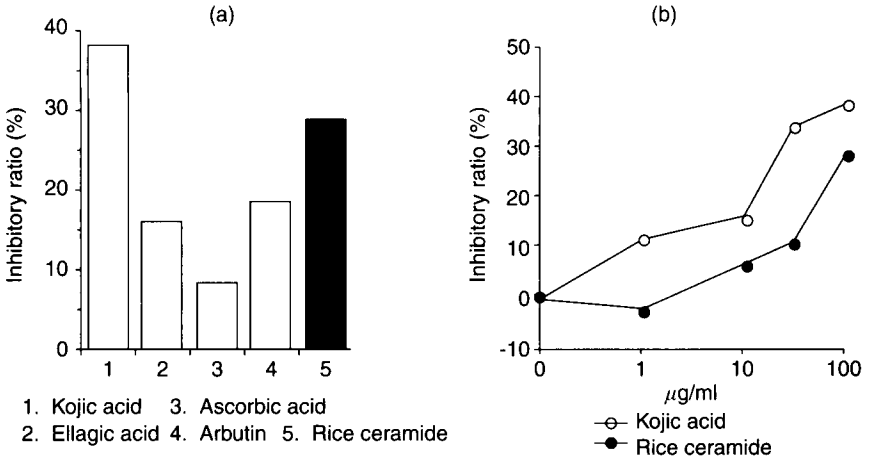


Figure 16.7 Inhibitory effect of rice ceramide on melanin production in melanoma. (a) Comparison of rice ceramide with positive control at 100 μg/ml. (b) Dose response of kojic acid and rice ceramide. B16 melanoma cells (2×10^3 cells/ml) were pre-incubated for 24 hours and the medium was replaced with new media containing 100 μg/ml emulsified glucosphingolipids (>90% of purity) or kojic acid. After 2-day incubation, the medium was replaced with fresh media, followed by another 2-day incubation. Cells were lysed in 2N NaOH, and absorbance was measured at 450 nm. The value was normalized by the cell number.

proliferation at 300 μg/ml whereas rice ceramides exhibited the most potent proliferative effect. The proliferative effect of ceramides seems to be mainly via the mitogenic activity of sphingosine-1-phosphate [16,17]. In contrast, apoptotic activity on murine keratinocytes has been reported for ceramide [18]. Marchell et al. [19] also reported that ceramide inhibits mitosis and induces terminal differentiation and apoptosis in keratinocytes. However, they also found mitogenic activity in glucosylceramide. Rice ceramides contain large amount of glucosylceramides (Fig. 16.5) and are thus expected to have an overall proliferative effect for keratinocytes.

We described in a previous section that topical and oral application of ceramides is effective against atopic dermatitis. Besides their moisturizing effect and barrier function, ceramides exhibit antiallergic and anti-inflammatory activities. We evaluated the effect of rice ceramide against itch in mice induced by compound 48/80 via mast cell degranulation histamine release in skin [20].

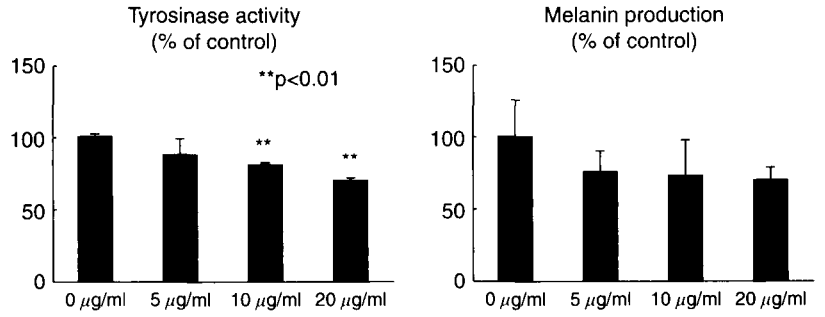


Figure 16.8 Effects of rice glucosphingolipid on tyrosinase activity (left) and melanin production (right) in melanoma. Each column represents mean with the SE. For determination of tyrosinase activity, mouse melan-a cells (1×10^4 cells/well) were incubated for 24 hours in RPMI 1640 medium containing 200 nM phorbor-12-myristate-13-acetate. The medium was replaced with new medium containing ceramide (glucosphingolipids: >95% of purity) and cultured for 2 days. Cells were lysed with PBS and the tyrosinase activity of lysate was determined using L-DOPA as a substrate. For evaluation of melanin production ability, melan-a cells (3×10^5 cells/well) were cultured with ceramide under the same culture condition described above. Cells were lysed in 1 N NaOH, and absorbance was measured at 405 nm.

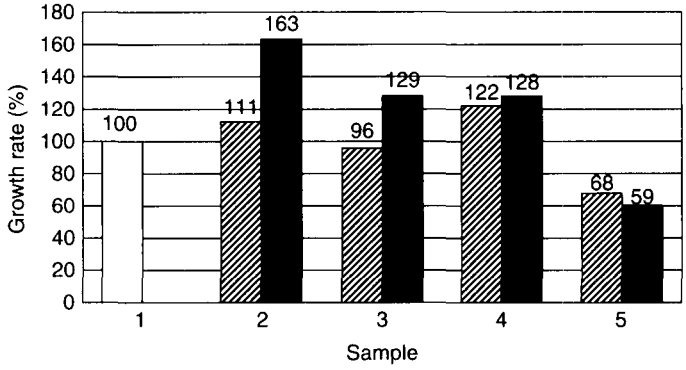


Figure 16.9 Proliferative effect of various glucosphingolipids on human dermal fibroblasts. (1) Control, (2) rice ceramide, (3) elephant foot ceramide, (4) corn ceramide, (5) wheat ceramide. All ceramides contain more than 95% glucosphingolipids. Hatched and solid column represent 100 μg/ml and 300 μg/ml, respectively. Human dermal fibroblasts (HS-K, 1×10^5 cells) were cultured with various ceramides for 72 hours. Cell growth was determined by MTT assay.

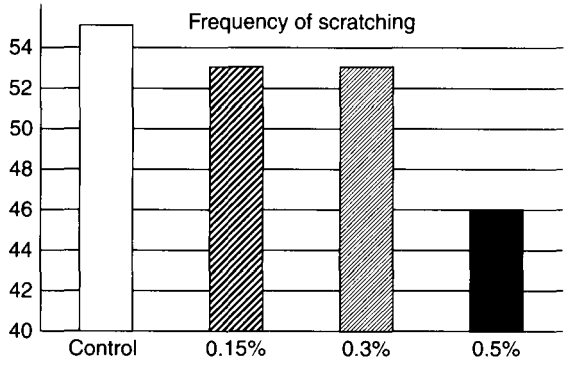


Figure 16.10 Effect of rice ceramide on scratching behavior in mice induced by compound 48/80. Mice (ddY strain, male) were fed with rice ceramide (0.15–0.5%) freely for 3 days. Compound 48/80 solution (3%) was injected into the cervical skin. The scratching action was counted for 30 minutes after they started to scratch.

Table 16.1 Inhibitory Effect of Ceramide on Degranulation from RBL-2H Cells

Origin	Inhibition (%)
Rice	87.3 ± 2.2
Wheat	82.2 ± 5.9
Elephant foot	70.8 ± 5.9
Corn	64.2 ± 5.4

Sample concentration: 1 mg/ml, mean ± S.E., n = 6.

In the model, continuous oral administration of rice ceramide decreased scratching action in mice in a dose-dependent manner (Fig. 16.10), suggesting an antiallergic effect of rice ceramide. We then examined the effect of plant-derived ceramides on degranulation in sensitized mast cells (RBL-2H3). All of the plant ceramides inhibited mast cell degranulation at 1 mg/ml, with rice ceramide being the most potent (Table 16.1). The inhibitory mechanism of ceramides on mast cell degranulation likely involves suppression of phosphorylation of ERK1/2 and p38 mitogen-activation of protein kinase [21]. In contrast, several glycosphingolipids are reported to be mast cell activators. Prieschl et al. [22] reported that galactosylsphingosine enhanced relocation of the tyrosine kinases such as Lyn and Syk, leading to tyrosine phosphorylation followed by mast cell activation. Phosphorylated ceramide (ceramide 1-phosphate) has been reported to play an important role in mast

cell degranulation [23]. Masini et al. concluded that ceramide is a pro-inflammatory agent and that reducing ceramide levels is effective against allergic disease [24]. Principal ceramides contained in plants are glucosyl-sphingolipids, which are different from ceramides in the above studies. Plant ceramides may thus not act as inducers of allergic responses. However, further investigations are required concerning pro- and antiallergic activities of various ceramides.

16.3 Other Plant Extracts as Inhibitors for Skin Component Degradation

Strength and elasticity of skin are maintained by a balance of collagen, elastin, and hyaluronic acid [25,26]. Distributed in the entire dermis of the skin, collagen constitutes 90% of the dermis [27,28]. Hyaluronic acid is widely distributed in tissues such as skin, synovial fluid, vitreous body, and ligaments [29]. This skin tissue component is involved in cellular adhesion, in cell protection, and in maintenance of moisture and flexibility of the tissue. Skin loses moisture and tension, developing wrinkles and sagging as the level of hyaluronic acid decreases. Elastin is distributed in the dermis and is essential for maintaining appropriate elasticity and strength of the skin [30]. These skin constituents are degraded by collagenase, hyaluronidase, and elastase, respectively.

We have developed several plant extracts with inhibitory activities on these enzymes (Table 16.2). Litchi seed extract is extracted from crushed seed of *Litchi chinensis* with aqueous ethanol and contains saponins [31],

Table 16.2 Inhibitory Effects of Plant Extracts on the Enzyme Activities Related to Skin Degradation

	Specification	IC_{50} (μg/ml)		
		Collagenase	Hyaluronidase	Elastase
Litchi seed extract	Polyphenols: 24%	59	290	45
Purple rice extract	Polyphenols: 30% Anthocyanins: 10%	>1,000	>2,500	180
Grape extract	Polyphenols: 40% Resveratrol: 10%	130	150	5

tannins [31], flavonoid (leucocyanidin [32]), and anthocyanins (cyanidin 3-*O*-glucoside and malvidin 3-*O*-glucoside). The biological effect of litchi seed extract has not yet been well studied. Extract of seeds and pericarps of red grapes contain polyphenols including flavonoids, anthocyanidins, and resveratrol. Purple rice extract contains anthocyanins (cyaniding 3-*O*-glucoside and malvidin 3-*O*-glucoside) as the major constituents. Whereas the former two extracts inhibit all three skin-component-degrading enzymes [33–35], purple rice extract selectively inhibits elastase.

Litchi seed extract exhibited the highest collagenase inhibitory activity (IC_{50} = 59 μg/ml), which is also higher than that of persimmon leaf extract (IC_{50} < 100 μg/ml) [36] and comparable to that of procyanidins isolated from grape (*Vitis vinifera*) seeds (IC_{50} value = 38 μM) [37].

For hyaluronidase, the extract of seeds and pericarps of red grapes was the most potent inhibitor, with an IC_{50} of 150 μg/ml. Litchi seed extract also inhibits this enzyme with an IC_{50} of 290 μg/ml. For comparison, the IC_{50} of myrrha (oleoresin from the *Commiphora mukul* tree), a traditionally natural product used for treatment of arthritis, for inhibiting hyaluronidase is as high as 1,000 μg/ml [38]. Hederagenin and oleanolic acid, sapogenins isolated from horse chestnut (*Aesculus hippocastanum*), have been reported to inhibit hyaluronidase with IC_{50} values of 280.4 μM and 300.2 μM, respectively [39]. Similar types of saponins contained in litchi seeds are likely the principal compounds for the hyaluronidase inhibitory activity. In the case of grape extract, constitutive polyphenols including flavonoids, anthocyanidins, and resveratrol seem to account for the hyaluronic inhibitory activity because this activity has been reported for flavonoid [40].

All three extracts exhibited inhibitory activity for elastase, but litchi seed extract and grape extract are extremely potent (IC_{50} value: 45 μg/ml and 5 μg/ml). For comparison, the IC_{50} of elastase-inhibitory activity in the extract from black currant (*Ribes nigrum* L.) and lady's mantle (*Alchemilla vulgaris* L.) was 560 μg/ml and 160 μg/ml, respectively, as reported by Jonadet et al. after evaluating a number of plant extracts [41–43]. Hence, each of these three extracts is likely effective for elastin stabilization in skin.

16.4 Conclusion

Plant-derived ceramides consist mainly of glucosphingolipids, which are conjugated with glucose. In contrast, skin ceramides distributed in the

stratum corneum are mainly sphingolipids. This difference in structures has been the concern for different biological functionalities of plant- and animal-derived and synthetic ceramides. We described the skin-health-promoting effect of rice ceramide in this chapter. In addition to the well-known barrier function and moisturizing effect, we found other novel activities in rice ceramide, such as inhibition of melanin synthesis, promotion of fibroblast proliferation, and anti-inflammatory and antiallergic effects. Supporting evidence for these skin-healthy effects of rice ceramide can also be obtained from a number of studies on various types of sphingolipids. Plant-derived ceramides can improve skin problems by topical and oral application. Further studies on plant ceramides are in progress that will provide more information supporting application of these skin-health-promoting components in development of new cosmetics. We also introduced several other plant extracts that inhibit collagenase, hyaluronidase, and elastase. These plant extracts are expected to be able to improve disturbed skin turnover and suppress excessive degradation of skin components. Finally, as extracts from common crops or cultivated plants, these natural materials are safe, ecological, and environment-friendly. We believe that these plant materials can be widely applied for various skin care products.

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