[Short Communication]

Potential Use of *Bischofia javanica* as an Active Ingredient of Functional Foods and Cosmeceutical Products Possessing Hyaluronidase, Collagenase, Tyrosinase and Urease Inhibitory Effects

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[ABSTRACT]

Bischofia javanica leaf extract exhibited inhibitory activities against hyaluronidase, collagenase, tyrosinase and urease, suggesting that it is useful as an active ingredient for functional foods and cosmeceutical products. In particular, its 50% inhibitory concentration for hyaluronidase was comparable to that of disodium cromoglycate, which is a well-known hyaluronidase inhibitor used as an anti-inflammation and anti-allergy agent. In addition, the extract also inhibited urease with the almost the same potential as acetohydroxamic acid, which is reported to suppress *Helicobacter pylori*-induced gastritis by inhibiting urease.

[Key words]

Hyaluronidase inhibition, Urease inhibition, *Bischofia javanica*

INTRODUCTION

The spurge family, Euphorbiaceae, is a large family of flowering plants with 326 genera and 7750 species. Many plants in the family are used for their therapeutic effects as medical plants. *Mallotus japonicus* (named Akamegashiwa in Japan) is one of well-known Chinese medicinal plants involved in the family of Euphorbiaceae, and is distributed over temperate zone and widely in the tropical zone around many south-east Asian countries. The bark of this plant, which is commercially available in Japan, is infused and used for the prevention of gastric and duodenal ulcer, gastric hyperacidity, and as a folk remedy in many Asian countries. Several published reports have demonstrated that *M. japonicus* has notable biological activities, such as anti-tumor, anti-viral, and hepatoprotective effects^{1,2}. Bergerin, a major constituent of the leaf and bark extracts of this plant, has been reported to be a physiologically active compound possessing anti-ulcer and hepatoprotective effects^{2,3}.

Like *M. japonicus*, *Bischofia javanica* is also a plant species of the family of Euphorbiaceae, and is distributed over Polynesia, Australia, and tropical zone of Asia including Okinawa. *Bischofia* leaf and bark extracts have been used as traditional folk medicine in the limited areas of Southeastern Asia and

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Polynesia^{4,5}). It has been reported that the leaf extract of this plant has anti-inflammatory and anti-microbial effects^{6,7}). However, *B. javanica*, compared with *M. japonicus*, has not yet been well characterized regarding physiological activities. To evaluate the availability of *B. javanica* as functional foods and cosmeceutical products, in this report, we have determined the inhibitory activities against hyaluronidase, collagenase, tyrosinase and urease in the leaf, bark and fruit extracts of this plant, and compared with those in the leaf and bark extracts of *M. japonicus*.

MATERIALS AND METHODS

The leaves, bark and fruits of *B. javanica*, and the leaves of *M. japonicus* were collected on September 2004 from the botanical garden, inside the campus of Osaka Prefecture University, washed with water, and stored at -80°C until use. *M. japonicus* bark was purchased from Uchida Wakan-Yaku Co. Ltd (Osaka, Japan). Plant materials were cut up and extracted with 35% (v/v) aqueous ethanol under reflux for 2 h, and then filtered through a filter paper. The filtrate was concentrated and dried *in vacuo* to give a powdery extract.

Hyaluronidase inhibitory activity was examined as described by Ono et al.⁸) with some modifications. Briefly, 160 units of hyaluronidase (from bovine testes) (Nacalai Tesque, Kyoto, Japan) was incubated with a sample (the *Bischofia* and *Mallotus* extracts) dissolved in 10 μl of 40% dimethyl sulfoxide (DMSO) and 70 μg of compound 48/80 in 0.1 M sodium acetate buffer (pH 4.0), in a total volume of 0.25 ml at 37°C for 20 min. Then, 0.275 mg of hyaluronic acid sodium salt (from rooster comb) (Sigma) dissolved in 0.25 ml of 0.1 M sodium acetate buffer (pH 4.0) was added to the enzyme solution to start the hyaluronidase reaction, and the mixture was incubated for an additional 40 min. The reaction was stopped by the addition of 0.1 ml of 1 M NaOH, and the hyaluronidase activity was estimated by measuring an increase in the *N*-acetyl-D-glucosamine residue at the reducing end by Morgan-Elson method.

Collagenase inhibitory activity was determined by using Pz-peptide (Pz-Pro-Leu-Gly-Pro-D-Arg-OH) (Bachem, Bubendorf, Switzerland) as a substrate according to the method of Henández et al.⁹) with some modifications. The reaction mixture (0.5 ml) contained 2.5 μg of collagenase (from *Clostridium histolyticum*) (Sigma), a sample (the *Bischofia* and *Mallotus* extracts) dissolved in 25 μl of 10% DMSO, 0.5 mg of Pz-peptide, 20 mM CaCl_2 , and 0.1 M Tris-HCl buffer (pH 7.1).

After incubating at 37°C for 15 min, the enzyme reaction was stopped by the addition of 25 mM citric acid (1 ml), and then the reaction product was extracted by 5 ml of ethyl acetate. The collagenase activity was estimated by measuring absorbance at 320 nm in the ethyl acetate extract.

Tyrosinase inhibitory activity was determined by using L-3,4-dihydroxyphenylalanine (L-DOPA) as a substrate according to the method of Mason and Peterson¹⁰) with some modifications. The assay mixture (1 ml) contained 5 units of tyrosinase (from mushroom) (Nacalai Tesque), a sample (the *Bischofia* and *Mallotus* extracts) dissolved in 0.1 ml of 20% DMSO, 1.25 mM L-DOPA, and 60 mM HEPES-NaOH buffer (pH 7.0). The tyrosinase activity was estimated by following an absorbance change at 475 nm due to the formation of dopaquinone at 25°C .

Urease inhibitory activity was determined according to the method of Matsubara et al.¹¹) with some modifications. The assay mixture (0.1 ml) contained 0.2 unit of urease (from jack bean) (Nacalai Tesque), a sample (the *Bischofia* and *Mallotus* extracts) dissolved in 10 μl of 20% DMSO, 12.5 mM urea, and 20 mM sodium phosphate buffer (pH 7.4). After incubating at 37°C for 10 min, the enzyme reaction was stopped by the addition of 4% trichloroacetic acid (0.6 ml). The concentration of NH_4^+ was measured by using a commercial kit (Ammonia-test Wako) (Wako Pure Chemical Industries, Osaka, Japan).

RESULTS AND DISCUSSION

The aqueous ethanolic extract was prepared from *Bischofia* leaves, bark and fruits, and from *Mallotus* leaves and bark, and their inhibitory effects against hyaluronidase (from bovine testis), collagenase (from *C. histolyticum*), tyrosinase (from mushroom) and urease (from jack bean) were examined. The inhibitory activities of these extracts were compared with respective positive controls; disodium cromoglycate for the hyaluronidase inhibition¹²), EDTA for the collagenase inhibition¹³), kojic acid for the tyrosinase inhibition¹⁴), and acetohydroxamic acid and epigallocatechin 3-*O*-gallate (EGCG) for the urease inhibition^{11,15}).

As shown in Fig. 1-A, these *Bischofia* and *Mallotus* extracts exhibited a potent inhibitory effect (over 50% inhibition) on the hyaluronidase reaction at 1 mg/ml, and the most potent effect (over 80% inhibition) was observed with the *Bischofia* leaf extract. The 50% inhibitory concentration (IC_{50}) of the *Bischofia* leaf extract was estimated to be 370 $\mu\text{g}/\text{ml}$, which was comparable to that of disodium cromoglycate (230 $\mu\text{g}/\text{ml}$;

Fig. 1 Inhibitory effects of the *Bischofia* and *Mallotus* extracts on the hyaluronidase (A), collagenase (B), tyrosinase (C) and urease (D) reactions. The *Bischofia* and *Mallotus* extracts were added at 1 mg/ml in the hyaluronidase and collagenase reactions and at 0.1 mg/ml in the tyrosinase and urease reactions. L, B and F mean leaves, bark and fruits, respectively.

450 μ M). It is thus indicated that the *Bischofia* leaf extract inhibits hyaluronidase with almost the same extent as the well-known inhibitor, disodium cromoglycate, which is used for an anti-inflammatory and anti-allergy agent¹².

These *Bischofia* and *Mallotus* extracts also exhibited a significant inhibitory effect (30–70% inhibition) on the collagenase reaction at 1 mg/ml (Fig. 1-B). Among them, the *Bischofia* leaf extract was the strongest inhibitor, and its IC_{50} value was estimated to be 590 μ g/ml; this value was about 3.5-fold as that of EDTA (170 μ g/ml; 580 μ M).

The tyrosinase activity was inhibited about 50% by the *Bischofia* leaf extract at 0.1 mg/ml (Fig. 1-C), although the inhibitory effect of the extract (IC_{50} : 140 μ g/ml) was considerably weaker than that of kojic acid (IC_{50} : 3.8 μ g/ml; 27 μ M). The tyrosinase inhibitory effect was also observed in the *Bischofia* bark and fruit extracts, but the *Mallotus* extracts (both leaf and bark) were virtually inactive.

A strong inhibitory effect (70–90% inhibition) against urease was observed either with the *Bischofia* leaf, bark or fruit extract at 0.1 mg/ml (Fig. 1-D). The IC_{50} value of the leaf extract was estimated to be 4.9 μ g/ml; this value was comparable to or lower than those of acetohydroxamic acid (2.8 μ g/ml; 37 μ M) and EGCG (87 μ g/ml; 190 μ M). The *Mallotus* leaf and bark extracts also inhibited the urease reaction, but the inhibitory effects were considerably weaker than those of *Bischofia* extracts.

These results demonstrate that the extract of *B. javanica*, in particular the leaf extract, exhibits a potent inhibitory activity against hyaluronidase, collagenase, tyrosinase and urease, and these inhibitory activities of the *Bischofia* leaf extract are greater when compared with those observed in the leaf and bark extracts of *M. japonicus*, which is well-known as a Chinese medical plant.

Hyaluronidase, which decomposes hyaluronic acid, induces histamine release from mast cells during inflammation reactions, and some hyaluronidase inhibitors, including disodium cromoglycate, have been used as an anti-inflammation and anti-allergy agent^{12,16}. Thus, the inhibitory effect on hyaluronidase demonstrated by the *Bischofia* leaf extract substantiates its potential use to suppress inflammation and allergies. Indeed, Lingadurai et al. reported that the leaf extract exhibited anti-inflammatory effects against acute carrageenan-induced paw edema, sub-chronic cotton pellet-induced granuloma, and Freund's adjuvant-induced arthritis in rats⁷.

Hyaluronic acid and collagen are found in the extracellular matrix in the skin, and hyaluronidase and collagenase play important roles in the turnover and remodeling of the skin^{17,18}. When the level of hyaluronic acid decreases, the moisture and tension of the skin are reduced¹⁷. The collagen content in the skin decreases greatly during the aging process and due to long-term exposure to ultraviolet radiation^{19,20}. Tyrosinase catalyzes the transformation of tyrosine to dopaquinone, and is responsible for melanization in animals, which leads to the coloring of the skin²¹. Thus, the *Bischofia* leaf extract with the hyaluronidase, collagenase and tyrosinase inhibitory effects is thought to be a useful cosmeceutical ingredient as they have anti-wrinkle, anti-aging and whitening effects on the skin.

Helicobacter pylori colonizes the mucous layer of the gastric epithelium, and is thought to cause gastritis, peptic ulcer disease, gastric adenocarcinoma, and gastric lymphoma²². Most patients, specifically with peptic ulcer disease, can be cured by killing *H. pylori*²³. *H. pylori* is able to survive in the stomach by releasing urease which converts urea into ammonia that neutralizes the stomach acid. Thus, the *Bischofia* leaf extract with a potent urease inhibitory activity is thought to be useful as a therapeutic agent for *H. pylori*-related disease. Indeed, other urease inhibitors, including acetohydroxamic acid and EGCG, have been reported to suppress *H. pylori*-induced gastritis in Mongolian gerbils^{11,15}.

In summary, data obtained in the present paper show that the *Bischofia* leaf extract has potential as a novel ingredient for use in a wide range of functional foods and cosmeceutical prod-

ucts, although it remains to elucidate whether the extract is effective *in vivo*.

GRANT

None

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要 旨

ヒアルロニダーゼ，コラゲナーゼ，チロシナーゼ，ウレアーゼ阻害活性を有する機能性食品素材や薬用化粧品の活性成分としてのアカギの有用性について

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アカギ葉抽出物にはヒアルロニダーゼ，コラゲナーゼ，チロシナーゼ，ウレアーゼに対する阻害活性が存在し，機能性食品や薬用化粧品の活性成分としての有用性が示唆された．特に，ヒアルロニダーゼとウレアーゼにはそれぞれの強い阻害剤であるジソジウムクロモグリケートとアセトヒドロキサム酸と同等の阻害が見られた．

キーワード：ヒアルロニダーゼ阻害，ウレアーゼ阻害，アカギ (*Bischofia javanica*)