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Original article

Evaluation of effectiveness of *Prunus mume* extract such as anti-glycation

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Abstract

***Subjective*:** Since the skin is constantly exposed to the external environment, it is susceptible to environmental factors. This may cause morphological changes due to skin aging, and the apparent age may occasionally exceed the chronological age. In the glycation reaction, a risk factor of skin aging, abnormal crosslinks are formed in collagen and elastin in the dermis, resulting in reduction of flexibility and elasticity. It is useful to impart an anti-glycation effect to cosmetics from the viewpoint of preventing skin aging. In this study, to develop a cosmetic material targeting inhibition of glycation reactions, we examined the approach by ume (*Prunus mume*), which is generally used in foods and health foods.

***Methods*:** The test samples were prepared by extracting from each site including the shoots (young branches), flowers, branches, leaves, bark, and roots of Ume, the two varieties of Bungo and Shirakaga. Using this extracts, we verified the activities of SOD (superoxide dismutase)-like action, DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging, elastase inhibition, and Maillard reaction crosslinking inhibition.

***Results*:** The effects of elastase inhibiting, anti-oxidant, and anti-glycation were observed in ume root extracts of both varieties; Bungo and Shirakaga.

***Conclusion*:** It has been found that the beneficial effect is on the plum root, of which extracts can be expected to be a useful raw material for preventing skin aging.

***KEY WORDS*:** ume (Japanese apricot; *Prunus mume*), Maillard reaction, anti-oxdation, aging care, skin, root

Introduction

The skin is located in the outermost layer of human and is the largest organ in the body that covers the internal organs. The skin protects the inside of the body from any external environment such as invasion of foreign matter, and thus plays a role in maintaining homeostasis. To exert these functions, skin is composed of various cells and has a complex structure equipped with a variety of appendages [1]. However, their functions decline with age and changes occur in each tissue.

With age, the skin loses its ability to retain water. Besides, due to the decreased ability of regeneration and sebum secretion, aging phenomena appear in the appearance induced by drying and roughening [2].

Glycation reaction is one of the factors that accelerate skin aging. Glycation, also called Maillard reaction, has recently attracted attention as it is an important factor for aging care [3]. Once glycation reaction produces advanced glycation end products (AGEs), protein molecules lose flexibility and become sclerotic. Skin aging caused by glycation also affects the appearance, resulting that the apparent age may exceed the chronological age [4].

The appearance characteristics of aged skin include changes of color tone changes, *i.e.* spots (senile pigment spots) and dullness, and morphological changes, *i.e.* wrinkles, sagging.

This morphological change is considered to be due to structural changes in the skin dermis, which are caused by the decrease, degradation, or deterioration of collagen and elastin, the main components of the dermis [5]. Collagen and elastin form crosslinks via lysine and hydroxylysine residues during the process of fibrogenesis, thus forming a three-dimensional structure and maintaining the skin flexibility.

The amino group of these lysine residues or the guanidyl group of arginine residues non-enzymatically reacts with the carbonyl group of reducing sugar [6]. In the early stage of this non-enzymatic reaction, the reaction product is dehydrated

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to produce a Schiff base. Furthermore, intramolecular rearrangement occurs, thus forming Amadori rearrangement products [7]. In the late stage, amino compounds and carbonyl compounds react with Amadori products to form highly reactive dicarbonyl compounds, *i.e.* 3-deoxyglucosone [6, 7]. AGEs are produced in the late stages of the reaction and form abnormal crosslinks in the skin dermal tissue. The generation of AGEs in skin tissues influences the color tone and morphological changes of the skin, which causes the apparent age to exceed the chronological age.

Generation of AGEs by glycation involves oxidative reactions. Oxidation of sugars and Amadori compounds in the late stages produces compounds such as 3-deoxyglucosone, glyoxal, and methylglyoxal [8, 9]. In this process, O_2^- (superoxide anion radical), one of reactive oxygen species (ROS), is generated [10, 11]. ROS are physiologically active substances, and concurrently, once excessively produced, exert harmful effects on the body known as oxidative stress [12, 13].

Further, when abnormal cross-linking of collagen or elastin is formed by glycation, the cross-linked products are judged to be foreign substances, therefore increasing secretion of degrading enzymes, *i.e.* collagenase, elastase, thus resulting that normal collagen and elastin become easily decomposed [6]. It has been reported that skin aging due to glycatiin is likely to affect tissues that contain abundant proteins with long half-lives, *i.e.* collagen, elastin [14, 15].

Ume (Japanese apricot; *Prunus mume*) is a deciduous tree belonging to the family *Rosaceae* the genus *Prunus*, and is widely cultivated in East and Southeast Asian regions including China, Japan, South Korea, Taiwan, Thailand, and Vietnam. Its fruit has a long history of being used as an ornamental, edible, and traditional Chinese medicine since ancient times, and is rich in organic acids, *i.e.* citric acid [16].

In this study, for the purpose to develop a cosmetic material targeting the prevention of skin aging, by focusing on the flowers, roots, and branches of ume, which are widely used in foods and health foods, we elucidated the effects of anti-glycation, anti-oxidation and the inhibition effects on elastase activity of these extracts.

Methods

Sample preparation

For the two varieties of ume, Bungo and Shirakaga, each site of the shoots (young branches), flowers, branches, leaves, bark, and roots, adjusted their contents at 5%, was immersed in purified water or 95% ethanol as extractants, and the extraction was performed at 30°C for 3 days. Each extract obtained was filtered to remove insoluble matters and used as a test sample. Further, the samples confirmed to be effective were subjected to vacuum concentration and lyophilization for the additional test.

Evaluation for superoxide dismutase (SOD)-like activity

The SOD-like activity was measured according to previous reports [17, 18]. To a 96 well plate, 25 µL of test sample at the concentration pre-adjusted with purified water, 25 µL of 2 mM hypoxanthine (Sigma, St. Louis, MO, USA), 25 µL of 2.0 mM ethylenediaminetetraacetic acid (EDTA; Dojindo, Kumamoto, Japan), 25 µL of 0.5 mM nitro blue tetrazolium (NBT; Wako, Osaka, Japan), 100 µL of 0.1 M carbonate buffer, and 50 µL of 30 mU/mL SOD (Sigma) were added and then incubated at 37°C for 30 minutes, followed by measuring absorbance at 570 nm with a microplate reader. The scavenging rate was calculated using purified water as a control.

Evaluation for 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging effect

DPPH radical scavenging activity was evaluated according to previous reports [19, 20]. To the 96 well plate, 15 µL of test sample at the concentration pre-adjusted using purified water, 60 µL of 0.2 M 2-(N-morpholino)ethanesulfonic acid (MES) buffer, 50 µL of ethanol, and 25 µL of 0.6 mM DPPH radical (Wako) were added and then incubated at 30°C for 30 minutes, followed by measuring absorbance at 540 nm with a microplate reader. For the DPPH radical scavenging activity, the scavenging rate was calculated using purified water as a control.

Evaluation for elastase inhibition activity

The elastase inhibition activity was evaluated according to previous reports [21]. To the 96 well plate, 50 µL of test sample at the concentration pre-adjusted with purified water, 35 µL of 0.1 M Tris-HCl buffer, 100 µL of 1 mM Suc-Ala-Ala-Ala-PNA (Sigma), and 15 µL of 0.25 U/mL elastase (Wako) was added and incubated at 37°C for 30 minutes, followed by measuring absorbance at 405 nm with a microplate reader. The inhibitory rate was calculated using purified water as a control.

Inhibition tests on a Maillard reaction-induced crosslinking

The Maillard crosslinking inhibition test was evaluated according to a previous report [22, 23]. Into sample tubes, 40 µL of 0.5 M ribose (Wako) prepared with 800 µL of 0.1 M phosphate buffer (pH 7.4), 100 µL of 50 mg/mL lysozyme (Wako), and add 60 µL of test sample at the concentration pre-adjusted using purified water were added and then sterilized by filtration using a 0.45 µm filter in a clean bench, followed by incubation at 40°C for 7 days in an incubator. After the reaction, electrophoresis was performed by SDS-PAGE, stained with Coomassie Brilliant Blue R-250, and after decolorization, the dimer of lysozyme was visually determined. For judgement, the state when aminoguanidine sulfate was added was determined as a positive control and the other in which purified water was used instead of the test sample was used as a negative control. The band intensity of electrophoresis gel was quantified by performing image analysis on the same area using NIS-Elements (Nikon, Tokyo, Japan).

Results

Regarding two ume cultivars, Bungo and Shirakaga, each of the six sites was extracted with water and ethanol (***Figs. 1, 2***). The appearance, such as the color of the extract, differed depending on the variety, extractant, and site, that indicates the presence of difference in what was extracted. As a result of the examination, the anti-oxidant effect (SOD-like activity, DPPH radical scavenging activity) and the elastase inhibition activity were confirmed in the extracts from shoots (young branches) and roots (***Tables 1, 2***).

Among the ume extract liquids, the root-derived extract was found to have higher anti-oxidant activity and elastase inhibition activity than others. Particularly in the ethanol extract of ume roots, IC_{50} (the concentration of the sample in which 50% inhibition is shown) for active oxygen scavenging activity was less than 0.1%. Therefore, it was confirmed that the root extract has a higher anti-oxidant effect than other sites.

In addition, it was noted that the root ethanol extract had an elastase inhibition activity with IC_{50} values being 0.45% in Bungo and 0.24% in Shirakaga, which is lower compared to other sites.

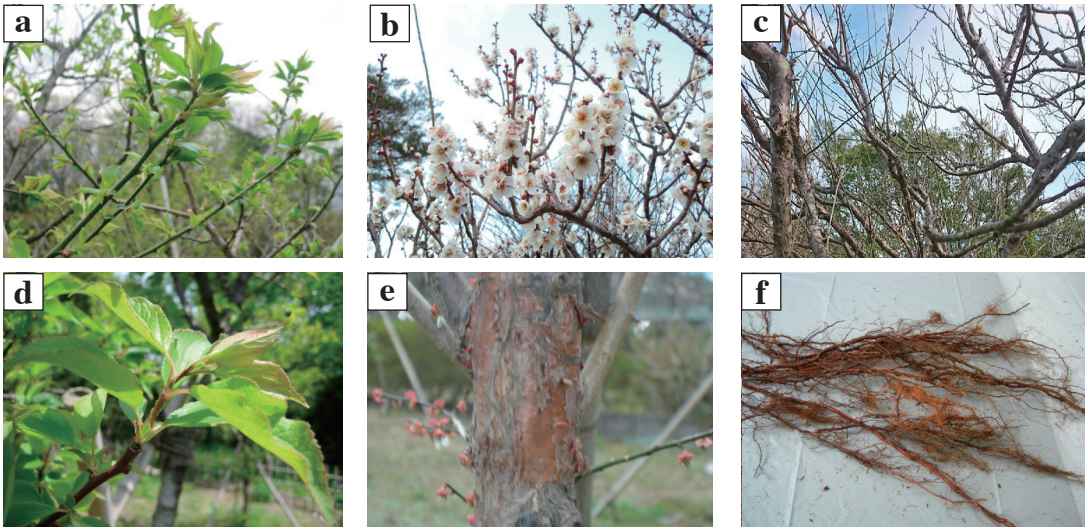

Fig. 1. **Photographs of each site of ume (*Prunus mume*).**
a) Shoots (young branches). **b)** Flowers. **c)** Branches. **d)** Leaves. **e)** Bark. **f)** Roots.

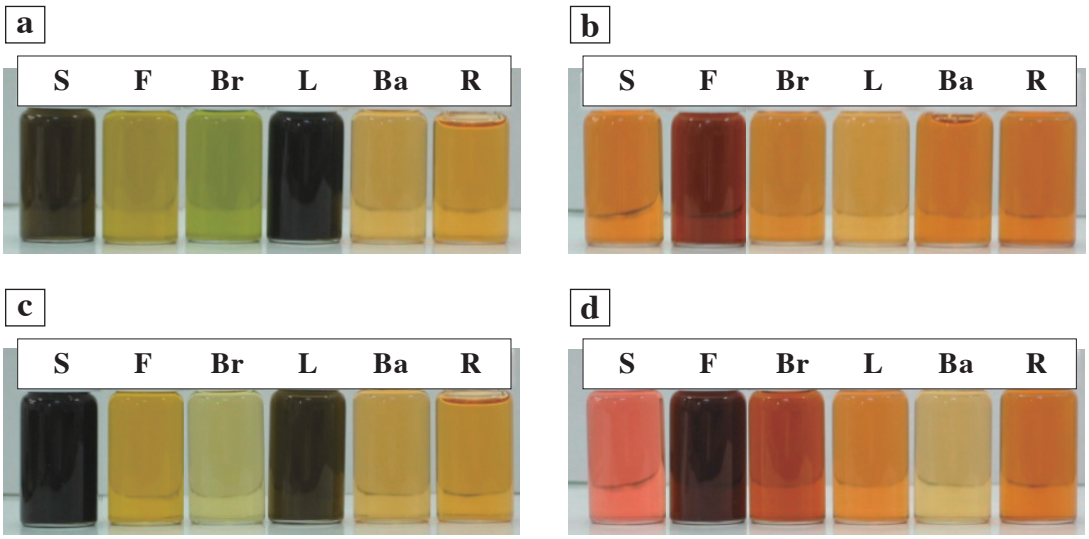

Fig. 2. **The appearance of 5% (w/w) extracts of each site by ume variety.**
a) Extract with ethanol / Shirakaga. **b)** Extract with water / Shirakaga. **c)** Extract with ethanol / Bungo. **d)** Extract with water / Bungo. S, shoots (young branches) ; F, flowers; Br, branches; L, leaves; Ba, bark; R, roots.

Table 1. SOD-like activity and DPPH radical scavenging effect.

Ume varieties	Extractants	Sites	SOD-like activity		DPPH scavenging	
			Effects	IC_{50}	Effects	IC_{50}
Bungo	Water	Shoots	84%	**0.05%**	88%	**0.04%**
Bungo	Ethanol	Shoots	62%	**0.14%**	70%	**0.08%**
Shirakaga	Water	Shoots	74%	**0.09%**	63%	**0.11%**
Shirakaga	Ethanol	Shoots	37%	> 0.2%	46%	> 0.2%
Bungo	Water	Flowers	34%	> 0.2%	54%	0.20%
Bungo	Ethanol	Flowers	−11%	> 0.2%	−13%	> 0.2%
Shirakaga	Water	Flowers	41%	> 0.2%	53%	0.20%
Shirakaga	Ethanol	Flowers	−31%	> 0.2%	13%	> 0.2%
Bungo	Water	Branches	4%	> 0.2%	−42%	> 0.2%
Bungo	Ethanol	Branches	14%	> 0.2%	−48%	> 0.2%
Shirakaga	Water	Branches	11%	> 0.2%	−42%	> 0.2%
Shirakaga	Ethanol	Branches	11%	> 0.2%	−16%	> 0.2%
Bungo	Water	Leaves	74%	0.08%	83%	0.11%
Bungo	Ethanol	Leaves	1%	> 0.2%	−17%	> 0.2%
Shirakaga	Water	Leaves	38%	> 0.2%	−3%	> 0.2%
Shirakaga	Ethanol	Leaves	−3%	> 0.2%	−27%	> 0.2%
Bungo	Water	Bark	−3%	> 0.2%	−55%	> 0.2%
Bungo	Ethanol	Bark	9%	> 0.2%	−54%	> 0.2%
Shirakaga	Water	Bark	3%	> 0.2%	−23%	> 0.2%
Shirakaga	Ethanol	Bark	29%	> 0.2%	−10%	> 0.2%
Bungo	Water	Roots	49%	**0.2%**	12%	> 0.2%
Bungo	Ethanol	Roots	75%	**0.06%**	77%	**0.10%**
Shirakaga	Water	Roots	37%	> 0.2%	17%	> 0.2%
Shirakaga	Ethanol	Roots	77%	**0.07%**	83%	**0.08%**

All tests were performed at 0.2% extract concentration. IC_{50} value indicates the concentration that inhibits the SOD-like activity or that eliminates the generated active oxygen by 50%. SOD, superoxide dismutase; DPPH, 2,2-Diphenyl-1-picrylhydrazyl; Shoots, young branches.

Table 2. Elastase activity inhibitory effect.

Ume varieties	Extractants	Sites	Elastase inhibition	
			Effects (2.5%)	IC_{50}
Bungo	Water	Shoots	−8%	> 2.5%
Bungo	Ethanol	Shoots	2%	> 2.5%
Shirakaga	Water	Shoots	−24%	> 2.5%
Shirakaga	Ethanol	Shoots	9%	> 2.5%
Bungo	Water	Flowers	−13%	> 2.5%
Bungo	Ethanol	Flowers	−43%	> 2.5%
Shirakaga	Water	Flowers	−9%	> 2.5%
Shirakaga	Ethanol	Flowers	−18%	> 2.5%
Bungo	Water	Branches	69%	1.10%
Bungo	Ethanol	Branches	43%	2.53%
Shirakaga	Water	Branches	−7%	> 2.5%
Shirakaga	Ethanol	Branches	53%	2.21%
Bungo	Water	Leaves	−35%	> 2.5%
Bungo	Ethanol	Leaves	34%	4.47%
Shirakaga	Water	Leaves	−22%	> 2.5%
Shirakaga	Ethanol	Leaves	−2%	> 2.5%
Bungo	Water	Bark	11%	> 2.5%
Bungo	Ethanol	Bark	43%	3.74%
Shirakaga	Water	Bark	77%	0.49%
Shirakaga	Ethanol	Bark	51%	2.13%
Bungo	Water	Roots	53%	**2.35%**
Bungo	Ethanol	Roots	84%	**0.45%**
Shirakaga	Water	Roots	66%	**1.32%**
Shirakaga	Ethanol	Roots	83%	**0.24%**

All tests were performed at 2.5% extract concentration. IC_{50} value indicates the concentration that inhibits the elastase activity by 50%; Shoots, young branches.

Moreover, it was confirmed that the extract liquid of ume shoots had an anti-oxidant effect with IC_{50} values for active oxygen scavenging activity being less than 0.14% or less except that of Shirakaga.

Therefore, the root extracts from the root and shoots, confirmed to be effective, were treated by the vacuum concentration and freeze-dried to obtain samples of the dry extracts, of which the effectiveness was then examined. In order to verify the inhibitory effect of glycative stress, one of the factors of skin aging, a Maillard crosslinking inhibition test was conducted. The test was performed by adjusting the roots dry extract to 0.15 mg/mL with 95% ethanol, and the results are shown in ***Fig. 3***. Also, integrated values of the optical density were calculated from the electrophoretic image (***Table 3***).

From the above measurements, the root extract was confirmed to have an anti-oxidant effect and an elastase inhibition activity, and its dry extract showed a Maillard crosslinking inhibition activity.

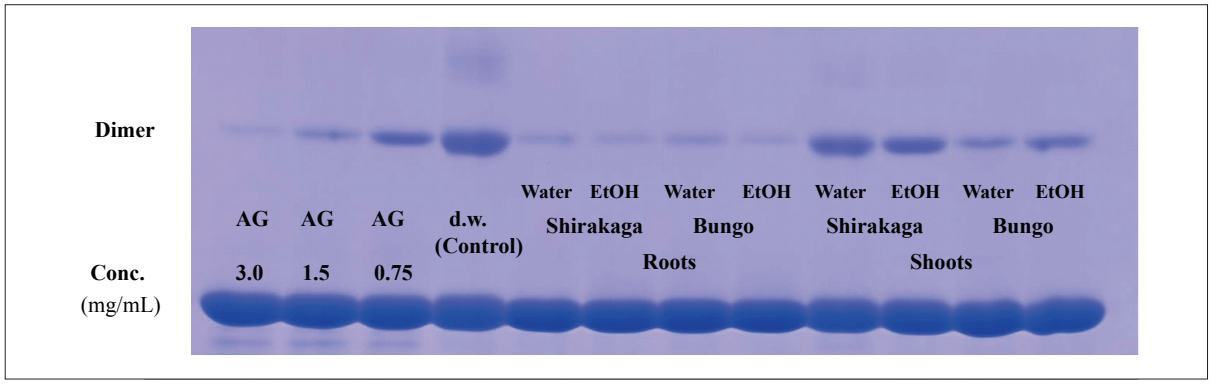

Fig. 3. **The electrophoresis gel image in the inhibition test of a Maillard reaction crosslinking by ume extract.**

Lysozyme dimers are formed by the Maillard reaction-induced crosslinking. Sample No.1-3, aminoguanidine sulfate (AG) as positive control, concentrations (Conc.) at 3.0, 1.5, 0.75 mg/mL, respectively; No.4, distilled water (d.w.) as control; No.5-8, root extracts; No.9-12, shoot (young branches) extracts. Two varieties, Shirakaga (No.5, 6, 9, 10) and Bungo (No.7, 8, 11, 12), were tested. Extractants were water in No. 5, 7, 9, 11 or 95% ethanol (EtOH) in No.6, 8. 10, 12.

Table 3. The optical band intensity of electrophoresis gel images in the inhibition test of a Maillard reaction crosslinking by ume extract.

The sample number in Fig. 3	Samples	Sites	Concentration (mg/mL)	Integrated values of the optical density
1	Aminoguanidine sulfate	Water	3.0	542.2 ± 7.5
2			1.5	604.2 ± 6.4
3			0.75	681.8 ± 6.4
4	Purified water	—	—	881.3 ± 7.3
5	Roots (Shirakaga)	Water	0.15	583.0 ± 3.4
6		95% ethanol	0.15	567.3 ± 6.6
7	Roots (Bungo)	Water	0.15	587.4 ± 6.5
8		95% ethanol	0.15	555.6 ± 6.8
9	Shoots (Shirakaga)	Water	0.15	768.6 ± 0.3
10		95% ethanol	0.15	733.1 ± 6.5
11	Shoots (Bungo)	Water	0.15	630.1 ± 0.3
12		95% ethanol	0.15	659.0 ± 5.4

Results are expressed as mean ± standard deviation by repeated measurement (n = 3). The optical band intensity of electrophoresis gel is quantified by using NIS-Elements (Nikon, Tokyo, Japan). Shoots, young branches.

Discussion

Regarding the efficacy of ume, it has been revealed that phenolic compounds prepared from ume vinegar, a by-product when making salting ume fruit (ume-boshi; salted ume), have anti-bacterial activity against intestinal bacteria [24]. Besides, the anti-viral effects of HSV-1 and HSV-2 have been confirmed in ume products [25].

Ume has been confirmed to be effective in clinical trials. In the comparison study of oral ammonia concentration between the group in which saliva secretion was induced by ume extracts and the group without them, ammonia concentration rise was reported to be suppressed to about half [26]. This suggests that ume extract can be an effective method for maintaining the oral hygiene. It has also been reported that substances such as mumeose which is isolated and purified from ume flower buds, as well as fruits, inhibit aldose reductase [27]. This aldose reductase has a function of converting surplus glucose, *i.e.* generated in diabetes, into sorbitol and fructose (polyol metabolism), thus the accumulation of sorbitol causes neuropathy. On the contrary, the inhibition of aldose reductase can be expected to moderate the neuropathy.

As described above, the Maillard reaction has a non-oxidative reaction route and an oxidative reaction route. Concurrently through these two routes, the reaction proceeds. It remains unclear which reaction pathway the root extract is involved in, however, it can be inferred that the inhibition of O_2^--activity is involved in the anti-glycation effect since a high anti-oxidant effect was observed in the root extract.

It is already mentioned that abnormal cross-linking of collagen and elastin increases the secretion of degrading enzymes. Also the root extract had an inhibitory effect on elastase as well as on the crosslinking formation by the Maillard reaction. Therefore, ume extract is expected to be a useful raw material for suppressing skin aging such as wrinkles, sagging and dullness which are caused by reduction of skin firmness and elasticity.

Conclusion

The effectiveness of each site of shoots, flowers, branches, leaves, bark, and roots was evaluated for two Ume varieties of Bungo and Shirakaga. Among them, the anti-oxidant effect (SOD-like effect, DPPH radical scavenging effect) and elastase inhibition activity were confirmed in the root extract. Additionally, the root extract was confirmed to have a Maillard crosslinking inhibition activity. From these findings, the ume root extracts are expected to have an inhibitory effect on the manifestation of senescence due to glycative stress. Furthermore, the results suggest that that ume root could be a useful raw material for cosmetics with various effects. We are planning to further study, among various ingredients contained in ume roots, which ingredient the confirmed effect is derived from.

Conflict of Interest Statement

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