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Inhibition of Advanced Glycation End Products Formation by *Mangifera indica* Leaf Extract

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Abstract

The purpose of this study was to examine an inhibitory effect of mango leaf extracts on advanced glycation end products (AGEs) formation and to identify these active ingredients, and also to investigate a relationship between leaves maturation and the inhibitory activity. A methanolic extract of old dark green mango leaf extract (OML-ext) exhibited an inhibitory activity of AGEs formation in nonenzymatic glycation of albumin. The inhibitory activity of OML-ext was attributable to 3-C-*β*-D-glucosyl-2,4,4',6-tetrahydroxybenzophenone (**1**), mangiferin (**2**) and chlorophyll. Inhibitory effect of young dark reddish brown mango leaf extract (YDL-ext) on AGEs formation was similar to that of OML-ext. The inhibitory activity of YDL-ext was attributable to **1** and **2**, in addition, a part of the the activity of YDL-ext due to anthocyanins whose content is highest in young dark reddish brown mango leaves. Considering the amounts of leaves obtained from pruning, old dark green leaves may be a reasonable natural resource for the preparation of ingredients with inhibitory activity of AGEs formation.

Keywords: advanced glycation end products (AGEs) formation inhibition, anthocyanin, chlorophyll, 3-C-*β*-D-glucosyl-2,4,4',6-tetrahydroxybenzophenone, glycation, *Mangifera indica*, mangiferin

1. Introduction

Glycation is a non-enzymatic browning reaction caused by amino-carbonyl reactions between reducing sugars and amino groups of proteins and lipids (Tsuji-Naito, Saeki, & Hamano, 2009). By glycation of these compounds, advanced glycation end products (AGEs) are irreversibly synthesized in the body (Huebschmann, Regensteiner, Vlassara, & Reusch, 2006), and the accumulation of AGEs in organs is induced by hyperglycemia and is one of the causes of diabetic complications (Sourris, Harcourt, & Forbes, 2009). Moreover, AGEs accumulate in the skin of non-diabetics and are correlated with skin aging (Dyer et al., 1993). The AGEs accumulation in the skin induce cross-linking of collagen and reduce skin degradability and dermal regeneration (Wondrak, Roberts, Jacobson, & Jacobson, 2002). In addition, AGEs induce fibroblast apoptosis by adducting to AGE receptors on the cell (Pageon, Bakala, Monnier, & Asselineau, 2007). These phenomena are also thought to be related to skin aging (Lohwasser, Neureiter, Weigle, Kirchner, & Schuppan, 2006). Therefore, in recent years, the role of AGEs has been increasingly discussed in the skin aging, and the inhibition of AGEs formation can be one of the effective strategies for direct alleviation of the development of novel antiaging cosmeceutical ingredients.

An AGEs formation inhibitor, namely aminoguanidine (Pimagedine®) (Dyer et al., 1993) was under development in U.S.A. as a drug for the treatment of diabetic complications such as diabetic nephropathy, however the clinical trial on aminoguanidine has been discontinued due to adverse reactions such as anemia, liver injury and vitamin B6 deficiency. In order to find new and safe AGEs formation inhibitors from natural resources, pharmacological screening of plant is considered as one of strategies. Hitherto, several plant, such as *Thymus vulgaris* whole grass (Morimitsu, Yoshida, Esaki, & Hirota, 1995), *Chrysanthemum morifolium* and

Chrysanthemum indicum corolla (Tsuji-Naito et al., 2009), *Alpinia zerumbet* rhizomes (Chompoo, Upadhyay, Kishimoto, Makise, & Tawata, 2011), *Derris indica* stem bark (Anusiri, Choodej, Chumriang, Adisakwattana, & Pudhom, 2014) and *Ribes nigrum* fruit (Chen et al., 2014, Xu et al., 2016), have been reported to have AGEs formation inhibitory activity.

In our preceding paper (Itoh et al., 2016), we reported that mango (*Mangifera indica* Linne) leaf extracts exhibited pancreatic lipase inhibitory activities, and a part of the activity of leaf extract was attributable to C-glucosyl-polyphenols, such as 3-C-*β*-D-glucosyl-2,4,4',6-tetrahydroxybenzophenone (**1**) and mangiferin (**2**), and that dark green mango leaf which was obtained by summer pruning may be a reasonable natural resource for the preparation of ingredients with lipase inhibitory activity. For finding another utility value of pruned mango leaves, we examined the inhibitory effects of mango leaf extracts on AGEs formation. In this paper, we report AGEs formation inhibitory activity of mango leaf extracts, and also discuss a relationship between leaves maturation and the inhibitory activity.

2. Materials and Methods

2.1 Plant Materials

Three kinds of Mango leaves (old dark green leaf, young dark reddish brown leaf, and young yellow leaf, Figure 1) of *M. indica* (cv. Irwin) were collected according to the preceding paper (Itoh et al., 2016). In order to describe accurately the color of young mango leaves, we describe the color of the leaves as dark reddish brown in this paper instead of dark brown in the preceding paper (Itoh et al., 2016).

1-a; Young dark reddish brown leaves 1-b; Young yellow leaves 1-c; Old dark green leaves

Figure 1. Photographs of typical mango leaves at various stages of development

2.2 Extraction

Methanolic extracts of young dark reddish brown leaves (this extract is abbreviated as YDL-ext throughout this paper), young yellow leaves (YYL-ext), and old dark green leaves (OML-ext) were obtained according to the preceding paper (Itoh et al., 2016).

2.3 Reagents

Aminoguanidine hydrochloride (Lot #: MKCB3580), Bovine serum albumin (BSA, fraction V, Lot #: SLBQ4710V) and authentic **1** and **2** were purchased from Sigma-Aldrich Japan (Tokyo, Japan). Authentic chlorophyll was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Other chemical and biochemical reagents were of reagent grade and were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and/or Nacalai Tesque, Inc. (Kyoto, Japan) unless otherwise noted.

2.4 In vitro AGEs Formation Inhibition Assay by Incubation of Glucose and Albumin

AGE formation activity was measured according to the method of Shimoda et al. (2011) with minor modification. The test sample was dissolved with dimethyl sulfoxide (DMSO) and diluted with sodium phosphate buffer (PBS, 0.2 M KH_2PO_4, 0.2 M NaOH, pH 7.2) to a final DMSO concentration of 5% v/v. The reaction mixture of glucose (10% w/v) and bovine serum albumin (BSA, 1% w/v) dissolved in PBS (900 μl) was incubated for 48 h at 60℃ in microtube (2 ml) with or without a test solution. After incubation, the reaction mixture was diluted with distilled water (1:7 v/v), the fluorescence (***F***) associated with AGEs was monitored at an excitation wavelength of 370 nm and emission of 450 nm using a multi-label counter (PerkinElmer 2030 ARVO X4，PerkinElmer Life and Analytical Sciences). Aminoguanidine hydrochloride was used as a reference agent. The inhibitory ratio of the sample was calculated using the following formula:

$$\% \text{ inhibition} = 1 - [(\boldsymbol{F} \text{ sample} - \boldsymbol{F} \text{ sample blank}) / (\boldsymbol{F} \text{ control} - \boldsymbol{F} \text{ normal})] \times 100$$

where ***F*** control is the fluorescence with PBS containing glucose and BSA; ***F*** normal is the fluorescence with PBS containing glucose and BSA without incubation (stored at 4 ℃); ***F*** sample is the fluorescence with sample solution in PBS containing glucose and BSA, ***F*** sample is blank the fluorescence with sample solution in PBS.

Each assay was performed in triplicate (P value < 0.01). IC_{50} value represents the concentration required to inhibit 50% of AGEs formation by incubation of glucose and albumin.

*2.5 Isolation of 3-C-β-D-glucosyl-2,4,4',6-tetrahydroxybenzophenone (**1**) and Mangiferin (**2**)*

The OML-ext (5.4 g) was submitted to a silica gel column chromatography with a stepwise gradient elution with $CHCl_3$/MeOH (10:0 v/v) to (0:10 v/v). Inhibition of AGEs formation at 25 μg/ml of each fraction was assayed. Fraction I (fr. 1-6) eluted firstly with $CHCl_3$ showed a significant activity. Fraction I was dark green, and it seemed to contain green pigments, such as chlorophyll as one of active constituents. Fraction II (fr. 7-25) eluted with $CHCl_3$/MeOH 30:1 v/v, 10:1 v/v, 5:1 v/v and 0:10 v/v also showed potent activity. Further purification of fraction II by a preparative HPLC led to isolation of **1** and **2** as described in the preceding paper (Itoh et al., 2016).

*2.6 Content of Chlorophyll, Total Anthocyanin, **1** and **2** in Mango Leaf Extracts*

In this paper, determination of each compound in leaf extracts was performed in triplicate, and values represent the mean ± standard deviation. Spectrophotometric determination of the content of chlorophyll (mg/g extract) was carried out with 80% acetone aqueous solution containing 2.5 mM sodium phosphate buffer (pH 7.8) according to the method of Porra et al. (Porra, Thompson, & Kriedemann, 1989). The content of total anthocyanin (μg/g extract) in each leaf extract was determined by the following HPLC analysis and shown as μg/g extract in which weight (μg) was calculated as that of an external standard, cyanidin-3-*O*-glucoside chloride. The extract was dissolved in 10% MeOH aqueous solution containing 10% acetic acid, followed by centrifugation at 12,000 G for 10 min. Resulting supernatant was applied to a HPLC system. The HPLC system consisted of LC-20A pump and SPD-20A photodiode array detector (Shimadzu, Kyoto, Japan). The samples were analyzed by using an Inertsil ODS-3 reverse phase column (4.6 ×150 mm, GL Sciences, Tokyo, Japan) and gradient elution with MeOH aqueous solution at a constant flow rate of 0.8 ml/min. The elution was carried out using linear gradient condition as follows; initial condition was set at 5% MeOH and maintained for 5 min, followed by a linear gradient from 5% to 40% MeOH for 35 min. The column temperature was set at 40 ℃, and eluted compounds were detected at 520 nm. Total anthocyanins content was determined using total 520 nm peak areas from linear calibration curves made from an external standard, cyanidin-3-*O*-glucoside chloride. Linear calculation curves in the range of 0.02 to 0.1 nmol were made from the peak areas analyzed at 520 nm, and the correlation coefficient was 0.992. The HPLC determination of **1** and **2** in each leaf extract was described in the preceding paper (Itoh et al., 2016).

2.7 Statistical Analysis

The experimental data were evaluated for statistical significance using Bonferroni/Dunn's multiple-range test with GraphPad Prism for Windows, Ver. 5 (GraphPad Software Inc., 2007).

3. Results and Discussion

3.1 Identification of AGEs Formation Inhibitory Active Ingredients of OML-ext

In the preliminary evaluation of mango leaf extract on inhibitory activity of AGEs formation in nonenzymatic glycation of albumin, the OML-ext inhibited AGEs formation with the IC_{50} value of 43 μg/ml. To identify the active constituents, we carried out activity-guided fractionation of OML-ext using AGEs formation inhibitory assay. Silica gel column chromatographic fractionation of OML-ext gave two active fractions, fraction I and fraction II. The dark green color of fraction I suggested that this fraction may contain green pigments such as chlorophyll as an active ingredient.

Table 1. Inhibitory activities of 3-C-*β*-D-glucosyl-2,4,4',6-tetrahydroxybenzophenone (**1**), mangiferin (**2**), chlorophyll and cyanidin-3-*O*-glucoside chloride on AGEs formation

Samples	**IC_{50} values [a] (μM or μg/ml or mM)**
3-C-*β*-D-glucosyl-2,4,4',6-tetrahydroxybenzophenone (**1**)	85 μM
Mangiferin (**2**)	18 μM
Chlorophyll	41 μg/ml
Cyanidin-3-*O*-glucoside chloride	32 μM
Aminoguanidine hydrochloride	0.9 mM

Aminoguanidine hydrochloride was used as reference compound. a); IC_{50} value represents the concentration required to inhibit 50% of AGEs

formation.

Further purification of another active fraction II led to isolation of **1** and **2** as active constituents. The IC_{50} values (Table 1) of **1** and **2** were 85 and 18 μM, respectively. As shown in Table 1, the IC_{50} value of aminoguanidine hydrochloride as a reference compound was 0.9 mM (= 99 μg/ml) in accordance with the reported IC_{50} value (138 μg/ml) (Shimoda et al., 2011). Thus, a part of the AGEs formation inhibitory activity of OML-ext is attributable to these two compounds. To the best of our knowledge, this is the first report on AGEs formation inhibitory activity of **1**. Mahali et al. (Mahali, Verma, & Manna, 2014) reported **2** inhibited AGE-mediated reactive oxygen intermediate generation and inhibited ERK and IKK activity, thereby suppression of sterol regulatory element binding protein activity and lipogenesis. Hou et al. (2016) described mangiferin reduced AGE formation and decreased the mRNA and protein expression of receptor for AGEs in diabetic cardiomyopathy model rats. In addition, Suchal et al. (2017) reported **2** attenuated ischemia-reperfusion induced myocardial injury in streptozotocin-induced diabetic rats by modulation of AGE-receptor/MAPK pathways which further prevented oxidative stress, inflammation and apoptosis in the myocardium. From the view point of structure-activity relationship, we will attempt to examine whether **1** and **2** have a similar inhibitory mechanism of AGEs formation because **1** and **2** belong to C-glucosyl-polyphenols.

3.2 A Relationship between Leaves Maturation and AGEs Formation Inhibitory Activity

We examined a relationship between leaves maturation and inhibitory activity of AGEs formation. The collected leaves were visually classified by the color of leaf into three groups as shown in Figure 1.

As shown in Table 2, OML-ext exhibited an inhibitory activity of AGEs formation with the IC_{50} value of 43 μg/ml. The IC_{50} values of young dark reddish brown mango leaf extract (YDL-ext) and young yellow leaf extract (YYL-ext) were 40 and 66 μg/ml, respectively (Table 2). The activity of YDL-ext was similar to that of OML-ext. The inhibitory activity of YYL-ext showed slightly decreased compared to those of OML-ext and YDL-ext. HPLC analysis revealed that the contents of **1** and **2** in these leaf extracts were high as described in the preceding paper (Itoh et al., 2016). Taking account of the inhibition data of **1** and **2**, and high contents of **1** and **2** in the extracts, the inhibitory activities of these extracts would be partly attributable to these compounds.

Table 2. Inhibitory activities of MeOH extracts of young dark reddish brown and young yellow leaves and old dark green mango leaves on AGEs formation

Samples	**IC_{50} values [a] (μg/ml or mM)**
Young dark reddish brown leaf extract (= YDL-ext)	40 μg/ml
Young yellow leaf extract (= YYL-ext)	66 μg/ml
Old dark green leaf extract (= OML-ext)	43 μg/ml
Aminoguanidine hydrochloride	0.9 mM

Aminoguanidine hydrochloride was used as reference compound. a); IC_{50} value represents the concentration required to inhibit 50% of AGEs formation.

On the other hand, we can not exclude a hypothesis that other ingredients may also contribute to the activity. Ali et al. (1999) reported that young dark reddish brown mango (cv. Irwin) leaves contain anthocyanin, however, anthocyanin rapidly disappears and chlorophyll content increases with an increase in area after unfolding. Sami & Shakoori (2011) have isolated cyanidin-3-*O*-glucoside as an anthocyanin with cellulase inhibitory activity from mango leaves. These reports prompted us to evaluate inhibitory effects of chlorophyll and cyanidin-3-*O*-glucoside on AGEs formation, considering with the assumption that the dark green fraction I contained green pigments, such as chlorophyll as one of active ingredients as described above. As shown in Table 1, the IC_{50} values of chlorophyll and cyanidin-3-*O*-glucoside chloride were 41 μg/ml, 32 μM, respectively. Although AGEs formation inhibitory activity of anthocyanins including its inhibitory mechanism has been reported (Chen et al. 2014), there is no report on AGEs formation inhibitory effect of chlorophyll. The content of chlorophyll in each leaf extract was spectrophotometrically determined by the method of Porra et al. (1989). The content of total anthocyanin in each leaf extract was determined by a HPLC analysis. As a result, the content of chlorophyll in YDL-ext was 0.85 mg/g. The corresponding content data of other two leaf extracts were as follows; YYL-ext, 2.18 mg/g, and OML-ext, 4.34 mg/g. The content of chlorophyll in leaf extract were increased

with leaves enlargement. The contents of total anthocyanins in these leaf extracts were as follows; YDL-ext, 7.38 ± 0.24 μg/g; YYL-ext, 5.80 ± 0.59 μg/g; OML-ext, not detected of any anthocyanins. These data are in accordance with the reported data of Ali et al. (Ali, Koeda, & Nii, 1999). Considering with the content of chlorophyll and total anthocyanin in leaf extracts, the inhibitory activity of OML-ext was attributable to **1**, **2** and chlorophyll. The inhibitory activities of YDL-ext and YYL-ext were attributable to **1** and **2**, in addition, a part of the inhibitory activity of YDL-ext and YYL-ext was due to anthocyanins whose content is high in young dark reddish brown and young yellow mango leaves. On the other hand, to fully identify other active ingredients and to reveal the inhibitory mechanisms of **1** and chlorophyll, further studies are required, and now undergoing.

From the view point of utility of mango leaves, old dark green leaves obtained by summer pruning may be a reasonable natural resource for the preparation of ingredients with inhibitory activity of AGEs formation.

4. Conclusion

YDL-ext, YYL-ext and OML-ext exhibited inhibitory activities of AGEs formation in nonenzymatic glycation of albumin. The inhibitory activity of YDL-ext was similar to that of OML-ext, and YYL-ext was less potent than YDL-ext and OML-ext, this is the first report to reveal a relationship between leaves maturation and inhibitory activity of AGEs formation. The inhibitory activity of OML-ext was attributable to 3-C-*β*-D-glucosyl-2,4,4',6-tetrahydroxybenzophenone (**1**), mangiferin (**2**) and chlorophyll. Whereas the inhibitory activity of YDL-ext and YYL-ext were attributable to **1** and **2**, in addition, a part of the inhibitory activity of YDL-ext and YYL-ext was due to anthocyanins whose content is high in young dark reddish brown and young yellow mango leaves. This is the first report on AGEs formation inhibitory activity of **1** and chlorophyll. Hitherto, pruned mango leaves were unworthy and discarded during the cultivation process of mango fruit, these findings suggested that pruned mango leaves may be a useful resource for the preparation of ingredients for skin aging or diabetic complications such as diabetic nephropathy with having lipase inhibitory activity. However, further investigations are required to examine administration safety and the mechanisms involved and to reveal other active constituents.

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References

Ali K., Koeda K., & Nii, N. (1999). Changes in anatomical features, pigment content and photosynthetic activity related to age of 'Irwin' mango leaves. *Journal of the Japanese Society for Horticultural Science*, *68*(6), 1090-1098. https://doi.org/10.2503/jjshs.68.1090

Anusiri, P., Choodej, S., Chumriang, P., Adisakwattana, S., & Pudhom, K. (2014). Inhibitory effects of flavonoids from stem bark of *Derris indica* on the formation of advanced glycation end products. *Journal of Ethnopharmacology*, *158*, 437-441. https://doi.org/10.1016/j.jep.2014.10.053

Chen, X. Y., Huang, I. M., Hwang, L. S., Ho, C. T., Shiming, L., & Lo, C. Y. (2014). Anthocyanins in blackcurrant effectively prevent the formation of advanced glycation end products by trapping methylglyoxal. *Journal of functional foods*, *8*, 259-268. https://doi.org/10.1016/j.jff.2014.03.025

Chompoo, J., Upadhyay, A., Kishimoto, W., Makise, T., & Tawata, S. (2011). Advanced glycation end products inhibitors from *Alpinia zerumbet* rhizomes. *Food Chemistry*, *129*, 709-715. https://doi.org/10.1016/j.foodchem.2011.04.034

Dyer, D. G., Dunn, J. A., Thorpe, S. R., Bailie, K. E., Lyons, T. J., McCance, D. R., & Baynes, J. W. (1993). Accumulation of Maillard reaction products in skin collagen in diabetes and aging. *Journal of Clinical Investigation*, *91*, 2463-2469. https://doi.org/10.1172/JCI116481

Hou, J., Zheng, D., Fung, G., Deng, H., Chen, L., Liang, J., … Hu, Y. (2016). Mangiferin suppressed advanced glycation end products (AGEs) through NF-κB deactivation and displayed anti-inflammatory effects in streptozotocin and high fat diet-diabetic cardiomyopathy rats. *Canadian Journal of Physiology and Pharmacology*, *94*(3), 332-340. https://doi.org/10.1139/cjpp-2015-0073

Huebschmann, A. G., Regensteiner J. G., Vlassara H., & Reusch J. E. B. (2006). Diabetes and advanced glycoxidation end products. Diabetes Care, *29*(6), 1420-1432. https://doi.org/10.2337/dc05-2096

Itoh, K., Murata, K., Nakagaki, Y., Shimizu, A., Takata, Y., Shimizu, K., … Matsuda, H. (2016). Inhibitory activity of *Mangifera indica* leaf extract on pancreatic lipase. Journal of Plant Studies, *5*(2), 72-78. https://doi.org/10.5539/jps.v5n2p72

Lohwasser, C., Neureiter, D., Weigle, B., Kirchner, T., & Schuppan, D. (2006). The receptor for advanced glycation end products is highly expressed in the skin and upregulated by advanced glycation end products and tumor necrosis factor-alpha. *Journal of Investigative Dermatology*, *126*(2), 291-299. https://doi.org/10.1038/sj.jid.5700070

Mahali, S. K., Verma, N., & Manna, S.K. (2014). Advanced glycation end products induce lipogenesis: regulation by natural xanthone through inhibition of ERK and NF-κB. *Journal of Cellular Physiology*, *229*(12), 1972-1980. https://doi.org/10.1002/jcp.24647

Morimitsu, Y., Yoshida, K., Esaki, S., & Hirota, A. (1995). Protein glycation inhibitors from thyme (*Thymus vulgaris*). *Biosci Biotechnol Biochem.*, *59*(11), 2018-2021. https://doi.org/10.1271/bbb.59.2018

Pageon, H., Bakala, H., Monnier, V. M., & Asselineau, D. (2007). Collagen glycation triggers the formation of aged skin in vitro. *European Journal of Dermatology*, *17*(1), 12-20. https://doi.org/10.1016/j.plantsci.2013.12.014

Porra R. J., Thompson W. A., & Kriedemann P. E. (1989). Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls *a* and *b* extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochimica et Biophysica Acta*, *975*, 384-394. https://doi.org/10.1016/S0005-2728(89)80347-0

Sami, A. J., & Shakoori, A. R. (2011). Cellulase activity inhibition and growth retardation of associated bacterial strains of *Aulacophora foviecollis* by two glycosylated flavonoids isolated from *Mangifera indica* leaves. *Journal of Medicinal Plants Research*, *5*(2), 184-190.

Shimoda, H., Nakamura, S., Morioka, M., Tanaka, J., Matsuda, H., & Yoshikawa, M. (2011). Effect of cinnamoyl and flavonol glucosides derived from cherry blossom flowers on the production of advanced glycation end products (AGEs) and AGE-induced fibroblast apoptosis. *Phytotherapy Research*, *25*, 1328-1335. https://doi.org/10.1002/ptr.3423

Sourris, K. C., Harcourt, B. E., & Forbes, J. M. (2009). A new perspective on therapeutic inhibition of advanced glycation in diabetic microvascular complications: common downstream endpoints achieved through disparate therapeutic approaches? *American Journal of Nephrology*, *30*, 323-335. https://doi.org/10.1159/000226586

Suchal, K., Malik, S., Khan, S. I., Malhotra, R. K., Goyal, S. N., Bhatia, J., … Arya, D. S. (2017). Protective effect of mangiferin on myocardial ischemia-reperfusion injury in streptozotocin-induced diabetic rats: role of AGE-RAGE/MAPK pathways. *Scientific Reports*, *7*, 1-11. https://doi.org/10.1038/srep42027

Tsuji-Naito, K., Saeki, H., & Hamano, M. (2009). Inhibitory effects of *Chrysanthemum* species extracts on formation of advanced glycation end products. *Food Chemistry*, *116*, 854-859. https://doi.org/10.1016/j.foodchem.2009.03.042

Wondrak, G. T., Roberts, M. J., Jacobson, M. K., & Jacobson, E. L. (2002). Photosensitized growth inhibition of cultured human skin cells: mechanism and suppression of oxidative stress from solar irradiation of glycated proteins. *Journal of Investigative Dermatology*, *119*, 489-498. https://doi.org/10.1046/j.1523-1747.2002.01788.x

Xu, Y., Liu, G., Yu, Z., Song, X., Li, X., Yang, Y., … Dai, J. (2016). Purification, characterization and antiglycation activity of a novel polysaccharide from black currant. *Food Chemistry*, *199*, 694-701. https://doi.org/10.1016/j.foodchem.2015.12.078