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ORIGINAL ARTICLE

Antioxidant, inhibition of α-glucosidase and suppression of nitric oxide production in LPS-induced murine macrophages by different fractions of *Actinidia arguta* stem

Jaehak Lee [a,1], Kandhasamy Sowndhararajan [b,1], Mihae Kim [a], Jaehun Kim [c], Daeho Kim [c], Sunpyo Kim [c], Gur-Yoo Kim [a], Songmun Kim [b], Jin-Woo Jhoo [a,*]

[a] *Kangwon National University, Department of Animal Products and Food Science, Chuncheon, Gangwon 200-701, Republic of Korea*
[b] *Kangwon National University, Department of Biological Environment, Chuncheon, Gangwon 200-701, Republic of Korea*
[c] *Ildong Foodis Co., Ltd., R&D Center, #1153 Geodoo-ri, Dongnae-myun, Chuncheon, Gangwon 200-881, Republic of Korea*

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Abstract In traditional systems of medicine, fruits, leaves, and stems of *Actinidia arguta* (Sieb. et Zucc.) Planch. ex Miq. have been used to treat various inflammatory diseases. The present study determined the proximate composition, antioxidant, anti-inflammatory, and hypoglycemic potential of *A. arguta* stem. Phenolic composition of hot water extract and its sub-fractions was determined by Folin–Ciocalteu's reagent method. *In vitro* antioxidant activities of the samples were evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical scavenging assays. Anti-inflammatory activity of different fractions was investigated through the inhibition of nitric oxide (NO) production in lipopolysaccharide (1 μg/ml) stimulated RAW 264.7 cells. In addition, inhibition of α-glucosidase activity of hot water extract was determined using *p*-nitrophenyl-α-D-glucopyranoside (*p*NPG) as a substrate. Ethyl acetate (557.23 mg GAE/g) fraction contains higher level of total phenolic content. The antioxidant activity evaluated by DPPH radical scavenging assay showed a strong activity for ethyl acetate (IC_{50} of 14.28 μg/ml) and *n*-butanol fractions (IC_{50} of 48.27 μg/ml). Further, ethyl acetate fraction effectively inhibited NO production in RAW 264.7 cells induced by

* Corresponding author. Address: Kangwon National University, Department of Animal Products and Food Science, KNU Ave. 1, Chuncheon, Gangwon-do 200-701, Republic of Korea. Tel.: +82 33 250 8649; fax: +82 33 251 7719.
E-mail address: jjhoo@kangwon.ac.kr (J.-W. Jhoo).
[1] These authors contributed equally.

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lipopolysaccharide (LPS) than other fractions (nitrite level to 32.14 μM at 200 μg/ml). In addition, hot water extract of *A. arguta* stem exhibited appreciable inhibitory activity against α-glucosidase enzyme with IC_{50} of 1.71 mg/ml. The obtained results have important consequence of using *A. arguta* stem toward the development of effective anti-inflammatory drugs.

1. Introduction

Actinidia arguta (Sieb. et Zucc.) Planch. ex Miq. (Actinidaceae) is a perennial, fast growing and deciduous twining vine. It is native to northern China, Korea, Siberia and Japan and commercially available in New Zealand, USA and many European countries. This plant bears smooth-skinned, grape-sized kiwifruit and commonly known as hardy kiwi (Matich et al., 2003). Traditionally, fruits, leaves, and stems of *A. arguta* are used for the treatment of various inflammatory diseases (Choi et al., 2008). This plant has been reported to contain various bioactive compounds such as quercetin, kaempferol, catechins, and volatile compounds. Anti-cancer and anti-allergic properties were reported from the stem of this plant (Webby, 1991; Matich et al., 2003; Ravipati et al., 2012). Takano et al. (2003) isolated (+)-catechin and (−)-epicatechin from methanol extract of *A. arguta* stem. These compounds effectively promoted the bone marrow cell proliferation and stimulated the formation of myeloid colonies. Several studies have been conducted in relation to the biochemical and pharmacological properties of the fruits and flowers. However, only limited studies have examined on the phytochemical and pharmacological potential of other parts of *A. arguta*. Additionally, the safety of this plant is well understood based on the long history of human utilization.

In the last few decades, numerous studies have been documented for the utilization of natural antioxidants as potential disease preventing agents to reduce the risk of cardiovascular diseases, neuro-degenerative diseases, inflammations, diabetes and cancers (Anwar et al., 2007; Yangthong et al., 2009). The protective effects of the plants are mostly related to the antioxidant components such as phenolics, carotenoids, phytates, isothiocyanates, phytosterols, phytoesterogens and organosulfur (Halvorsen et al., 2002). Hence, the search continues for the novel and effective antioxidants from the plant source to reduce the risk of free radical mediated disorders.

Inflammation is the normal response of a living tissue to injury caused by physical or noxious chemical stimuli or microbiological toxins. Macrophages are the main pro-inflammatory cells responsible for invading pathogens by releasing many pro-inflammatory molecules such as nitric oxide (NO), prostaglandin E_2 (PGE_2), and of cytokines, like interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). Among these, NO is a short-lived biomolecule that mediates many biological functions, including host defense, vasoregulation, platelet aggregation and neurotransmission (Moncada et al., 1991). However, overproduction of NO has been concerned in the development of various inflammatory diseases, such as arthritis, asthma, multiple sclerosis, inflammatory bowel disease, and atherosclerosis (MacMicking et al., 1997; Rankin, 2004). Hence, this pro-inflammatory mediator, NO has been considered as an important therapeutic target for the development of anti-inflammatory drugs (MacMicking et al., 1997).

Based on the above knowledge, the present investigation was undertaken to evaluate the proximate composition, antioxidant, anti-inflammatory (through the inhibition of NO production in RAW 264.7 cells activated with lipopolysaccharide), and the inhibition of α-glucosidase activities of hot water extract and its sub-fractions of *A. arguta* stem.

2. Materials and methods

2.1. Chemicals and materials

All solvents were of HPLC grade from J.T. Baker (Phillipsburg, NJ, USA). 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), Folin–Ciocalteu's reagent, Trolox, gallic acid, and yeast α-glucosidase were purchased from Sigma–Aldrich (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and phosphate buffered saline (PBS) were from WelGENE (Daegu, Republic of Korea). The authentic stem material of *A. arguta* was purchased from local herbal store (Gangwon, Republic of Korea) and deposited at Ildong Foodis (Chuncheon, South Korea) with batch No. IL-2012-0023.

2.2. Proximate composition

The moisture, ash, crude lipid and crude protein contents of *A. arguta* stem were determined by following the standard AOAC Methods (1990).

2.3. Extract preparation

The sample of dried stem chips (10 mm × 10 mm, 400 g) was refluxed in 2000 ml distilled water for 6 h in a round bottomed flask three times. The resulting aqueous extract was filtered with No. 2 filter paper. The combined hot water extract was evaporated under reduced pressure to get crude extract. The crude extract was dissolved in water and the aqueous solution was successively partitioned with ethyl acetate and *n*-butanol. The solvents were evaporated using a rotary vacuum-evaporator at 40 °C. The obtained fractions, in addition to the aqueous solution remained after the extraction were filtered and freeze dried (FD-1000, Eyela, Tokyo, Japan). The crude extract and fractions were used for the assessment of free radical scavenging activity and inhibition of nitric oxide production assays. The extract yield obtained from different fractions was expressed as percent of dry weight of the hot water extract.

2.4. Determination of total phenolic content

The total phenolic content of the samples was determined spectrophotometrically at 760 nm after reaction with the

Folin–Ciocalteu's reagent (Chew et al., 2009). The analysis was performed in triplicate and the results were expressed as the gallic acid equivalents (GAE).

2.5. Free radical scavenging activity on DPPH

The DPPH radical scavenging activity of the samples was determined according to the slightly modified method of Doughari et al. (2012). IC_{50} values of the extract i.e., concentration of extract necessary to decrease the initial concentration of DPPH by 50% were calculated.

2.6. Antioxidant activity by the $ABTS^{\cdot+}$ assay

Radical scavenging activity of crude extract and its sub-fractions of *A. arguta* stem was assessed spectrophotometrically by $ABTS^{\cdot+}$ cation decolorization assay (Re et al., 1999).

2.7. Cell culture

The murine macrophage RAW 264.7 cell line was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, 100 μg/ml streptomycin, and 100 IU/ml penicillin at 37 °C in a 5% CO_2 atmosphere (HERAcell 150, Thermo Electron Corp., Waltham, MA, USA). Cells count and viability were performed using a standard trypan blue cell counting technique. The cells were seeded at a density of 5×10^5 cells/well in the same medium and incubated for 12 h. Then media of each well were aspirated and fresh FBS-free media were replaced. Different concentrations of the samples were prepared from the stock solutions by serial dilution in FBS-free DMEM to give a volume of 100 μl in each well of a microtiter plate. Then cells were stimulated with 1 μg/ml of LPS and incubated for 24 h.

2.8. Inhibition of nitric oxide (NO) production

The presence of nitrite, a stable oxidized product of NO, was determined in cell culture media by Griess reagent (iNtRON, Sungnam, South Korea). 100 μl of cell culture medium with an equal volume of Griess reagent in a 96-well plate was incubated at room temperature for 10 min. Then the absorbance was measured at 540 nm in a microplate reader (Biotek, Winooski, VT, USA). Fresh culture medium was used as the blank in all experiments. The amount of nitrite in the samples was determined with reference to a sodium nitrite standard curve.

2.9. α-Glucosidase inhibition assay

Inhibition of α-glucosidase enzyme assay was determined spectrophotometrically in a 96-well microtiter plate based on *p*-nitrophenyl-α-D-glucopyranoside (*p*NPG) as a substrate (Li et al., 2005). In brief, 25 μl of enzyme solution [0.1 U/ml of α-glucosidase in 0.1 M potassium phosphate buffer (pH 6.8)] and 50 μl of the test sample in 0.01 M potassium phosphate buffer were mixed, and was pre-incubated at 37 °C prior to initiation of the reaction by adding the substrate. After 15 min of pre-incubation, *p*NPG solution (25 μl) [5.0 mM PNPG in 0.1 M potassium phosphate buffer (pH 6.8)] was added and then incubated together at 37 °C. After 30 min of incubation, 0.2 M Na_2CO_3 (100 μl) in 0.1 M potassium phosphate buffer was added to stop the reaction. The amount of *p*-nitrophenol released from *p*NPG was quantified using a microplate reader at 405 nm (Biotek, Winooski, VT, USA). A unit of enzyme activity was defined as 1 μmole of *p*-nitrophenol produced per min. The molar extinction coefficient for *p*-nitrophenol used for the determination of enzyme activity was $1.78 \times 10^4\ M^{-1}\ cm^{-1}$.

2.10. Statistical analysis

The values expressed are means of three replicate determinations ± standard deviation. The statistical analysis was carried out by analysis of variance (ANOVA) followed by Tukey's test. The data were evaluated with SPSS 20.0 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.2. Proximate composition, extract yield and total phenolic content

A. arguta stem contains substantial levels of proximate components. The values obtained for moisture, ash, crude lipid, and crude protein from the present study are 5.45%, 2.25%, 0.63%, and 2.46%, respectively.

Phenolic compounds have been reported to have multiple biological effects, including antioxidant activity and abundantly present in plants. Therefore, it is a common practice to determine both phenolic content and antioxidant activities when investigating the antioxidant potential of plants. In the present study, the percent yield and total phenolic content obtained from hot water extract and its sub-fractions of *A. arguta* stem are presented in Table 1. Hot water extraction gave better recovery percentage of 10.91. The hot water extract of *A. arguta* stem contains a moderate level of total phenolic content (40.78 mg GAE/g extract). Hot water extract was further separated to ethyl acetate, *n*-butanol and water fractions and the yields obtained are 1.0%, 9.9%, and 89% respectively. It was noticed that the total phenolic content of ethyl acetate (557.23 mg GAE/g extract) and *n*-butanol (194.13 mg GAE/g extract) fractions was significantly higher ($P < 0.05$) than the crude extract. Polyphenol compounds play an important role in scavenging of ROS by adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides. The total phenolic content of skin and flesh was determined among the cultivars of hardy kiwifruit in Japan. The average values of the total phenolic content in the skin and flesh were 2.66 and 0.18 g/100 g fresh weight, respectively (Kim et al., 2009). Mikulic-Petkovsek et al. (2012) determined that the phenolic content of hardy kiwi fruit was 784 mg GAE/kg.

3.3. DPPH radical scavenging activity

The antioxidant properties of plant extracts or compounds can be determined using various *in vitro* assays. In these, the DPPH radical scavenging assay constitutes a quick and inexpensive method, which has frequently been used for the evaluation of the antioxidant potential of various natural products. The

Table 1 Extraction yield, total phenolic content, DPPH and ABTS radical scavenging activities of crude extract and its sub-fractions of *Actinidia arguta* stem.

Sample	Extract yield (%)*	Total phenolics (mg GAE/g extract)	IC_{50} of DPPH (μg/ml)	ABTS (TEAC)
ACE	10.91	40.78 ± 0.09^{c}	127.85 ± 0.72^{b}	0.0387 ± 0.0002^{c}
AEF	1.0	557.23 ± 1.48^{a}	14.28 ± 0.16^{d}	0.3081 ± 0.0072^{a}
ABF	9.9	194.13 ± 1.36^{b}	48.27 ± 0.68^{c}	0.0989 ± 0.0023^{b}
AWF	89	32.87 ± 0.13^{d}	296.31 ± 2.38^{a}	0.0277 ± 0.0007^{d}
Ascorbic acid	–	–	1.73 ± 0.01^{e}	–

Values are mean of three replicate determinations ($n = 3$) ± standard deviation. Mean values followed by different superscripts (a–e) in a column are significantly different ($P < 0.05$).
ACE, crude hot water extract; AEF, ethyl acetate fraction; ABF, butanol fraction; AWF, water fraction.
* Extract yield was expressed on the basis of dry weight of the sample for ACE and dry weight of the extract for AEF, ABF and AWF.

scavenging effects of the crude extract and its sub-fractions of *A. arguta* stem on DPPH were examined at different concentrations. The IC_{50} values for DPPH assay of the samples along with ascorbic acid as standard are given in Table 1. In the DPPH radical scavenging activity of crude extract and its sub-fractions of *A. arguta* stem, a dose dependent inhibition was observed. The ethyl acetate (IC_{50} of 14.28 μg/ml) and *n*-butanol fractions (IC_{50} of 48.27 μg/ml) registered appreciable DPPH radical scavenging activity. When compared to the standard ascorbic acid (IC_{50} of 1.73 μg/ml), all the samples showed significantly lower ($P < 0.05$) radical scavenging activity. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen-donating ability. Similar order to the total phenolic contents of the extract and fractions that showed the extent of free radical scavenging activity is in accordance with the amount of phenolic content present in that extract or fractions. The correlation between antioxidant activity and the content of phenolic compounds has also been reported by many researchers (Sun and Ho, 2006; Liu et al., 2009).

3.4. ABTS radical scavenging activity

In the Trolox equivalent antioxidant capacity (TEAC) has attracted much interest because it enables high-throughput screening of potential antioxidant activity of functional food materials (Tyrakowska et al., 1999; Gliszczynska-Swiglo 2006). The efficacy of crude extract and its sub-fractions of *A. arguta* stem on ABTS radical cation scavenging activity is presented in Table 1. The results revealed that all the samples tested were able to scavenge $ABTS^{\bullet+}$ radical cation. Similar to DPPH radical scavenging activity, ethyl acetate (TEAC value of 0.3081) and *n*-butanol (TEAC value of 0.0989) fractions exhibited significantly higher ($P < 0.05$) ABTS radical scavenging activity than crude extract and water fraction. The results of the present study revealed that the ethyl acetate fraction registered higher TEAC value than the vitamins such as folic acid (0.06), pyridoxine (0.03), pyridoxal (0.03) and pyridoxamine (0.03) previously reported by Gliszczynska-Swiglo (2006).

3.5. Inhibition of nitric oxide production in LPS-stimulated RAW 264.7 cells

Macrophages play important roles in inflammation via the production of many pro-inflammatory molecules, including NO. The excessive and uncontrolled production of NO can be harmful and results in various inflammatory diseases. NO has been recognized as a mediator and regulator in pathological reactions, especially in acute inflammatory responses (Terao, 2009). Thus, inhibition of the production of NO is essential to alleviate various diseases mediated by excessive and protracted activation of macrophages. Nicholas et al. (2007) stated that the LPS is a cell wall component of gram-negative bacteria that is known to induce the activation of macrophages and production of pro-inflammatory mediators.

In the present study, we demonstrated that the different fractions of hot water extracts of *A. arguta* stem inhibit LPS-induced NO production in RAW 264.7 cells, a widely used macrophage-like cell line. RAW 264.7, a murine macrophage cell line, provide excellent means of screening anti-inflammatory drugs and for evaluating the inhibitors of the pathways that lead to the inductions of pro-inflammatory enzymes and cytokines. For this purpose, cells were simultaneously treated with 1 μg/ml LPS and different concentrations of fractions. The macrophages were activated by LPS, and NO production was measured as nitrite concentration in the culture medium. Different fractions of hot water extract of *A. arguta* stem exhibited the inhibition of LPS-induced NO production in RAW 264.7 cells in a dose-dependent manner (Fig. 1A–C). Among the different fractions, ethyl acetate fraction exhibited a significant reduction of nitrite level to 32.14 μM at the concentration of 200 μg/ml. The results reveal that fractions of hot water extract of *A. arguta* may act as efficient anti-inflammatory agents by inhibiting the production of NO.

The inhibitory activity of NO production in macrophages by different fractions was probably due to the presence of higher level of phenolic content. Previous studies have also reported that phenolic compounds act as excellent anti-inflammatory agents and they play an important role between oxidative stress and inflammation (Yen et al., 2008). Recent studies have indicated that regulation and control of nuclear transcription factor (NF)-*k*B activation may be a key molecular target for the anti-inflammatory therapy. In addition, phenolic compounds have been reported to have strong antioxidant activity and exhibit a wide range of biological activities such as inhibition of cyclooxygenase (COX), induction of CD95 signaling dependent apoptosis, effects on cell division cycle and the modulation of NF-*k*B activation (Falchetti et al., 2001; Lin and Tang, 2008).

3.6. α-Glucosidase inhibition assay

Diabetes mellitus is a chronic disorder caused by altered metabolism of carbohydrate, and thus creating the hyperglycemic state (Zheng et al., 2007). One therapeutic approach in the

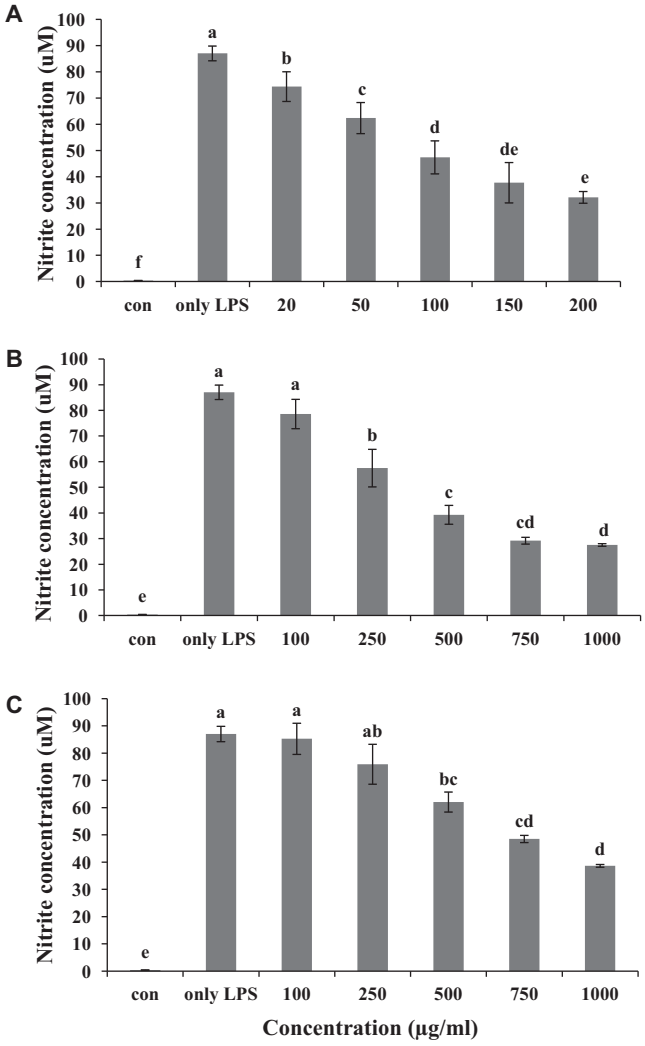

Figure 1 Inhibitory effects of different fractions of *Actinidia arguta* stem on NO production by LPS-stimulated RAW264.7 macrophages. (A, ethyl acetate fraction; B, butanol fraction; C, water fraction). Values are mean of three replicate determinations ($n = 3$) ± standard deviation. Bars having different letters are significantly different ($P < 0.05$).

early stage diabetes is to decrease post-prandial hyperglycemia. It is well-known that complex polysaccharides are hydrolyzed by amylases to dextrins which are further hydrolyzed to glucose by intestinal α-glucosidase before entering blood circulation through intestinal epithelium absorption. For this reason, inhibition of these enzymes could reduce the high post-prandial blood glucose levels in diabetics (Tundis et al., 2007). The enzyme reaction was performed under *in vitro* using *p*-nitrophenyl-α-D-glucopyranoside (pNPG) as the substrate, which was hydrolyzed by α-glucosidase to release *p*-nitrophenol, a color agent that can be monitored at 405 nm (Babu et al., 2004).

In this context, the hypoglycemic potential of *A. arguta* hot water extract was evaluated by the α-glucosidase inhibition assay. The hot water extract exhibited a good activity against α-glucosidase with IC_{50} value of 1.71 mg/ml (Fig. 2). Fig. 3 shows a Lineweaver–Burk plot of α-glucosidase inhibitory activities in the presence of hot water extract at the

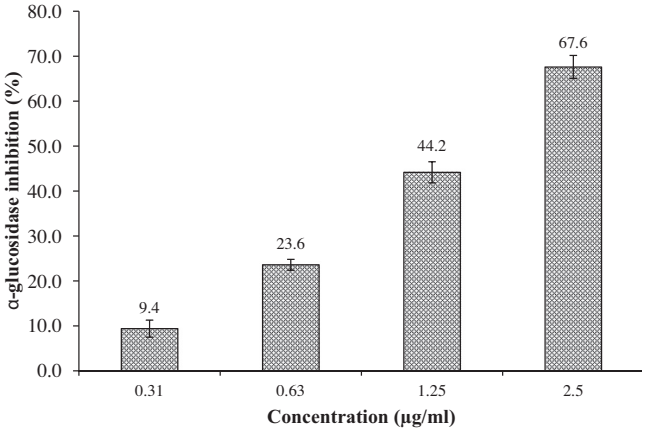

Figure 2 Effect of hot water extract of *Actinidia arguta* stem on α-glucosidase inhibition. Values are mean of three replicate determinations ($n = 3$) ± standard deviation.

concentrations of 1.00 and 1.25 mg/ml. This result demonstrated that the velocity of the reaction catalyzed by α-glucosidase was reduced with increasing the concentration of hot water extract. Dong et al. (2012) reported that the inhibitory effect of α-glucosidase by trilobatin isolated from *Lithocarpus polystachys* Rehd showed a non-competitive pattern of enzyme inhibition. The results indicated that *A. arguta* hot water extract produced a mixed-type inhibition, which is characterized by a combination of competitive and non-competitive inhibition. Previous studies have reported that the retardation of α-glucosidase enzyme by inhibitors would be one of the most effective ways to control Type II diabetes (Chethan et al., 2008; Islam, 2006).

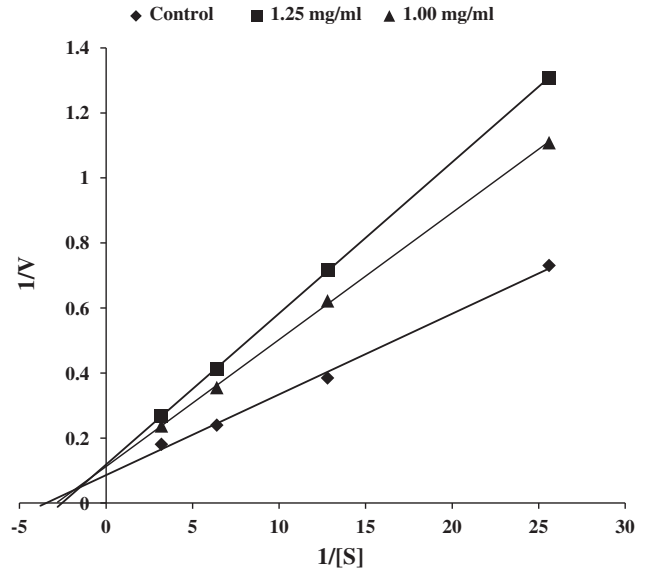

Figure 3 Lineweaver–Burk plot of α-glucosidase inhibition in the presence of *Actinidia arguta* stem extracts at different concentrations of substrate. Values are mean of three replicate determinations ($n = 3$).

4. Conclusions

This study would propose the use of stems as an accessible possible source of phenolic compounds with remarkable free radical scavenging potentials. Further, ethyl acetate fraction of hot water extract has high potential to reduce the level of NO production. In addition, hot water extract markedly inhibits the α-glucosidase enzyme. The results concluded that *A. arguta* stem extract could be useful in preventing inflammation-mediated diseases. Further studies related to hypoglycemic potential and isolation of bioactive compounds from the fractions of *A. arguta* stem are under progress.

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References

Anwar, F., Latif, S., Ashraf, M., Gilani, A.H., 2007. *Moringa oleifera*: a food plant with multiple medicinal uses. Phytother. Res. 21, 17–25.

Association of Official Analytical Chemists (AOAC), 1990. Official methods of analysis, 15th ed. Association of Official Analytical Chemists, Washington, DC.

Babu, K., Tiwari, A.K., Srinivas, P.V., Ali, A.Z., Raju, B., Rao, J.M., 2004. Yeast and mammalian α-glucosidase inhibitory constituents from Himalayan rhubarb *Rheum emodi* Wall. Ex Meisson. Bioorg. Med. Chem. Lett. 14, 3841–3845.

Chethan, S., Sreerama, Y.N., Malleshi, N.G., 2008. Mode of inhibition of finger millet malt amylases by the millet phenolics. Food Chem. 111, 187–191.

Chew, Y.L., Goh, J.K., Lim, Y.Y., 2009. Assessment of *in vitro* antioxidant capacity and polyphenolic composition of selected medicinal herbs from Leguminosae family in Peninsular Malaysia. Food Chem. 116, 13–18.

Choi, J.J., Park, B., Kim, D.H., Pyo, M.Y., Choi, S., Son, M., Jin, M., 2008. Blockade of atopic dermatitis-like skin lesions by DA-9102, a natural medicine isolated from *Actinidia arguta*, in the Mg-deficiency induced dermatitis model of hairless rats. Exp. Biol. Med. 233, 1026–1034.

Dong, H.Q., Li, M., Zhu, F., Liu, F.L., Huang, J.B., 2012. Inhibitory potential of trilobatin from *Lithocarpus polystachyus* Rehd against α-glucosidase and α-amylase linked to type 2 diabetes. Food Chem. 130, 261–266.

Doughari, J.H., Ndakidemi, P.A., Human, I.S., Benade, S., 2012. Antioxidant, antimicrobial and antiverotoxic potentials of extracts of *Curtisia dentate*. J. Ethnopharmacol. 141, 1041–1050.

Falchetti, R., Fuggetta, M.P., Lanzilli, G., Tricarico, M., Ravagnan, G., 2001. Effects of resveratrol on human immune cell function. Life Sci. 70, 81–96.

Gliszczynska-Swiglo, A., 2006. Antioxidant activity of water soluble vitamins in the TEAC (trolox equivalent antioxidant capacity) and the FRAP (ferric reducing antioxidant power) assays. Food Chem. 96, 131–136.

Halvorsen, B.L., Holte, K., Myhrstad, M.C.W., Barikmo, I., Hvattum, E., Remberg, S.F., et al, 2002. A systematic screening of total antioxidants in dietary plants. J. Nutr. 132, 461–471.

Islam, S., 2006. Sweetpotato (*Ipomea batatas* L.) leaf: its potential effect on human health and nutrition. J. Food Sci. 71, R13–R18.

Kim, J.G., Beppua, K., Kataokaa, I., 2009. Varietal differences in phenolic content and astringency in skin and flesh of hardy kiwifruit resources in Japan. Sci. Hort. 120, 551–554.

Li, Y., Wenb, S., Kotaa, B.P., Penga, G., Lia, G.Q., Yamaharac, J., Roufogalisa, B.D., 2005. *Punica granatum* flower extract, a potent α-glucosidase inhibitor, improves postprandial hyperglycemia in Zucker diabetic fatty rats. J. Ethnopharmacol. 99, 239–244.

Lin, J.Y., Tang, C.Y., 2008. Strawberry, loquat, mulberry, and bitter melon juices exhibit prophylactic effects on LPS-induced inflammation using murine peritoneal macrophages. Food Chem. 107, 1587–1596.

Liu, S.C., Lin, J.T., Wang, C.K., Chen, H.Y., Yang, D.J., 2009. Antioxidant properties of various solvent extracts from lychee (*Litchi chinenesis* Sonn.) flowers. Food Chem. 114, 577–581.

MacMicking, J., Xie, Q.W., Nathan, C., 1997. Nitric oxide and macrophage function. Annu. Rev. Immunol. 15, 323–350.

Matich, A.J., Young, H., Allen, J.M., Wang, M.Y., Fielder, S., McNeilage, M.A., MacRae, E.A., 2003. *Actinidia arguta*: volatile compounds in fruit and flowers. Phytochemistry 63, 285–301.

Mikulic-Petkovsek, M., Schmitzer, V., Slatnar, A., Stampar, F., Veberic, R., 2012. Composition of sugars, organic acids, and total phenolics in 25 wild or cultivated berry species. J. Food Sci. 77, C1064–C1070.

Moncada, S., Palmer, R.M.J., Higgs, E.A., 1991. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol. Rev. 43, 109–142.

Nicholas, C., Batra, S., Vargo, M.A., Voss, O.H., Gavrilin, M.A., Wewers, M.D., Guttridge, D.C., Grotewold, E., Doseff, A.I., 2007. Apigenin blocks lipopolysaccharide-induced lethality in vivo and proinflammatory cytokines expression by inactivating NF-kappa B through the suppression of p65 phosphorylation. J. Immunol. 179, 7121–7127.

Rankin, J.A., 2004. Biological mediators of acute inflammation. AACN Clin. 15, 3–17.

Ravipati, A.S., Zhang, L., Koyyalamudi, S.R., Jeong, S.C., Reddy, N., Bartlett, J., Smith, P.T., Shanmugam, K., Munch, G., Wu, M.J., Satyanarayanan, M., Vysetti, B., 2012. Antioxidant and anti-inflammatory activities of selected Chinese medicinal plants and their relation with antioxidant content. BMC Complement. Altern. Med. 12, 173 (1–14).

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26, 1231–1237.

Sun, T., Ho, C.T., 2006. Antioxidant activities of buckwheat extracts. Food Chem. 90, 743–749.

Takano, F., Tanaka, T., Tsukamato, E., Yahagi, N., Fushiya, S., 2003. Isolation of (+)-catechin and (−)-epicatechin from *Actinidia arguta* as bone marrow cell proliferation promoting compounds. Planta Med. 69, 321–326.

Terao, J., 2009. Dietary flavonoids as antioxidants. Forum Nutr. 61, 87–94.

Tundis, R., Loizzo, M.R., Statti, G.A., Menichini, F., 2007. Inhibitory effects on the digestive enzyme α-amylase of three *Salsola* species (Chenopodiaceae) *in vitro*. Pharmazie 62, 473–475.

Tyrakowska, B., Soffers, A.E.M.F., Szymusiak, H., Boeren, S., Boersma, M.G., Lemanska, K., Vervoort, J., Rietjens, I.M.C.M., 1999. TEAC antioxidant activity of 4-hydroxybenzoates. Free Radic. Biol. Med. 27, 1427–1436.

Webby, R.F., 1991. A flavonol triglycoside from *Actinidia arguta* var. *giraldii*. Phytochemistry 30, 2443–2444.

Yangthong, M., Nongporn, H.T., Phromkunthong, W., 2009. Antioxidant activities of four edible seaweeds from the southern coast of Thailand. Plant Foods Hum. Nutr. 64, 218–223.

Yen, G.C., Duh, P.D., Huang, D.W., Hsu, C.L., Fu, T.Y.C., 2008. Protective effect of pine (*Pinus morrisonicola* Hay.) needle on LDL oxidation and its anti-inflammatory action by modulation of iNOS and COX-2 expression in LPS-stimulated RAW 264.7 macrophages. Food Chem. Toxicol. 46, 175–185.

Zheng, J., He, J., Ji, B., Li, Y., Zhang, X., 2007. Antihyperglycemic effects of *Platycodon grandiflorum* (Jacq.) A. DC extract on streptozotocin-induced diabetic mice. Plant Foods Hum. Nutr. 62, 7–11.