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### **Original Article**

# Nutritional and nutraceutical characteristics of Sageretia theezans fruit



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#### ABSTRACT

The fruit of Sageretia theezans is one of many underutilized edible fruits that grow along the southern seashores of East Asia. In this study, to evaluate the nutritional and nutraceutical values of S. theezans fruit, the composition of minerals, organic acids, and proximate and fatty acids, the total phenolic, total flavonoid, and total anthocyanin content, and the antioxidant and antidiabetic activities of S. theezans fruit were analyzed. The results indicate that S. theezans fruit could be classified as a potential potassium-, malic acid-, and linoleic/oleic acid-rich fruit. In addition, The ethyl acetate (EtOAc) fraction of the 70% ethanol (EtOH) crude extract exhibited strong antioxidant activities including free radical scavenging and reducing power activities compared with the same concentration of butylated hydroxytoluene. Furthermore, the EtOAc fraction showed significant inhibition of  $\alpha$ -glucosidase activity. The analysis of the total phenolic and flavonoid content suggested that the remarkable antioxidant and antidiabetic activities of the EtOAc fraction are due to the presence of high levels of polyphenolic compounds.

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#### 1. Introduction

Sageretia theezans, commonly known as Chinese sweet plum, alternatively named Chinese bird plum, is an evergreen tender shrub of the Rhamnaceae family [1]. Although S. theezans is widely used to create bonsais, it has been used in traditional herbal medicine for the treatment of hepatitis and fevers in Korea and China [1-3]. Since friedeline, syringic acid, betasitosterol, daucosterol, gluco-syringic acid, and taraxerol were isolated from S. theezans [4], flavonol glycosides from leaves, named 7-O-methylmearnsitrin, myricetrin, kaempferol 3-O-R-L-rhamnopyranoside, europetin 3-O-R-L-rhamnoside, and 7-Omethyl quercetin 3-O-R-L-rhamnopyranoside, have been isolated [1]. Based on the in vitro analysis of free radical scavenging activity, myricetrin and 7-O-methylmearnsitrin have shown stronger antioxidant activities than ascorbic acid and atocopherol [1]. In addition, flavonoid-rich fractions from S. theezans leaves exhibited a protective effect on low-density lipoprotein against oxidative modification [1]. This indicates the considerable potential of S. theezans as a resource for functional applications in the cosmetic and pharmaceutical industries.

In developing countries, numerous types of edible wild plants are exploited as sources of supplementary nutrition including carbohydrates, protein, fat, vitamins, and minerals [5]. The Food and Agricultural Organization reported that at least one billion people are thought to use wild food in their diet [6,7]. In the past decades, numerous studies have demonstrated the value of wild fruits to provide nutritional and medicinal needs and suggested that the kinds and amounts of minerals, organic acids, and dietary fibers they include are important factors for determining whether wild fruits and vegetables are potential sources for dietary health supplements [8]. In addition, the World Health Organization emphasizes that the antioxidant activity of phenolic compounds from small colorful fruits plays an important role in the protection of cellular compounds such as lipids, membranes, proteins, and nucleic acids from oxidative damage, resulting in preventing many health problems, such as, cancer, diabetes, cardiovascular diseases, and obesity [9,10]. In the case of S. theezans, the fruit is hexose-dominated (glucose, 12.7% of pulp dry weight; fructose, 11.8% of pulp dry weight [11]), and a flavonoid-rich fraction of leaves has been suggested as a candidate material for use in functional foods and dietary supplements [1]. Although the fruit is known to be edible, the nutritional information for the raw fruit is unavailable.

Therefore, in this study, we evaluate the nutritional value of S. theezans fruit by analyzing its minerals, organic acids, proximate composition, and fatty acid content. In addition, the pharmaceutical properties of the fruit such as its antioxidant and antidiabetic activities were determined. These results will help consumers and researchers to make it a regular source of food and a dietary health supplement.

#### 2. Methods

#### 2.1. Plant materials

S. theezans fruits were collected in Jeju Island, Republic of Korea, in May 2012. The collected samples were washed under

running tap water and rinsed in distilled water. Fresh or freeze dried samples were used for experiments.

# 2.2. The measurement of pH, total soluble solids, and total acidity

The pH and total soluble solids (in degrees Brix) of the juice of S. theezans fruits were determined using a pH meter (Metrohm-827, Metrohm AG, Herisau, Switzerland) and a refractometer (PAL-1, Atago, Tokyo, Japan).

The total acidity of the juice was determined using a titration method; 10 mL of the diluted juice (10% solution of the sample) was titrated against 0.05 N of NaOH using a phenolphthalein indicator. The end point was noted (the color changed from colorless to pale pink). The results were expressed as a percentage of malic acid according to AOAC [12].

#### 2.3. Proximate analysis

The moisture content was determined after drying the sample at 105°C until a constant weight was attained, calculated as the loss in weight of the original sample and expressed as the percentage of moisture content [12]. The crude protein was determined by the macro-Kjeldahl method [12]. The percent protein was calculated by multiplying the percent nitrogen by the conversion factor 6.25. Crude lipids were extracted using the Soxhlet extraction method, and the ash content was determined according to methods outlined in AOAC [12]. Crude fiber was estimated by acid-base digestion with H<sub>2</sub>SO<sub>4</sub> (1.25%) and NaOH (1.25%) solution as described by Association of Analytical Chemists International (AOAC) [12].

#### 2.4. Mineral composition

S. theezans fruits (freeze dried materials) were digested with H<sub>2</sub>SO<sub>4</sub> and H<sub>2</sub>O<sub>2</sub>. Then, their mineral composition (e.g., K, Ca, Mg, Na, Mn, Fe, P, Mo, B, and Cu) was determined by inductively-coupled argon plasma optical emission spectrometry, using a Perkin-Elmer Optima 7300 DV system (PerkinElmer, Waltham, MA, USA).

#### 2.5. The analysis of organic acids

First, 1 g of the freeze dried sample was mixed with 100 mL of distilled water. After centrifugation at 6500 g for 10 minutes, the supernatant was diluted with ultra-pure water. The diluted sample was loaded on a Sep-Pack C18 cartridge (Waters Millipore, Milford, MA, USA) and then filtered through a  $0.2~\mu m$  membrane filter. To determine the concentration of organic acids including oxalic acid, tartaric acid, malic acid, lactic acid, citric acid, and succinic acid, a Grace prevail organic acid column (150 mm  $\times$  46 mm, 3  $\mu$ m) was applied, with 0.5mM of KH<sub>2</sub>PO<sub>4</sub> serving as the mobile phase at a flow rate of 0.5 mL/min. The high-performance liquid chromatography-photodiode array apparatus consisted of a Waters Alliance 2695 separations module and a Waters 2996 photodiode array detector with Empower 2 software for data acquisition (Waters Millipore). The standard calibration curves of organic acids were constructed in the concentration range of 50– $400 \,\mu g/mL$ . The quantification was performed by peak integration using the external standard method.

#### 2.6. The analysis of fatty acid composition

The lipid of *S. theezans* fruits was extracted with ethyl ether in a Soxhlet apparatus for 24 hours. The extraction was carried out at  $40^{\circ}$ C. The solvent was removed with a rotary vacuum evaporator. The lipid sample was hydrolyzed with 0.5N of NaOH in methanol at  $100^{\circ}$ C for 10 minutes. Then, fatty acid methyl esters were prepared by adding 14% BF<sub>3</sub> in methanol at  $37^{\circ}$ C for 20 minutes. Water was added to the solution, and then the methyl esters were recovered from the aqueous phase by extracting them three times with 1 mL hexane. The organic phase was then washed three times with 1 mL water and dried by adding Na<sub>2</sub>SO<sub>4</sub>. After vortexing and centrifugation, the hexane was evaporated and the residue dissolved in a small amount of hexane for gas chromatography analysis.

Fatty acid methyl esters were determined by gas chromatography using a Varian CP-3800 (Varian Analytic Instruments, Walnut Creek, CA, USA) gas chromatograph equipped with a flame ionization detector and SP-2560 fused silica capillary column (100 mm  $\times$  0.25 mm, 0.2  $\mu$ m, Supelco, Bellefonte, PA, USA). The oven temperature was maintained at 150°C for 2 minutes, and then increased to 200°C at 2°C/min. Injector and detector temperatures were 270°C and 290°C, respectively. Nitrogen gas was used for the carrier. Fatty acid methyl esters were identified by comparing the retention time of the samples and appropriate fatty acid methyl ester standards, purchased from Sigma-Aldrich (St. Louis, MO, USA).

#### 2.7. Analysis of anthocyanin contents

The total anthocyanin content was determined using the pH differential method described by Chen et al [13], and 1 g of the freeze dried sample was soaked in acidified methanol (0.1N HCl in methanol) for 24 hours in the dark. The anthocyanin extract was diluted with potassium chloride buffer (0.025M, a pH of 1.0) or sodium acetate buffer (0.4M, a pH of 4.5) by using a predefined dilution factor. After 15 minutes of incubation, absorbencies were read at 510 nm and 700 nm. The anthocyanin content was calculated as cyanidin 3-glucoside (C3G) with an extinction coefficient of 26,900 L/cm·mg and molecular weight of 449.2. The results were expressed as mg of C3G equivalents/g of extract.

### 2.8. Preparation of extract and fractions

The crude methanolic extract and fractions from *S. theezans* fruits were prepared as described previously [14]. The freeze dried fruits were ground into a fine powder. The ground material was soaked in methanol [1:10 dry weight material to 70% EtOH (mL)] for 24 hours and sonicated at 55°C in an ultrasonic bath (Power Sonic 520, Hwashin Co., Yeongcheon, Korea). After filtration, the 70% EtOH extract was evaporated using a rotary vacuum evaporator. The crude 70% EtOH extract was suspended in water, and then sequentially partitioned with equal volume of *n*-hexane, ethyl acetate (EtOAc), and *n*-butanol (BuOH). The remaining aqueous extract was used as an aqueous fraction. Each fraction was evaporated using a

vacuum evaporator. Then,  $1000~\mu g$  of 70% EtOH extract and its fractions was redissolved in 1mL of 70% EtOH or hexane (only for hexane fraction) for further analysis.

#### 2.9. Analysis of total phenolic and flavonoid contents

The total phenolic content (TPC) and the total flavonoid content (TFC) were determined according to the Folin-Ciocalteu method and colorimetric method, respectively, described by Hyun et al [15]. The TPC in the 70% EtOH extract and its fractions were calculated in mg of gallic acid equivalents (GAE)/g of extract using the equation obtained from the standard gallic acid graph. In addition, the TFC was determined as mg of quercetin equivalent (QE)/g of extract.

#### 2.10. Antioxidant activities by chemical-based assays

The antioxidant activity of the crude 70% EtOH extract and its fractions was determined by monitoring the disappearance of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) at 517 nm and the reducing power assay at 700 nm, according to the procedures of Hyun et al [14]. The DPPH free radical scavenging activity was calculated as percentage inhibition (%).

To determine the total antioxidant capacity, the ammonium molybdate reduction method [16] was used. Different concentrations of each sample (100  $\mu g/mL$ , 200  $\mu g/mL$ , and 300  $\mu g/mL$ ) were mixed with 3 mL of reagent solution including 0.6M of sulfuric acid, 28mM of sodium phosphate, and 4mM of ammonium molybdate. The reaction mixture was incubated in a water bath at 95°C for 90 minutes, and then the mixture was cooled to room temperature. The absorbance was measured at 695 nm. Ascorbic acid and butylated hydroxytoluene (BHT) were used to compare all the antioxidant activity tests.

#### 2.11. $\alpha$ -Glucosidase inhibition assay

The inhibition of  $\alpha$ -glucosidase activity was performed according to a previously described method [17]. For  $\alpha$ -glucosidase inhibitory activity, the half maximal inhibitory concentration (IC<sub>50</sub>) value was calculated by linear regression analysis of the activity under the assay conditions.

#### 2.12. Statistical analysis

All of the experiments were conducted for three independent replicates, and data were expressed as mean  $\pm$  standard deviation. Statistical analyses were performed by analysis of variance. Duncan's test was used to determine the significance of differences between the groups. Differences at p < 0.05 were considered significant.

#### 3. Results and discussion

# 3.1. Physical and chemical characteristics of S. theezans fruits

Although S. theezans fruit is known as a wild food, the nutrition value of this fruit has not been investigated. Therefore,

we first determined the fruit's quality defined by different parameters. The pH, total acidity (as malic acid %), and total soluble solids in the S. theezans fruit were  $4.17 \pm 0.05$ ,  $0.83 \pm 0.03\%$  and  $22.2 \pm 0.06$  Brix, respectively (Table 1). The total acidity and total soluble solids (Brix) are important factors related to food flavor, whereas the pH of food indicates its resistance to microbial attack [18,19]. In addition, pH plays an important role in giving juice its color. At a pH < 2, anthocyanin existed in the form of red flavylium cation, whereas a rapid proton loss occurred to yield blue quinonoidal forms at a pH > 4.5 [19].

The proximate analysis showed the moisture content of S. theezans fruit to be  $75.04 \pm 0.01$  (w/w) (Table 2). The moisture content indicates the water content in samples, and the moderate level of moisture content indicated that it can be stored for a long time without developing mold [20]. The high level of moisture content in S. theezans fruit indicates the low shelf life of the fresh fruit. The level of crude fiber in food can be an indicator of the level of nondigestible carbohydrate and lignin [21]. The crude fiber obtained from S. theezans fruits was  $3.32 \pm 0.20\%$  of the fresh weight (Table 2), which is higher than blueberries (1.48  $\pm$  0.12%) and raspberries (0.85  $\pm$  0.05%) reported by Jeong et al [22]. The consumption of fruits containing high crude fiber content contributes to the prevention of diseases like cancer, high blood pressure, coronary heart diseases, diabetes, obesity, and digestive disorders [23,24]. Ash is the residue remaining after all the moisture and the organic material like fat, protein, carbohydrates, organic acid, and so on is removed. Therefore, it usually indicates the content of the total mineral in food [21,25]. The ash content of S. theezans fruit was  $0.48 \pm 0.03\%$  (Table 2), which is similar to pineapples (range, 0.36-0.76%) and oranges (range, 0.49-0.63%) reported by Ogoloma et al [26]. In addition, S. theezans fruit contained  $0.84 \pm 0.02\%$  of crude fat and  $2.01 \pm 0.48\%$  of crude protein (Table 2).

Minerals are required to form the body's structure and to regulate chemical reactions, although they are needed in only very small amounts. In S. theezans fruit, potassium  $(639.4 \pm 39.8 \text{ mg/}100 \text{ g, dry basis})$  was the most abundant element (Table 3). It was higher in content than spinach (470 mg/100 g [27]), which is known as a potassium-rich food. An increased level of dietary potassium reduces blood pressure, whereas reduced potassium intake raises blood pressure in animals and humans [28]. This indicates that S. theezans fruit is a potential source for controlling high blood pressure. In addition, the mineral content values in S. theezans fruit proceeded in the following order: Na (85.1  $\pm$  17.9 mg/100 g) > P  $(84.9 \pm 4.1 \text{ mg/}100 \text{ g}) > \text{Mg} (57.5 \pm 4.9 \text{ mg/}100 \text{ g}) > \text{Ca}$ (44.1  $\pm$  4.8 mg/100 g) (Table 3). Furthermore, this fruit could theoretically provide other nutritionally useful minerals including boron, iron, copper, molybdenum, and manganese (Table 3).

Table 1- Physicochemical characteristics of Sageretia theezans fruit.

рН	Total soluble solids (Brix)	Total acidity (%)
4.17 ± 0.05	$22.2 \pm 0.06$	$0.83 \pm 0.03$

Table 2 - Proximate composition of Sageretia theezans fruit.

Composition	Content (%)
Moisture	$75.04 \pm 0.01$
Crude protein	$2.01 \pm 0.48$
Crude fat	$0.84 \pm 0.02$
Crude fiber	$3.32 \pm 0.20$
Crude ash	$0.48 \pm 0.03$

Organic acids, which are primary metabolites found in all plants, have been used to maintain the quality and nutritive value of foods, whereas they are used as antioxidants, preservatives, and drug absorption modifiers in the pharmaceutical industry [29,30]. Citric, malic, and tartaric acids are commonly found in fruits and berries, while a significant amount of oxalic acid is found in green leaves [31]. In S. theezans fruit, malic acid (2631.5  $\pm$  80.9 mg/100 g, dry basis) was the dominant organic acid, and was typically present at concentrations two times higher than the second most abundant organic acid, lactic acid (1237.4 ± 44.9 mg/ 100 g, dry basis) (Table 3). Malic acid has protective effects against myocardial ischemia/reperfusion injury [32], and exhibits antioxidant activity [33]. In addition, this compound may be helpful to individuals suffering from chronic fatigue and fibromyalgia [34]. Although the profile and concentration of organic acids in fruits and vegetables depends on the species and environmental conditions [35], this finding indicates that S. theezans fruit is a good source of malic acid.

The quality and flavor of fruits can be affected by the fatty acid composition. In addition, plant fatty acids, which are the building blocks of most lipids, have important properties that could be used to treat a wide range of diseases [36]. Recently, it

Table 3 — Mineral, organic acid and total anthocyanin content of Sageretia theezans fruit.

Variables	_	Content (mg/100 g, dry basis)	
Minerals	K	639.4 ± 39.8	
	Ca	$44.1 \pm 4.8$	
	Mg	$57.5 \pm 4.9$	
	Na	85.1 ± 17.9	
	Mn	$0.8 \pm 0.0$	
	Fe	$2.1 \pm 0.5$	
	P	$84.9 \pm 4.1$	
	Mo	$1.4 \pm 0.2$	
	В	$3.6 \pm 1.2$	
	Cu	$1.6 \pm 0.2$	
	Total	1,264.90	
Organic acids	Oxalic acid	$51.8 \pm 2.1$	
	Tartaric acid	594.4 ± 7.7	
	Malic acid	2631.5 ± 80.9	
	Lactic acid	$1237.4 \pm 44.9$	
	Citric acid	$189.1 \pm 38.0$	
	Succinic acid	576.6 ± 119.5	
Total anthocyanins		Content (mg C3G/100g) <sup>a</sup>	
		520.8 ± 55.9	

 $<sup>^{\</sup>rm a}$  Total anthocyanins analyzed as the cyaniding 3-glucoside (C3G) mg/g of extract; values are the average of triplicates.

has been suggested that a diet rich in polyunsaturated fatty acids is effective in lowering serum cholesterol and reducing coronary heart disease [37,38], indicating the dietary importance of fatty acid composition. To investigate the composition of fatty acids in S. theezans fruit, 12 fatty acids, including saturated fatty acids and unsaturated fatty acids, were determined. Linoleic acid (C18:2n6c) was found to be the dominant fatty acid followed by oleic acid (C18:1n9c) and palmitic acid (C16:0) (Table 4). Linoleic acid, the primary dietary omega-6 polyunsaturated fatty acid (omega-6 PUFA), can be desaturated and elongated to form other omega-6 PUFAs including  $\gamma$ -linolenic and dihomo- $\gamma$ -linolenic acids, which are converted to the metabolically important omega-6 PUFA arachidonic acid [39]. Linoleic and  $\alpha$ -linolenic acids (omega-3 PUFA) are regarded as essential fatty acids, since they cannot be synthesized in humans and animals [40]. In addition, the amount of unsaturated fatty acids in S. theezans fruit (UFA, 74.96%) was higher than saturated fatty acids (SFA, 25.04%) giving an unsaturated to saturated ratio of 2.5 (Table 4). The UFA:SFA ratio indicates that S. theezans fruit could be classified as a potential linoleic/oleic-rich fruit having good nutritional

S. theezans has deep purple-colored drupe-shaped fruits (up to 1 cm in diameter), indicating that this fruit contains a high amount of anthocyanins. Anthocyanins, natural pigments, are one of the major flavonoid classes and are beneficial bioactive flavonoid compounds to protect individuals from many chronic diseases [41]. The major sources of anthocyanins in edible plants are the families Vitaceae (grape), Rosaceae (raspberry, strawberry, blackberry, apple, peach, etc.), and Ericaceae (blueberry and cranberry). Although the anthocyanin composition of blueberries varies depending on cultivars and the environmental growth conditions, it has been reported that blueberries contain anthocyanin ranging from 20 mg/ 100 g to 311 mg/100 g fresh weight [41]. In the case of S. theezans, the total anthocyanins found in fresh fruits were 520.8 ± 55.9 mg/100 g fresh weight, expressed as C3G equivalents (Table 3). The presence of a high level anthocyanin in S.

Table 4 — Fatty acid composition of Sageretia theezans

Fatty acids	g/100 g fatty acid	Proportion (%)
Lauric (C12:0)	0.0004 ± 0.0000	0.04
Myristic (C14:0)	$0.0012 \pm 0.0001$	0.14
Palmitic (C16:0)	$0.1525 \pm 0.0082$	17.09
Palmitoleic (C16:1)	$0.0029 \pm 0.0003$	0.33
Margaric (C17:0)	$0.0009 \pm 0.0001$	0.1
Stearic (C18:0)	$0.0684 \pm 0.0038$	7.67
Oleic (C18:1n9c)	$0.1989 \pm 0.0099$	22.3
Linoleic (C18:2n6c)	$0.2856 \pm 0.0148$	32.02
γ-linolenic (C18:3n6c)	$0.0278 \pm 0.0015$	3.12
cis-11-Eicosenoic acid ester (C20:1)	$0.1280 \pm 0.0016$	14.3
α-linolenic (C18:3n3)	$0.0156 \pm 0.0007$	1.75
cis-11.14.17-Eicosa trienoic (C20:3n3)	$0.0099 \pm 0.0003$	1.11
Sub total	0.892	100
Saturated fatty acid	0.2362	25.04
Unsaturated fatty acid	0.5856	74.96

theezans fruit would indicate its potential as a dietary health supplement.

# 3.2. Antioxidant and antidiabetic effects of S. theezans fruit

Oxidative stress due to the production of free radicals such as superoxide, hydroxyl, peroxide, and nitric oxide radicals is the major cause of numerous chronic diseases [42]. Since synthetic antioxidants such as BHT and butylated hydroxyanisole have been found to have side effects, plant phytochemicals have been widely studied as reactive oxygen species scavengers. In the case of S. theezans, flavonoid-rich fractions of leaves extract exhibited strong antioxidant activity [43]. In addition, the high level of anthocyanin in S. theezans fruit might indicate that S. theezans fruit should be used as an ingredient in antioxidant supplements. Therefore, to investigate the antioxidant activity of S. theezans fruit extract, we determined the antioxidant activities of the crude 70% EtOH extract and its fractions on the basis of DPPH free radical scavenging activity, reducing power, and total antioxidant capacity. As shown in Fig. 1A, the free radical scavenging activity of the extract increased in a dose-dependent manner. The scavenging effect of crude 70% EtOH extract and its fractions on DPPH free radical was in the following order: EtOAc fraction > aqueous fraction > BuOH fraction > 70% EtOH extract > hexane fraction (Fig. 1A). In addition, we determined Fe<sup>3+</sup>-Fe<sup>2+</sup> transformation in the presence of the crude 70% EtOH extract and its fractions using a reducing power assay. The EtOAc fraction (86.29  $\pm$  1.03% with 100  $\mu$ g/ mL extract) showed a higher reductive ability than BHT  $(36.02 \pm 2.53\% \text{ with } 100 \,\mu\text{g/mL BHT})$  (Fig. 1B). Furthermore, the BuOH fraction showed the highest total antioxidant capacity followed by the EtOAc fraction (Fig. 1C). The antioxidant activities of plant extracts are mediated by various mechanisms of action, such as radical scavenging, the reductive capacity on metals, the binding of heavy metal ion catalysts, the breakdown of peroxides, and the inhibition of chain initiation [44]. The BuOH fraction showed a relatively lower reductive ability and free radical scavenging activity than the EtOAc fraction (Figs. 1A and 1B), whereas the highest total antioxidant capacity was observed in the BuOH fraction (Fig. 1C). This indicates that the antioxidant activity of the BuOH fraction is mainly due to the redox property, which plays an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [45].

Folk plants and other sources of natural antidiabetic compounds have been increasingly used due to growing concerns over the safety of synthetic antidiabetic agents [17,46,47]. To identify the potential of S. theezans fruit as an antidiabetic supplement, we investigated the effect of crude 70% EtoH extract and its fractions on the activity of  $\alpha$ -glucosidase, an enzyme involved in the digestion of carbohydrates. As shown in Table 5, the EtoAc fraction (IC $_{50}=9.98\pm0.39~\mu g/$  mL) exhibited the highest inhibitory effect followed by the aqueous fraction (IC $_{50}=44.65\pm2.27~\mu g/$ mL), 70% EtoH extract (IC $_{50}=77.77\pm3.2~\mu g/$ mL), and BuOH fraction (IC $_{50}=78.05\pm0.81~\mu g/$ mL). The presence of a high level of these inhibitory activities in the EtoAc fraction indicates that

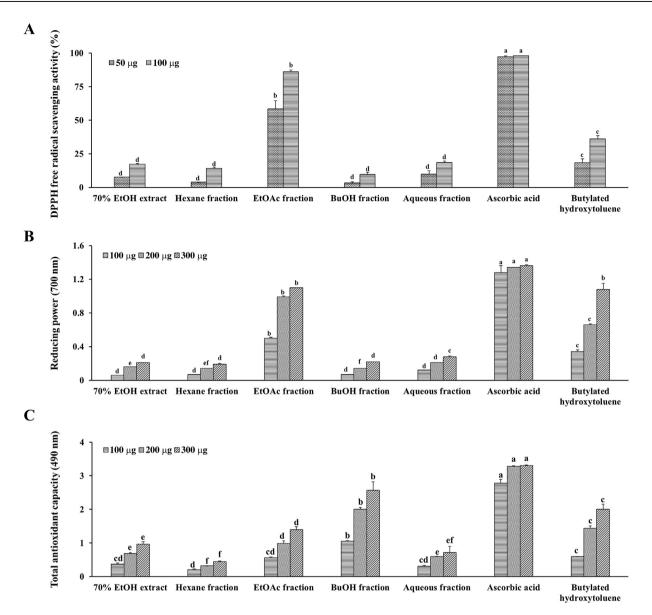


Fig. 1 — Antioxidant activities of Sageretia theezans fruit extract. Antioxidant activities were measured by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging activity (A), reducing power assay (B), and total antioxidant activity analysis (C). Values are the average of triplicate experiments and represented as mean  $\pm$  standard deviation. <sup>a-f</sup> Values in the same column with different superscripted letters are significantly different (p < 0.05).

the S. theezans fruit extract could potentially suppress the increase in postprandial glucose by starch.

Since various phenolic compounds including flavonoids have been generally accepted as antioxidant agents [15,48], many of these compounds have shown inhibitory activities against  $\alpha$ -glucosidase and  $\alpha$ -amylase [14]. These indicate that polyphenolic compounds are major contributors to antioxidant and antidiabetic activities. The total phenolic and flavonoid content (TPC and TFC, respectively) in the crude 70% EtOH extract of S. theezans fruit was 176  $\pm$  0.0 mg of GAE/g extract and 22.0  $\pm$  0.0 mg of QE/g extract, respectively (Table 5). The highest level of TPC and TFC was analyzed from the EtOAc fraction, which contained 2049  $\pm$  24 mg of GAE/g extract and 264.2  $\pm$  15.9 of QE/g extract, respectively. This indicates that the significant levels of antioxidant and antidiabetic activities

in the EtOAc fraction might be due to the presence of polyphenolic compounds, known to be soluble in polar solvents.

#### 4. Conclusion

In this study, we analyzed the nutritional compositions (the composition of proximate, minerals, organic acids, and fatty acids) and the nutraceutical composition (the antioxidant and antidiabetic activities) of S. theezans fruit, which is known as an edible fruit. The overall results of the present study suggest that S. theezans fruit contains nutrients and minerals, together with polyphenolic compounds, that are useful for human as well as animal health. S. theezans fruit is a good source of anthocyanin and potential linoleic/oleic-rich fruit, indicating

Table  $5 - \alpha$ -Glucosidase activity, total phenolic content and total flavonoid content of *Sageretia theezans* fruit crude 70% ethanolic extract and its fractions.

Extract and fractions	α-glucosidase activity	Total phenol	Total flavonoid
	$IC_{50} (\mu g/ml)^a$	(mg GAE/g) <sup>b</sup>	(mg QE/g) <sup>c</sup>
70% EtOH extract	$77.77 \pm 3.2c^{d}$	176 ± 0.0d	$22.0 \pm 0.0b$
Hexane fraction	>100e	$165 \pm 16d$	$1.4 \pm 0.0c$
EtOAc fraction	$9.98 \pm 0.39a$	$2049 \pm 24a$	264.2 ± 15.9a
BuOH fraction	$78.05 \pm 0.81c$	$211 \pm 14c$	$27.2 \pm 2.6b$
Aqueous fraction	$44.65 \pm 2.27b$	$394 \pm 18b$	$8.0 \pm 1.3c$
Acarbose	$118.34 \pm 6.091d$		

 $<sup>^{</sup>a}$  ICs0, 50% inhibition of  $\alpha\text{-glucosidase}$  activity under assay conditions.

the considerable potential of *S. theezans* fruit as a resource for dietary health supplement. In addition, the presence of notable antioxidant and antidiabetic activities in the EtOAc fraction indicates that *S. theezans* fruit has potential as a crude drug and dietary health supplement. It will be required to investigate the isolation of the bioactive compounds, mechanisms of action, and safety of bioactive compounds. Such information may be useful for further studies on *S. theezans* fruit for its applications in pharmaceutical industries. Additional studies on the antinutritive, enzymatic, and molecular effects on human as well as animal health will be needed to motivate further interest in the use of this fruit.

### Conflicts of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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<sup>&</sup>lt;sup>b</sup> Total phenolic content analyzed as the gallic acid equivalent (GAE) mg/g of extract; values are the average of triplicates.

<sup>&</sup>lt;sup>c</sup> Total flavonoid content analyzed as the quercetin equivalent (QE) mg/g of extract; values are the average of triplicates.

 $<sup>^{\</sup>rm d}$  Values in the same column with different letters are significantly different (p < 0.05).

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