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Antioxidant and phenolic contents in potatoes (*Solanum tuberosum* L.) and micropropagated potatoes

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Abstract

This work investigated the extraction efficacy of phenolic acids on the potato and its byproducts. Also, the compositions of bioactive compounds and antioxidants were evaluated in various parts of the potato, such as the tuber, microtuber, peel, and flesh. The chemical constituents were quantified by HPLC analysis, and the highest levels of phenolics (88.99 mg/L) were obtained in acetone extracts from a micropropagated potato. The micropropagated potato demonstrated that notable phenolic compounds were mainly a bound form of phenolic acids including caffeic acid and vanillic acid. The micropropagated extracts using acetone showed the higher radical scavenging activity, 94.3% and 95.5% at 5 mg/mL in 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azinobis-(3-ethylbenzothiazoline)-6-sulfonic acid ($ABTS^{\cdot+}$), respectively. In addition, the same extracts showed the highest (85.61%) β-carotene bleaching inhibition activity. A positive relationship existed between DPPH and either $ABTS^{\cdot+}$ ($r = 0.58$, $p < 0.05$), β-carotene bleaching ($r = 0.65$, $p < 0.05$), or total phenolics ($r = 0.63$, $p < 0.05$). However, $ABTS^{\cdot+}$ did not show a significant correlation between both total phenolics and β-carotene bleaching. The effective phenolic compounds contributing to antioxidant activity were caffeic acid and vanillic acid, which could be extracted in high amounts by acetone from potato peels and micropropagated potatoes.

Keywords: β-Carotene bleaching, Microtuber, Phenolics, Potato, Radical scavenging

Introduction

The potato (*Solanum tuberosum* L.) is a root vegetable and one of the most widely consumed crops, followed by wheat and rice [1]. World potato production is estimated at about 400 million tons every year [2], and the value of production accounts for $3.7 billion annually in the United States [3]. Indeed, potato production has great socioeconomic impacts in society. Currently, less than 50% of potatoes are consumed fresh; the rest are used for processed potato products, animal feed, and seed tubers for the next season's crops [4]. Also, the food processing industry benefits more from French fries and chips than fresh potatoes [1]. Potatoes supply key nutrients—potassium, fiber, protein, calcium, and magnesium—and are also known as a consolidated source of functional ingredients—vitamins B6, C, and E and various polyphenols and phenolic acids [5].

Previous research has investigated the agricultural and health benefits of potatoes and related implications, such as antibiotic, anticancer, and antioxidant properties [6–8]. In particular, the potato peel contains a rich source of phenolic compounds [9]. Although bioactive compounds are present in both the flesh and peel, the peels are largely discarded during potato consumption and processing. Therefore, more investigation of the utilization of potato byproducts or waste, like the peels, is warranted.

Traditionally, potatoes are propagated by cutting the vegetative tissue, which is tubers containing nodes or eyes [10, 11]. Since the development of conventional techniques, such as tissue cultures, to obtain virus-free crops through mass propagation, micropropagated potatoes have been produced using tissue culture techniques

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[10, 12]. In addition, by using tissue culture techniques, the propagated potato has more peel units per gram of powder due to its small size. Despite the socioeconomic importance of micropropagated potatoes as seeds, comparisons and phytochemical investigations of the seed potato's chemical constituents are scant. Indeed, the health benefits of the propagated microtubers have been rarely examined.

In this context, the present study aimed to analyze the composition of bioactive compounds in both potatoes and micropropagated potatoes (microtubers) as well as different parts of the potato through reversed-phase high-pressure liquid chromatography (HPLC). Using tissue cultures in vitro, the seed potatoes were micropropagated and the bioactive compositions were investigated. Moreover, the total phenolic contents, radical scavenging activity of potato extracts, and extraction efficacy were evaluated. Finally, the correlation between the total phenolic contents and their potential antioxidant content in extracts using (1,1-diphenyl-2-picrylhydrazyl [DPPH], 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) [ABTS], and β-carotene bleaching assay) was assessed.

Materials and methods

Chemicals

All solvents used in this study were analytical grade (Duksan Co., Seoul, ROK). Folin–Ciocalteu, sodium carbonate, DPPH, ABTS, and β-carotene were purchased from Sigma-Aldrich (St Louis, MO, USA). Gallic acid was obtained from Bio Basic Inc. (Ontario, Canada). Linoleic acid was supplied by Wako pure chemical (Dallas, TX, USA). DL-α-tocopherol acetate (α-TA) was purchased from Daejung Chemicals (Siheung, ROK). Chlorogenic acid (purity $\geq$95%), caffeic acid, vanillic acid, quercetin glucoside, and *p*-Coumaric acid were supplied by Sigma-Aldrich (St Louis, MO, USA).

Plant materials

Potato cultivars of type Sumi (*Solanum tuberosum* L. cv. Sumi), supplied by Innoseed Systems (Chungbuk, ROK), were harvested in 2013. Microtubers of cv. Sumi were generated using the nodal cutting technique in vitro. The explants were placed in a specialized container with liquid Murashige and Skoog (MS) medium supplemented with 8.5% sucrose and vitamin supplements. All the liquid medium was adjusted to pH 5.8 and autoclaved at 121 °C, 15 psi, for 15 min. Stem segments (2–2.5 cm long) with many nodes were cultured into the liquid medium and incubated in a dark room at 20 °C. After 8 weeks, eight microtubers (0.2 g each) were obtained from each container (Fig. 1a).

Preparation of potato extracts

The Sumi tuber (6.5 kg) was washed with water, dried, and manually peeled to a depth of ~ 1 mm, and then the peel and the peeled potato (flesh) were blended separately with a commercial mixer. The tuber (unpeeled whole potato) and microtuber (1 kg) were washed with water and blended separately. The tuber, microtuber, peel, and flesh were dried and powdered using a freeze dryer (OPERON, ROK). Powdered potato (100 g) was added to 800 ml of acetone, methanol, and a mixture of methanol:water (80:20, v/v), respectively. After adding the solvent, the mixtures were shaken at 200 rpm overnight, and the extracts were filtered through filter paper. The collected extracts were concentrated in a rotary evaporator to remove the major amounts of the organic solvents and were further dried in a freeze dryer.

Chromatography conditions

Phenolic compounds were quantified using an Ultimate 3000 HPLC system (Dionex, California, USA) with a UV/VIS diode array detector coupled with an auto sampler and a quaternary pump. The separation was carried out using an Agilent Zorbax SB-C18 column (250 nm × 4.6 mm i.d. and 5 µm particle size) at a flow rate of 0.8 mL/min. The gradient elution was obtained using (A) 0.3% trifluoroacetic acid, and (B) acetonitrile as follows: 10–60% B (0–25 min), 100% B (26–35 min), and 10% B (36–40 min). The peaks were detected at 280 nm, and data acquisition was completed with Dionex Chromeleon 6.8 version software. Calibration curves were constructed for five standard substances, and the correlation coefficients of the calibration curves were found to be significant at $p < 0.01$. The structures of the standard compounds are presented in Fig. 1b.

Determination of total phenolic contents

The total phenolic contents in each extract were determined using a Folin–Ciocalteu reagent, according to a previously published procedure [13]. In brief, gallic acid (as a standard phenolic compound) and 100 µl of each extract were distributed in the test tube and mixed with 500 µl of the diluted Folin–Ciocalteu (1:1 ratio in distilled water). After a 10 min incubation at room temperature, 1 ml of sodium carbonate (7.5% w/v) was mixed in the same tube and allowed to react for 20 min at room temperature. The absorbance of the mixture was measured at 760 nm and calculated the percentage of phenolics. The content of total phenolics was expressed as gallic acid equivalent (GAE).

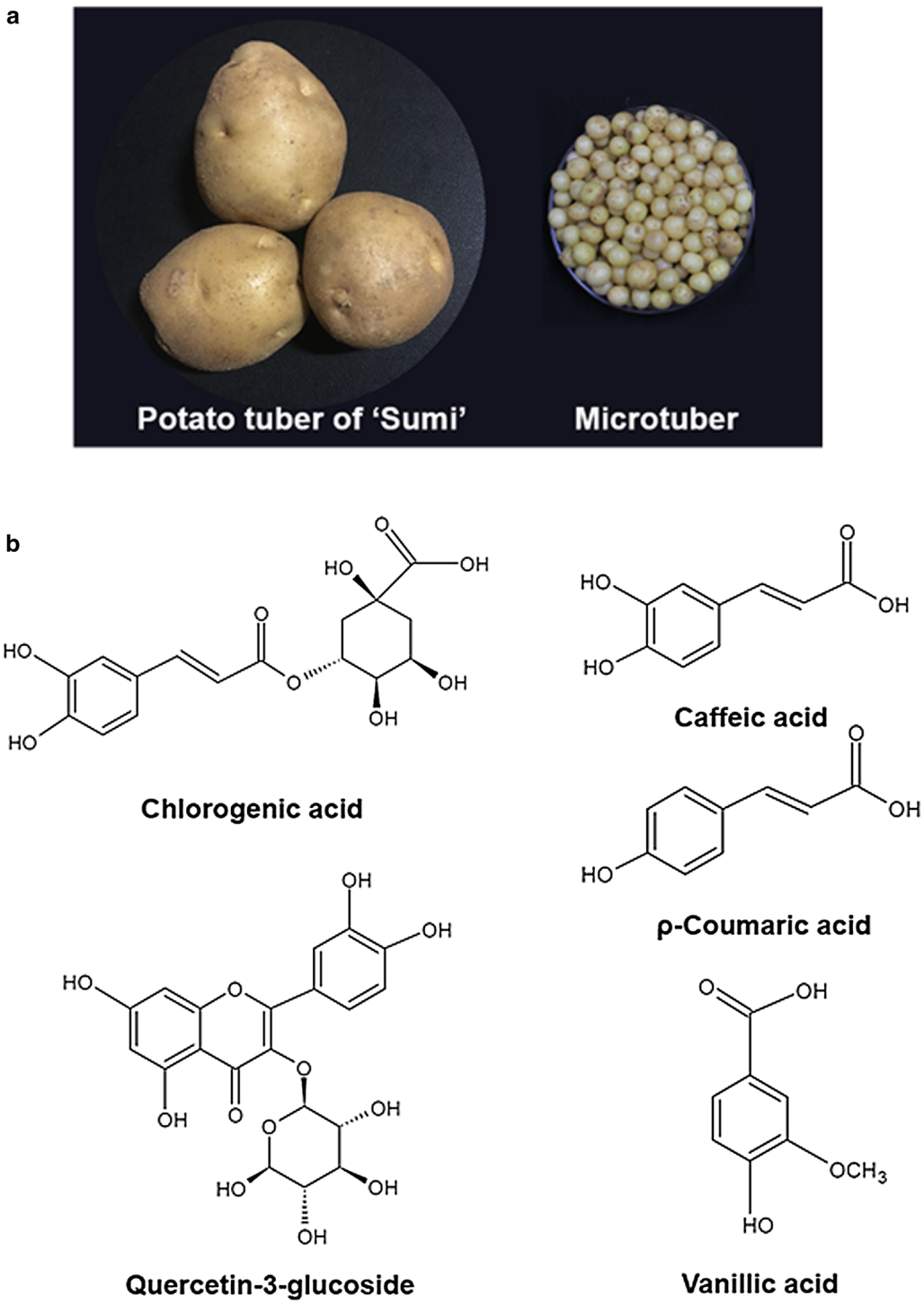

Fig. 1 **a** Sumi potato cultivar tuber and its micropropagated tuber (microtuber) using the tissue culture technique. **b** The structures of the tested phenolic compounds from the potato

DPPH radical scavenging activity

A DPPH radical solution (0.1 mM) was prepared with 38.35 mg of DPPH dissolved in 1000 ml of MeOH. Two different concentrations (2.5 and 5.0 mg/mL) of sample extracts and gallic acid as a control were presented into a 96-well plate and adjusted with MeOH to make 40 µl

of final volume. Two hundred microliters of DPPH solution was then added into and monitored for 33 min by absorbance at 518 nm using a FLUOstar Optima microplate reader (BMG Labtech, Durham, NC, USA).

ABTS radical scavenging activity

ABTS was dissolved in water to a 7 mM concentration, and 2.45 mM potassium persulfate was prepared. Two stock solutions were mixed and kept in the dark at room temperature for 16 h before use. The ABTS solution was diluted with methanol to an absorbance of 0.700 at 734 nm and equilibrated at 30 °C. After the addition of 200 µl of the diluted ABTS solution to 40 µl of the sample extracts (2.5 and 5.0 mg/mL concentration), the decrease in absorbance was measured for 1 min after mixing the solution, and the final absorbance reading was monitored for 33 min by absorbance at 734 nm using a FLUOstar Optima microplate reader (BMG Labtech, Durham, NC, USA).

β-Carotene bleaching activity

The current β-carotene bleaching activity was determined using a modification of the method described by Marco [14]. In this method, 5 mg β-carotene, 20 µl of linoleic acid, and 100 µl of Tween 20 were dissolved in 10 ml of chloroform in a round-bottom flask. After removing the chloroform, 10 ml of distilled water was added and stirred vigorously for 10 min. Thereafter, 240 µl of the emulsion was placed with 10 µl of extracts at various concentrations (1, 2, 3, 4, and 5 mg/mL) or butylated hydroxytoluene (BHT) as a positive control on 96-well plates. The absorbance was measured every 15 min for 120 min using the FLUOstar Optima microplate reader at 470 nm. The antioxidant activity of extracts in terms of β-carotene bleaching was calculated using the following formula: $AA\ \% = [1 - (Abs_0\ \text{sample} - Abs_{120}\ \text{sample})/(Abs_0\ \text{control} - Abs_{120}\ \text{control})] \times 100$.

Statistical analysis

was conducted using Microsoft Excel and SPSS 19.0 for Windows (Chicago, IL, USA). A one-way and a two-way analysis of variance (ANOVA) followed by the Tukey post hoc test were used to analyze statistical significance ($p < 0.05$). The Spearman correlation coefficient was applied to evaluate the degree of correlation between the different antioxidant activity categories, DPPH, ABTS, total phenolics, and beta-carotene bleaching. The analysis was carried out at least in triplicate. Significance and confidence level were estimated at $p < 0.05$.

Results and discussion

Total phenolic content and phenolic composition

Phenolics, which are well-known and important phytochemicals, are widely distributed in the plant kingdom, and variation in their chemicals occurs through different biosynthesis and chemical reactions such as hydroxylation, glycosylation, form of esters, and glycosides [15, 16]. Therefore, over the last 50 years, numerous research studies have focused on finding optimum extraction and identification techniques to obtain higher amounts of phenolic compounds from a source [17]. Phenolic compounds are secondary plant metabolites, and the most abundant phenolic acids, primarily chlorogenic acid, constitute up to 90% of the total phenolic compounds in potatoes [9]. For the current study, the total phenolic contents present in different extracts from various parts of the potato, such as the tuber, microtuber, flesh, and peel, are shown in Table 1. The amount of total phenolic

Table 1 Total phenolic content of respective extracts from different parts of the potato (tuber, flesh, and peel) and micropropagated potato tuber

Samples	Total phenolics (mg GAE per g of extract)		
	Acetone	Methanol	80% Methanol
Tuber	28.89 ± 0.28 aA	29.93 ± 1.28 aA	39.02 ± 2.96 bA
Microtuber	63.17 ± 4.59 aB	73.87 ± 7.74 bB	44.80 ± 4.26 cB
Flesh	22.83 ± 0.43 aC	28.58 ± 1.32 aA	27.74 ± 1.28 aC
Peel	38.99 ± 5.55 aD	29.24 ± 1.63 bA	24.08 ± 0.66 bC
Source	**Two-way ANOVA analysis**		
	df	**F**	***p***
Solvent	2	10.895	< 0.001
Part of potato	3	177.989	< 0.001
Solvent part of potato	6	22.022	< 0.001

Values represented as mean ± standard deviation in triplicate analysis. The values with lowercase letters (a–c) and capital letters (A–D) are significantly different in rows and columns, respectively, for the one-way ANOVA followed by Tukey post hoc test ($p < 0.05$)

content in various parts of the potato ranged from 79.14 to 181.85 mg GAE per gram of extract. The amount of total phenolic content was high in the microtuber and low in the potato flesh. The highest extraction yields of total phenolics in the microtuber, tuber, and peel were obtained using methanol, 80% methanol, and acetone, respectively. In the case of the flesh of the potato, extraction efficiency was not determined (Table 1). In order to understand the interaction between the extraction solvent and the part of the potato affecting total phenolic content extraction efficacy, a two-way ANOVA was conducted. Tukey post hoc analysis indicated 80% methanol was significantly different with both acetone ($p=0.01$) and methanol ($p=0.00$). In the Tukey test, acetone-methanol did not show any significant differences ($p=0.38$). In the analysis of the part of the potato, while the flesh-peel ($p=0.06$) and peel-tuber ($p=0.68$) were not significant, other parts of the potato indicated significant differences for total phenolic contents at $p<0.01$. This study showed the extraction efficiency by using the different extraction solutions, although many other studies focused on the various cultivars of potatoes to find total phenolics [18–20]. Our results indicate that the extraction efficiency of the extraction solutions varied in the tuber, microtuber, flesh, and peel of the potato.

In order to compare the phenolic composition in different extractions from the tuber, microtuber, flesh, and peel of the potato, phenolic acids and glucoside were identified and quantified (Table 2). Chlorogenic acid, caffeic acid, vanillic acid, quercetin glucoside, and *p*-coumaric acid were quantified using HPLC because total phenolic contents measured by the Folin–Ciocalteu procedure did not show any specification of phenolic composition. Calibration curves were obtained using the equations for chlorogenic acid, caffeic acid, vanillic acid, quercetin glucoside, and *p*-coumaric acid, respectively: $y=0.9994x+0.0441$ ($r^2=0.9998$), $y=0.9987x+0.0977$ ($r^2=0.9997$), $y=0.9995x+0.0363$ ($r^2=0.9999$), $y=0.9992x+0.0621$ ($r^2=0.999$), $y=0.9985x+0.1162$ ($r^2=0.9995$). Phenolic acids were found to be major phenolic compounds (Table 2), and these observations are in agreement with previous studies by Akyol et al. [9] and Zhu et al. [21]. In our results, vanillic acid, followed by caffeic acid, was identified as the predominant phenolic acid in potatoes (Table 2). However, several other authors have found chlorogenic acid to be the most abundant phenolic acid in potatoes [9, 22, 23]. Zhu et al. [21] quantified higher amounts of vanillic acid and caffeic acid than chlorogenic acid and attributed the finding to the high-pressure homogenization extraction method. Since the vanillic acid and caffeic acids are free forms of phenolic acid, which differ from chlorogenic acid, which is a bound form [24], in our extraction procedure, the blending process could apparently improve the yield of the non-bound form of the phenolic acid content.

Table 2 Quantification of the phenolic chemical composition in different parts of the potato (tuber, flesh, and peel) and micropropagated potato tuber

Extract	Phenolic compounds					Total (mg/L)
	Chlorogenic acid	Caffeic acid	Vanillic acid	Quercetin glucoside	*p*-Coumaric acid	
Tuber						
Acetone	0.15 ± 0.00^{a}	2.30 ± 0.01^{a}	3.03 ± 0.09^{a}	–	0.08 ± 0.01^{a}	5.55 ± 0.11
Methanol	1.47 ± 0.10^{b}	1.40 ± 0.04^{b}	–	1.26 ± 0.08^{a}	–	4.13 ± 0.22
80% Methanol	1.16 ± 0.06^{c}	0.85 ± 0.05^{c}	–	–	–	2.47 ± 0.11
Microtuber						
Acetone	–	47.81 ± 0.81^{d}	40.33 ± 1.36^{b}	–	0.85 ± 0.03^{b}	88.99 ± 2.20
Methanol	1.17 ± 0.06^{d}	17.28 ± 0.27^{e}	27.30 ± 0.46^{c}	–	0.33 ± 0.01^{c}	46.07 ± 0.80
80% Methanol	1.20 ± 0.04^{e}	10.81 ± 0.08^{f}	23.30 ± 0.46^{d}	–	0.16 ± 0.02^{d}	35.47 ± 0.61
Flesh						
Acetone	–	0.21 ± 0.00^{g}	2.68 ± 0.12^{a}	–	–	2.89 ± 0.12
Methanol	0.87 ± 0.00^{f}	–	–	–	–	0.87 ± 0.00
80% Methanol	1.00 ± 0.02^{g}	–	–	–	–	1.00 ± 0.02
Peel						
Acetone	0.43 ± 0.01^{h}	15.44 ± 0.24^{h}	6.57 ± 0.09^{e}	–	0.06 ± 0.00^{a}	22.51 ± 0.34
Methanol	3.25 ± 0.11^{i}	6.53 ± 0.01^{i}	8.87 ± 0.22^{f}	–	–	18.66 ± 0.34
80% Methanol	1.98 ± 0.06^{j}	1.78 ± 0.01^{j}	3.72 ± 0.08^{g}	–	–	7.49 ± 0.15

Data are expressed as mean ± standard deviation of triplicate analysis. Values marked with different letters are significantly different for the two-way ANOVA followed by Tukey post hoc test at $p<0.05$

Several authors have studied the optimization of the extraction method or food processing method that improves the health effects related to having antioxidants [23]. In this present study, in comparing the three extraction solutions, acetone extract showed the highest efficiency in all the tested potato extracts. Although vanillic acid (tuber-flesh) and *p*-coumaric acid (tuber-peel) in the acetone extract did not show significant differences, all other separate compounds were significantly different in a two-way ANOVA test. In addition, the total amounts of five compounds were significantly different in all the tested extraction solvents and parts of the potato at $p < 0.01$. Among the different parts of the potato, the microtuber contained the highest amount of phenolic compounds in acetone (88.99 mg/L), methanol (46.07 mg/L), and 80% methanol (35.47 mg/L) extraction solvents. Peel extracts ranked second in terms of phenolic compounds in acetone (22.51 mg/L), methanol (18.66 mg/L), and 80% methanol (7.49 mg/L). Flesh exhibited the lowest efficacy in all tested extracts. These results are in agreement with previous research findings that potato peels have the highest amount of phenolic compounds and flavonoids [25]. A study comparing major flavonoids and phenolic acid in the different parts (tuber, leaves, and flower) using 26 cultivars of *Solanum tuberosum* L. noted that the tuber (peel and flesh) contains a high percentage of total phenolic acid and relatively low flavonoids—about 79% and 9%, respectively [26]. By contrast, leaves contain about 36% and 64% of phenolic acid and flavonoids, respectively [26]. It should be noted that the phenolic acid pathway along with phenolic precursor compounds, rather than the flavonoid pathway, has been largely linked to the major phenolic acid in both the peel and flesh [26].

Radical scavenging activity

Reactive oxygen species, produced as a byproduct of metabolic reactions, are widely intercorrelated in biological system controlling processes such as growth, regulation of environmental stress, development, and defense mechanisms [27]. Due to the toxicity of these reactive molecules, the ameliorative effect of radical scavengers has become a targeted research area related to disease prevention. Indeed, potatoes have been nominated as a better source of phenolic compounds—as antioxidants—than other vegetables and fruits, such as carrots, onions, and tomatoes, due to their higher daily consumption [28]. Using the DPPH and ABTS radicals, which are the most popular radicals for measuring antioxidant activity [29], we measured the radical scavenging activity in different extracts from various potato parts, including the tuber, microtuber, flesh, and peel. In the comparison of the flesh and peel, the peel had higher antioxidant scavenging activities in the extract solution (Fig. 2b, d). Antioxidant activities using DPPH and ABTS were higher in acetone extracts, in which DPPH and ABTS were 97.9% and 96.6% at 5 mg/mL, respectively. DPPH and ABTS at 2.5 mg/mL were also higher in acetone extracts in potato peels. However, DPPH and ABTS radical scavenging activities were variable in the extraction solutions of the potato peel. The comparison of the antioxidant activity in the potato tuber and microtuber showed that the microtuber had higher DPPH and ABTS radical scavenging activities than the tuber (Fig. 2a, c). When comparing the potato extracts at the 5 mg/mL concentration, the acetone extraction of the microtuber exhibited high antioxidant capacity by DPPH (94.3%) and ABTS (95.5%) assay, while the tuber showed minimum radical scavenging activities by DPPH (13.7%) and ABTS (18.2%) at 5 mg/mL of acetone extract. These results showed that the most potent antioxidant activities were found in acetone extracts of the potato microtuber and peel, while the least antioxidant activities were observed in acetone extracts of the potato flesh and tuber. A number of studies investigated the higher antioxidant activity in potato peels, which contain 90% of phenolic compounds, versus the whole potato or flesh [9]. Similarly, our data showed that peels have higher antioxidants than the whole potato tuber and flesh.

Interestingly, microtubers showed the highest antioxidant activity in all the tested solvent extracts (acetone, methanol, 80% methanol) (Fig. 2), with consistent amounts of total phenolic contents and phenolic compounds (Tables 1, 2). It seems that the antioxidant capacity is greatly influenced by not only the extraction solution but also the potato part (tuber, microtuber, flesh, and peel). Also, these data clearly support the idea that the phenolic compounds act as antioxidants.

Even though microtuber chemical constituents have not yet been studied much, as previously reported for the phenolic contents in potatoes, the tuber peel is considered the predominant source for phenolic acid, rather than any other parts of the potato, such as the flesh, leaves, or flower [9, 26]. As noted previously, the microtuber has more peel units per gram of powder due to its small size; thus, our results are consistent with the literature in supporting the role of potato peels as a good source of bioactive compounds. A recent paper by Navarre et al. demonstrated that phenolic concentration is much higher in immature tubers than mature tubers, and is responsible for the increased phenylpropanoid content contributed by the high content of sugar in immature tubers [30]. The maturation difference between the micropropagated tuber and potato (peel, flesh) controls the phenylpropanoid biosynthesis via the acting sugar contents; therefore, the micropropagated potato tuber,

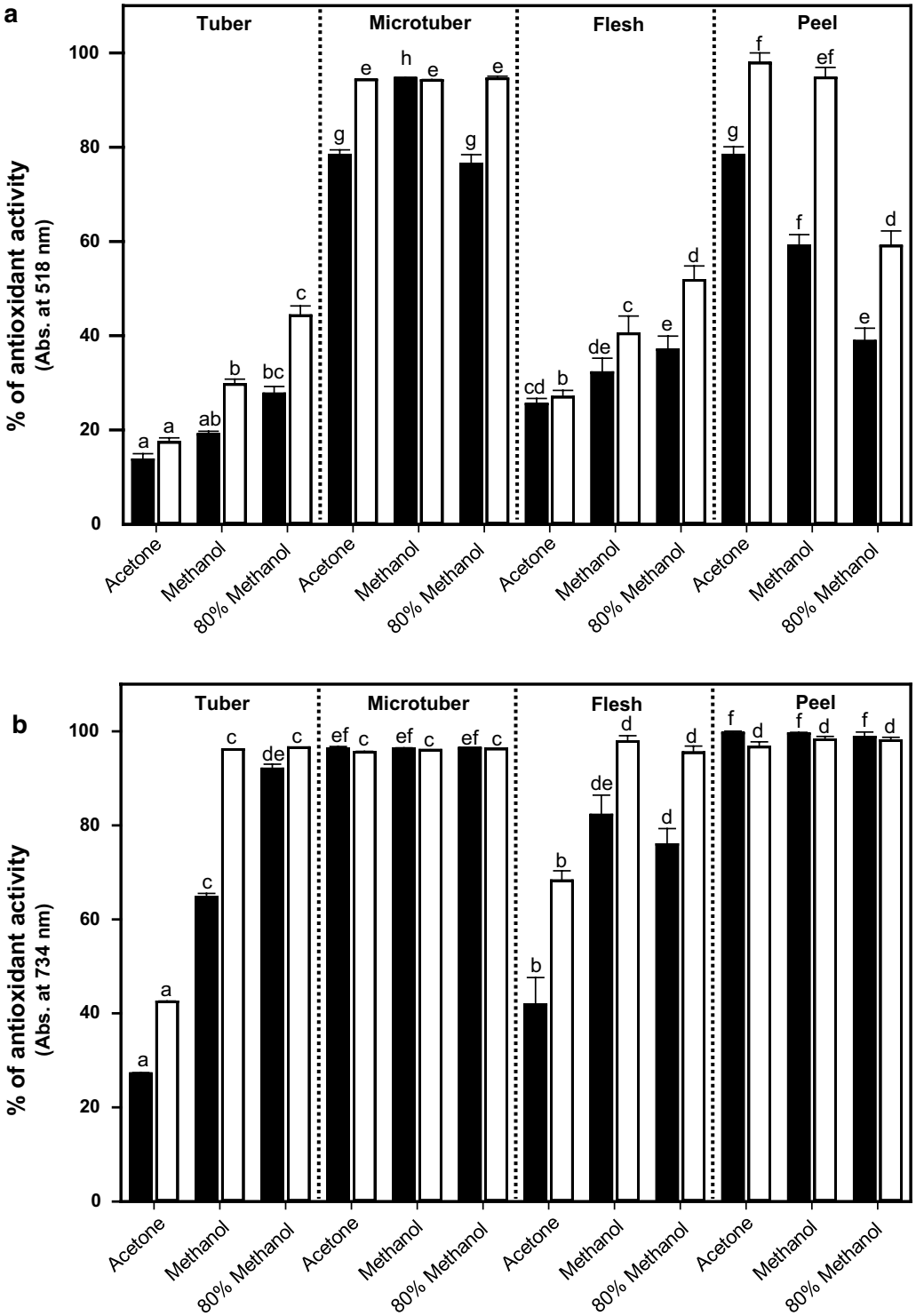

Fig. 2 **a** DPPH; and **b** ABTS free radical scavenging activity of the various solvent extracts from the tuber, microtuber, flesh, and peel of the potato at 2.5 mg/mL (black bar) and 5 mg/mL (white bar). The different lowercase letters indicate significant differences in all series of extracts at 2.5 and 5 mg/mL, respectively. One-way ANOVA followed by Tukey post hoc test was performed to analyze statistical significance ($p < 0.05$)

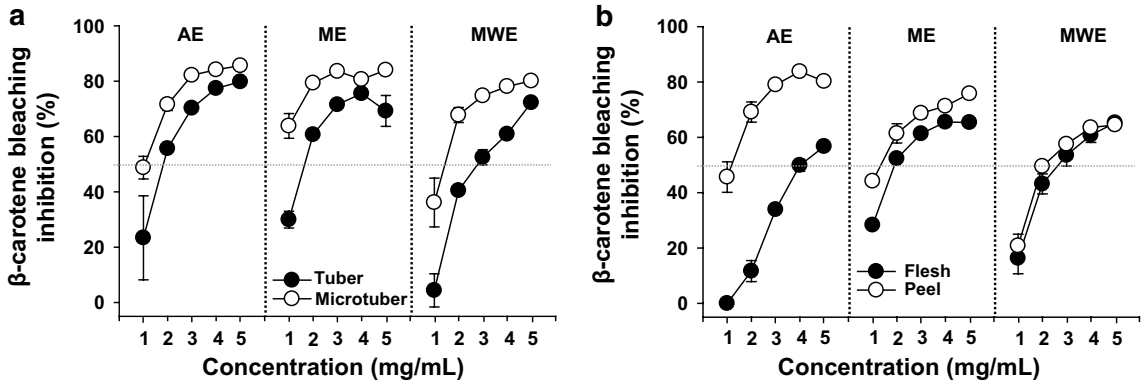

Fig. 3 β-Carotene bleaching activity was determined in different solvent extracts from the potato: **a** tuber and microtuber extracts, **b** flesh and peel extracts. The bars represent standard deviations of three independent experiments. The significance and confidence levels were estimated using one-way ANOVA ($p < 0.05$). *AE* acetone extract, *ME* methanol extract, *MWE* 80% methanol in water extract

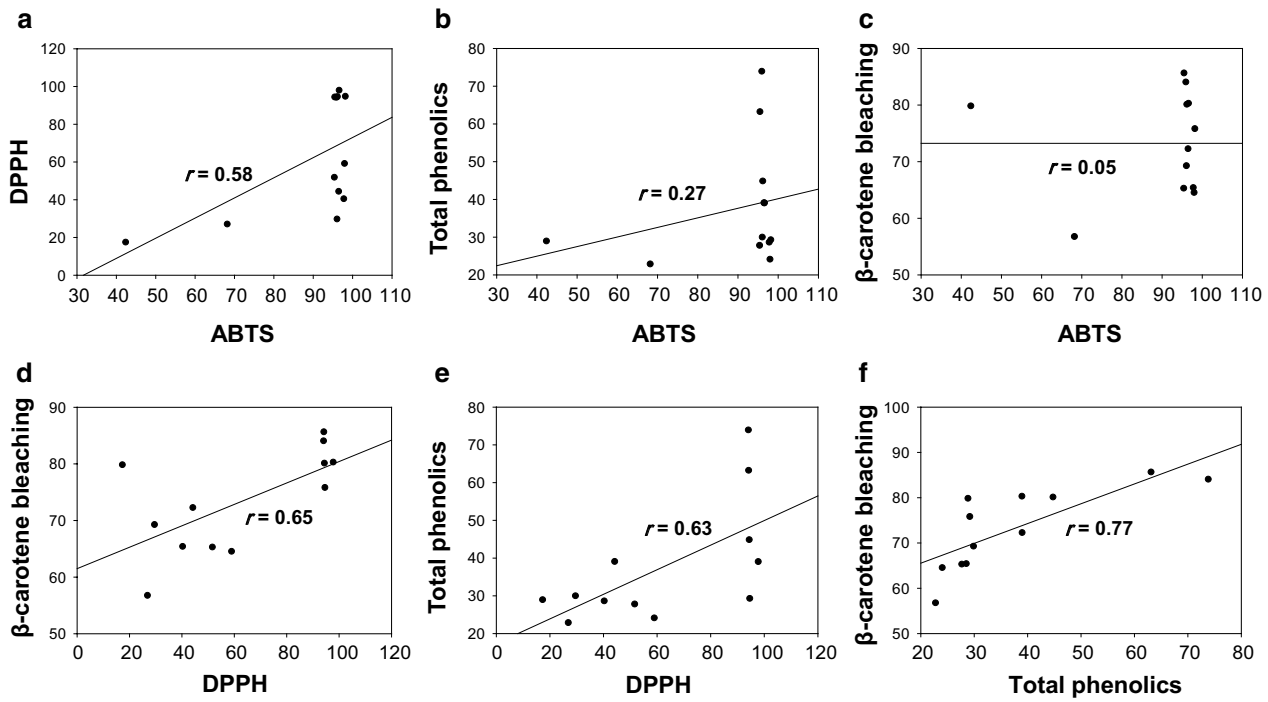

Fig. 4 Scatterplot of the interaction between the different methods to measure antioxidant activity. The correlation was determined by the Spearman coefficient of correlation method. Correlation coefficient between ABTS and either **a** DPPH ($r = 0.58$, $p < 0.05$) or **b** total phenolics ($r = 0.27$, $p > 0.05$). Correlation coefficient between β-carotene bleaching and either **c** ABTS ($r = 0.05$, $p > 0.05$) or **d** DPPH ($r = 0.65$, $p < 0.05$). Correlation coefficient between total phenolics and either **e** DPPH ($r = 0.63$, $p < 0.05$) or **f** β-carotene bleaching ($r = 0.77$, $p < 0.05$)

which is an immature tuber, contains the highest antioxidant activity reflecting phenolic contents [30, 31]. However, it is not clear whether the highest antioxidant potential is due to the contribution of the peel or immaturation. This maturation effect should be taken into consideration in future studies seeking to find the maximum extraction efficacy using optimized extraction of bioactive compounds in potato tubers.

β-Carotene bleaching inhibition

The antioxidant activities of potato parts using the β-carotene bleaching assay were evaluated by measuring the inhibition of potato extracts (Fig. 3). β-Carotene bleaching inhibition showed that maximum antioxidant activity was observed in the potato microtuber followed by the peel, tuber, and flesh. The order of antioxidant activity of the potato microtuber, peel, flesh, and tuber

was similar to the DPPH and ABTS radical scavenging activity. It was also clear that acetone extracts of most potato parts highly inhibited antioxidant activity compared to ME and MWE extracts.

Correlation coefficients between the total phenolic content and antioxidant activities

The Spearman correlation between the total phenolic content, radical scavenging activities, and bleaching activity was evaluated and is presented in Fig. 4. The correlation coefficient of the DPPH radial scavenging activity showed a significantly positive correlation with ABTS ($r=0.58$, $p<0.05$) (Fig. 4a), β-carotene bleaching ($r=0.65$, $p<0.05$) (Fig. 4d), and total phenolics ($r=0.63$, $p<0.05$) (Fig. 4e). Similarly, the total phenolic content was also significantly correlated to β-carotene bleaching ($r=0.77$) (Fig. 4f). However, the level of ABTS was not correlated to total phenolics and inhibition of β-carotene bleaching (Fig. 4b, c).

Authors' contributions

JK analyzed the data and wrote the paper. SYS provided technical assistance to JK and helped with the data collection. HB edited the manuscript. SN supervised the work. JK revised and edited the manuscript and supervised the work. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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