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*Full Length Research Paper*

# Antioxidant activity of different parts of eggplant

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**After preparing 70% ethanol (EE) and water extracts (WE) from different parts (calyx, leaf, peel, pulp, and stem) of eggplant, antioxidant activity of the extracts was evaluated. Total phenolic contents of EE and WE were the highest in peel (55.19 mg/g) and calyx (121.07 mg/g) extracts, respectively. Total flavanol contents of EE and WE were the highest in leaf (8.00 mg/g) and calyx (5.61 mg/g) extracts, respectively. The peel extract showed the highest anthocyanins content (138.05 mg %) followed by calyx (135.94 mg %), stem (110.38 mg %), leaf (97.29 mg %), and pulp (2.29 mg %) extracts. In both EE and WE, extracts of peel and calyx parts showed relatively higher DPPH radical scavenging activity and reducing power. The remarkable high SOD-like activity was detected in WE of calyx part ($IC_{50}$ = 0.39±0.01 µg/ml), which is about 1,700 times stronger than WE of pulp part ($IC_{50}$ = 0.69±0.01 mg/ml). The results indicate that antioxidant activity of eggplant varied by parts and solvents. This study also shows the calyx part had strong antioxidant activity.**

**Key words:** Eggplant, antioxidant activity, phenolics.

## INTRODUCTION

Eggplant (*Solanum melongena* L.) is a plant native in India, and many cultivars exhibiting different size, shape, and color are cultivated in tropical, subtropical, and temperate zones. Its fruit, commonly known as aubergine, melanzana, garden egg, brinjal, or patlican, also has same name and widely used as a vegetable in cooking. The most widely cultivated varieties are elongated ovoid or slender type in a dark purple skin. Eggplant is ranked as one of the top ten vegetables in terms of oxygen radical scavenging capacity due to the fruit's phenolic constituents (Cao et al., 1996). Anthocyanins, an important group of naturally occurring pigments of red and/or purple colored fruits, are the main phenolic compounds in eggplant peel (Mazza et al., 2004). Nasunin, a major component of anthocyanin pigment of eggplant, was isolated from the eggplant peels, and its antioxidant activity was evaluated (Igarashi et al., 1993; Noda et al., 2000). There are also many reports on the health benefits of eggplant. The research on the antioxidant activity of eggplant with different assays was reported by Huang et al. (2004). Stommel and Whitaker (2003) carried out a systematic examination of the phenolic acid content of the fruit flesh of seven commercial eggplant cultivars. Optimized extraction of phenolic acids from eggplant by different solvents mixtures (Luthria, 2006), and the relationship between phenolic content and antioxidant activity of eggplant pulp were also reported (Singh et al., 2009). However, information concerning the antioxidant activity and phytochemical composition of eggplant according to parts except peel and pulp is unavailable. In this study, the antioxidant activity and phenolic contents of five different parts (calyx, leaf, peel, pulp, and stem) of eggplant extracted by two different solvents (70% ethanol and water) were evaluated.

## MATERIALS AND METHODS

A slender type of eggplant (*Solanum melongena* L. cultivar Chikuyou) was purchased from a farm located at Jinju City, Korea. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), L-ascorbic acid, gallic acid, dimethyl sulfoxide (DMSO), trichloroacetic acid (TCA), and superoxide dismutase (SOD) assay kit were supplied from Sigma-Aldrich Co. (St. Louis, MO, USA). Folin-Ciocalteu reagent was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Ethanol and methanol were provided from Duksan Chemical Co. (Asan, Korea).

### Preparation of extracts from eggplant

Immediately after purchasing fresh eggplants, they were divided

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into five parts (pulp, peel, calyx, stem, and leaf), and then freeze-dried for 3 days with a lyophilizer (Freeze Dryer FD 5512, IlshinLab Co., Seoul, Korea). The dried samples were crushed using a grinder (Mixer MC 811C, Novita Co., Seoul, Korea), and each 30 g was extracted with 20 volumes of 70% ethanol and water, respectively.

After shaking at 100 rpm in a shaker (SHWB-30, Woori Science Co., Seoul, Korea) for overnight at room temperature, the solvent extracts were filtered through a Whatman No.1 filter paper. The filtrates were concentrated to dryness under reduced pressure on a rotary evaporator (Eyela N-1000, Tokyo Rikakikai Co., Tokyo, Japan) at 37℃. Each dried 70% ethanol (EE) and water extract (WE) samples was re-dissolved in DMSO with concentration of 50 mg/ml, and diluted with DMSO when needed. All samples were placed in a glass bottle with saturation of nitrogen gas and stored at 4℃ until used.

#### DPPH radical scavenging activity

The DPPH radical scavenging activity (RSA) of eggplant extract was determined according to the method of Lee et al. (2006). After mixing 0.1 ml of each concentration (1, 5, and 10 mg/ml) of the extracts dissolved in DMSO with 0.9 ml of 0.041 mM DPPH in ethanol for 30 min, the absorbance of the sample was measured at 517 nm by a spectrophotometer (Shimadzu UV-1601, Kyoto, Japan). All tests were carried out triplicate and RSA was expressed as a percentage of inhibition and it was calculated by using the following formula.

% DPPH RSA = [1 – (absorbance of sample / absorbance of control)] × 100

#### Reducing power

The reducing power of each extract was determined according to the method of Oyaizu (1986). 1 ml of the eggplant extract (concentration: 1, 5, and 10 mg/ml, respectively), 1.0 ml of sodium phosphate buffer (0.2 M, pH 6.6), and 1.0 ml of potassium ferricyanide (10 mg/ml) were mixed and incubated at 50℃ for 20 min.

Then, 1.0 ml of 10% TCA was added to the mixture and centrifuged at 13.400 × g for 5 min. 1 ml of supernatant was mixed with 1.0 ml of $H_2O$ and 0.1 ml of 0.1% ferric chloride, and then the absorbance was measured at 700 nm.

#### Total phenolic contents (TPC)

TPC of the extracts were determined according to the method of Gutfinger (1981). 1 ml of each extract with concentration of 1 mg/ml was mixed with 1 ml of 2% sodium carbonate followed by standing for 3 min. And then 0.2 ml of 50% Folin-Ciocalteau reagent was added to the mixture. After standing for 30 min, the mixture was centrifuged at 13,400 × g for 5 min. The absorbance was measured at 750 nm, and TPC were expressed as gallic acid equivalents (GAE).

#### Total flavanol contents (TFC)

TFC of the extracts were estimated by the vanillin method using catechin as a standard (Price et al., 1978). Each sample (1.0 ml) was mixed with 5.0 ml of 2.0% vanillin (8.0% methanolic HCl). The absorbance was measured at 500 nm after 20 min incubation in the dark at room temperature. TFC were expressed as (+)-catechin equivalent.

#### Anthocyanin contents

Each 3 g of dried eggplant powder was extracted with 100 ml of 1% methanolic HCl for 24 h at room temperature in darkness. The solution was filtered with Whatman No. 1 filter paper. The same extraction procedure was repeated by adding 40 ml of 1% methanolic HCl to the residues, and the filtrate was combined. The combined filtrate was centrifuged at 5,000 rpm (13400 × g) for 10 min at 4℃. The absorbance of the resulting solution was measures at 535 nm (Fuleki and Francis, 1968).

#### Superoxide dismutase (SOD)-like activity

SOD-like activity of the extracts was determined by SOD assay kit according to the manuals. In 96-well plate, 20 µl of the extract was added to sample and blank 2 well, and then 20 µl of double distilled water was added to blank 1 and blank 3 well. Two hundred µl of WST (water-soluble tetrazolium salt) working solution was added to each well, and 20 µl of dilution buffer was added to blank 2 and blank 3 well. 20 µl of enzyme working solution was added to each sample and blank 1 well and then mixed thoroughly. The plate was incubated at 37℃ for 20 min and then absorbance for the plate was measured by the multiplate reader at 450 nm. SOD-like activity was calculated according to the following equation:

$$\% \text{ SOD-like activity} = [(A_{blank1} - A_{blank3}) - (A_{Sample} - A_{blank2})]/ (A_{blank1} - A_{blank3})] \times 100$$

#### Statistical analyses

All measurements were performed in triplicate, and the SPSS package for Windows (Ver. 12) was used for the analyses of variance. A p-value less than 0.05 was considered statistically significant.

## RESULTS AND DISCUSSION

### Extraction yield

Ethanol and water are permitted as solvents in the food industry, and 70% ethanol has been widely used to extract physiological components from plant sources (Jeong et al., 2004; Oh et al., 2008). The extraction yields of five different parts (calyx, leaf, peel, pulp, and stem) of eggplant by 70% ethanol and water are presented in Table 1. The yields of the extracts significantly ($p<0.05$) varied by parts. The highest yield was obtained in pulp (53.5%) followed by peel (33.5%), calyx (25.8%), leaf (22.0%), and stem (21.1%) parts of ethanol extract (EE). Water extract (WE) also showed similar trends in yields with those of EE; pulp (58.3%), peel (36.1%), leaf (28.5%), calyx (23.7%), and stem (21.0%) parts.

### TPC, TFC, and anthocyanin contents of eggplant

It is well known that plant phenolics are highly effective free radical scavengers and antioxidants, and antioxidant activity of vegetables and fruits is derived largely from phenolic compounds, so there should be a close

**Table 1.** Extraction yield from eggplant with various solvents and different part (unit: %).

| Extraction solvents | Parts | | | | |
|---|---|---|---|---|---|
| | Calyx | Leaf | Peel | Pulp | Stem |
| 70% Ethanol | 25.8$^{d}$ | 22.0$^{e}$ | 33.5$^{c}$ | 53.5$^{b}$ | 21.1$^{f}$ |
| Water | 23.7$^{e}$ | 28.5$^{d}$ | 36.1$^{c}$ | 58.3$^{a}$ | 21.0$^{f}$ |

Extraction yield was calculated from the equation, (weight of extracts/ weight of dried eggplant parts) × 100.

**Table 2.** Total phenolic contents (TPC), total flavanol contents (TFC), and anthocyanin of eggplant parts depend on solvent.

| Content | Extraction solvents | Sample | | | | |
|---|---|---|---|---|---|---|
| | | Calyx | Leaf | Peel | Pulp | Stem |
| TPC (mg GAE[1)]/g extract) | 70% Ethanol | 32.02±2.24$^{c}$ | 37.86±1.95$^{d}$ | 55.19±1.30$^{e}$ | 10.82±0.19$^{a}$ | 28.73±0.44$^{b}$ |
| | Water | 121.07±5.00$^{d}$ | 25.39±5.71$^{b}$ | 54.94±8.26$^{c}$ | 13.83±0.13$^{a}$ | 31.56±0.96$^{b}$ |
| TFC (mg CE[2)]/g extract) | 70% Ethanol | 2.26±0.14$^{c}$ | 8.00±0.14$^{e}$ | 6.19±0.28$^{d}$ | 0.81±0.14$^{a}$ | 1.29±0.28$^{b}$ |
| | Water | 5.61±0.73$^{d}$ | 5.20±0.14$^{cd}$ | 3.57±0.14$^{b}$ | 0.40±0.14$^{a}$ | 4.87±0.25$^{c}$ |
| Anthocyanin contents (mg%) | Methanolic HCl | 135.94±0.66$^{d}$ | 97.29±0.21$^{b}$ | 138.05±0.67$^{e}$ | 2.29±0.60$^{a}$ | 110.38±0.19$^{c}$ |

[1)] GAE: Gallic acid equivalents, [2)] CE: (+)-catechin equivalents.

correlation between the content of phenolic compounds and antioxidant activity (Bravo, 1998). Singh et al. (2009) identified many kinds of phenolic compounds such as N-caffeoylputrescine, 5-caffeoylquinic acid, and 3-acetyl-5-caffeoylquinic acid from eggplant pulp. Noda et al. (2000) also reported that nasunin, delphinidin-3-(*p*-coumaroylrutinoside)-5-glucoside, was a representative anthocyanin in eggplant peel.

The results of three kinds of analysis for phenolic compounds of eggplant extracts are summarized in Table 2. TPC of EE were in the order of peel (55.19 mg/g), leaf (37.86 mg/g), calyx (32.02 mg/g), stem (28.73 mg/g), and pulp (10.82 mg/g). On the other hand, TPC of WE were calyx (121.07 mg/g), peel (54.94 mg/g), stem (31.56 mg/g), leaf (25.39 mg/g), and pulp (13.83 mg/g).

TFC of EE were in the order of leaf (8.00 mg/g), peel (6.19 mg/g), calyx (2.26 mg/g), stem (1.29 mg/g), and pulp (0.81 mg/g). On the other hand, the order of TFC of WE were calyx (5.61 mg/g), leaf (5.20 mg/g), stem (4.87 mg/g), peel (3.57 mg/g), and pulp (0.40 mg/g).

Table 2 also shows anthocyanin contents of eggplant extracts. The peel extract showed the highest anthicyanins content (138.05 mg %) followed by calyx (135.94 mg %), stem (110.38 mg %), leaf (97.29 mg %), and pulp (2.29 mg %) extract.

Generally, peel tissue of plant contains higher amounts of phenolics, anthocyanins, and flavonols than pulp tissue (Tomás-Barberán et al., 2001; Jang et al., 2010). Eggplant peel also showed higher amounts of those compounds than pulp. On the other hand, it is noticeable that eggplant calyx exhibited relatively high amounts of phenolics. In the case of persimmon, water extract of calyx showed about five times higher amount of TPC than that of flesh (Jang et al., 2010). These findings may answer why the calyx part of some plant have been used as a traditional medicine (Bruni et al., 2004; Hirunpanich et al., 2006), however, it has still not been elucidated how calyx contains high amount of phenolics.

## DPPH RSA of eggplant

The DPPH radical is usually used as a substrate to evaluate the antioxidative action of antioxidants by determining the free radical-scavenging ability of various samples (Amarowicz et al., 2004). Figure 1 shows the DPPH radical scavenging activity of eggplant extracts depend on solvent and part.

The DPPH radical scavenging activities of EE (Figure 1A) and WE (Figure 1B) showed increasing trends with increase of extract concentration. DPPH radical scavenging activities of EE except pulp part did not strongly increased between 5 and 10 mg/ml range, while those of WE significantly increased at same concentrations. In both EE and WE, extracts of peel and calyx showed the highest DPPH radical scavenging activity. As shown at Table 3, the half maximal inhibitory concentration ($IC_{50}$) values for DPPH radical scavenging activity of eggplant parts were the lowest in calyx WE (0.49±0.16 mg/ml), and EE (0.98±0.33 mg/ml) and WE (1.69±0.13 mg/ml) of peel also showed relatively lower $IC_{50}$ values.

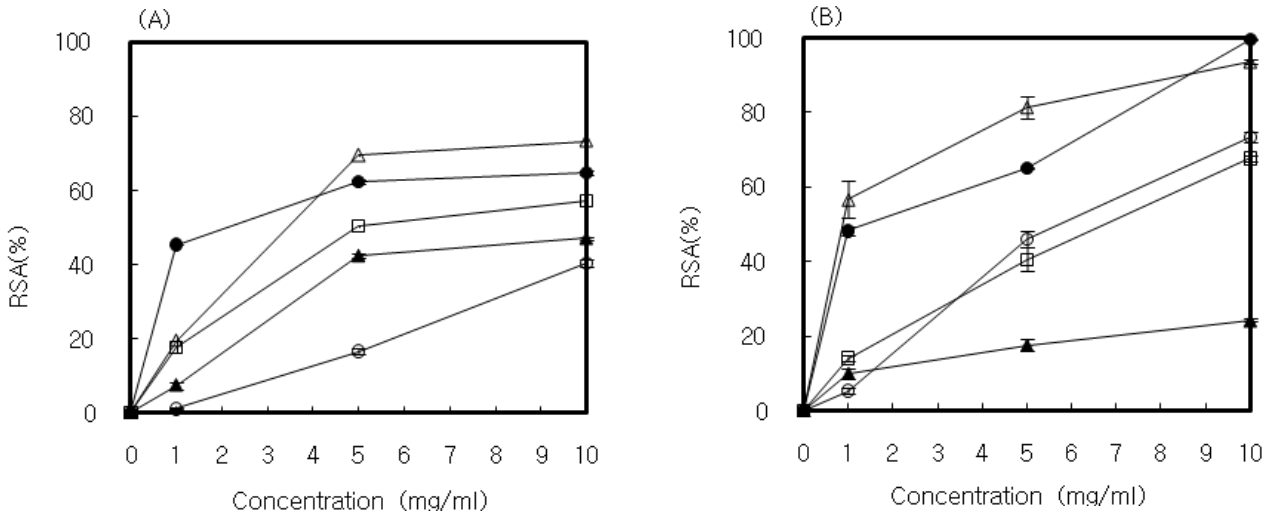


**Figure 1.** DPPH radical scavenging activity of different parts of eggplant extracts. (A) 70% ethanol extracts, and (B) water extracts. ○, pulp; ●, peel; △, calyx; ▲, stem; and □, leaf.

**Table 3.** Antioxidant activity, represented by $IC_{50}$ (mg/ml), of eggplant extracts depending on parts and extracting solvents.

| Antioxidant activity | Extraction solvents | Parts | | | | |
|---|---|---|---|---|---|---|
| | | Calyx | Leaf | Peel | Pulp | Stem |
| DPPH RSA | 70% ethanol | 3.45±0.02$^{b}$ | 4.94±0.03$^{c}$ | 0.98±0.33$^{a}$ | 12.30±0.27$^{d}$ | 13.13±0.06$^{e}$ |
| | Water | 0.49±0.16$^{a}$ | 6.89±0.20$^{b}$ | 1.69±0.13$^{a}$ | 6.47±0.18$^{b}$ | 26.20±1.96$^{c}$ |
| RP | 70% ethanol | 2.42±0.26$^{b}$ | 2.92±0.06$^{c}$ | 1.10±0.13$^{a}$ | 3.90±0.08$^{d}$ | 3.10±0.04$^{c}$ |
| | Water | 0.03±5.49$^{a}$ | 162.20±23.34$^{c}$ | 1.66±0.09$^{b}$ | 2.83±0.02$^{b}$ | 329.80±13.80$^{d}$ |
| SOD-like activity | 70% ethanol | 0.51±0.02$^{c}$ | 0.58±0.02$^{d}$ | 0.21±0.02$^{a}$ | 0.40±0.01$^{b}$ | 0.38±0.00$^{b}$ |
| | Water | 0.39±0.01$^{a1)}$ | 0.30±0.07$^{c}$ | 0.22±0.00$^{b}$ | 0.69±0.00$^{d}$ | 0.16±0.00$^{b}$ |

[1)] Only this value is μg/ml unit.

## Reducing power

The antioxidant activity has been reported to be concomitant with reducing power (Lee et al., 2003). In the assay, the presence of reductants in the antioxidant sample causes the reduction of the $Fe^{3+}$/ferricyanide complex to the $Fe^{2+}$/ferrous from (Gülçin, 2006), so the reducing power of the sample could be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Chung et al., 2002). The reducing power of EE (Figure 2A) and WE (Figure 2B) increased with concentration dependent manner except WE of leaf and stem. There were not much significant differences in reducing power among parts of EE. Likewise DPPH radical scavenging activity, peel and calyx showed relatively higher reducing power. Calyx WE also showed the lowest $IC_{50}$ values (0.03±0.01 mg/ml), and EE (1.10±0.13 mg/ml) and WE (1.66±0.09 mg/ml) of peel part also exhibited low values (Table 3). In the case of stem and leaf of eggplant, EE showed considerable reducing power, while WE did not. It is suggested that ethanol soluble not water soluble compounds of leaf part might have reducing power.

## SOD-like activity of eggplant

SOD is known to catalyze the conversion of $O_2^-$ to $H_2O_2$ plus $O_2$, and provide a defense system under oxidation conditions, in which $O_2^-$ appears to play an important role (Nagami et al., 2004). As shown in Figure 3, SOD-like activity of eggplant extracts increased with concentration. The highest SOD-like activity was detected in WE ($IC_{50}$ = 0.39±0.01 μg/ml) of calyx part WE, which is about 1,700 times higher than WE ($IC_{50}$ = 0.69±0.01 mg/ml) of pulp part. $IC_{50}$ values for SOD-like activity of eggplant parts

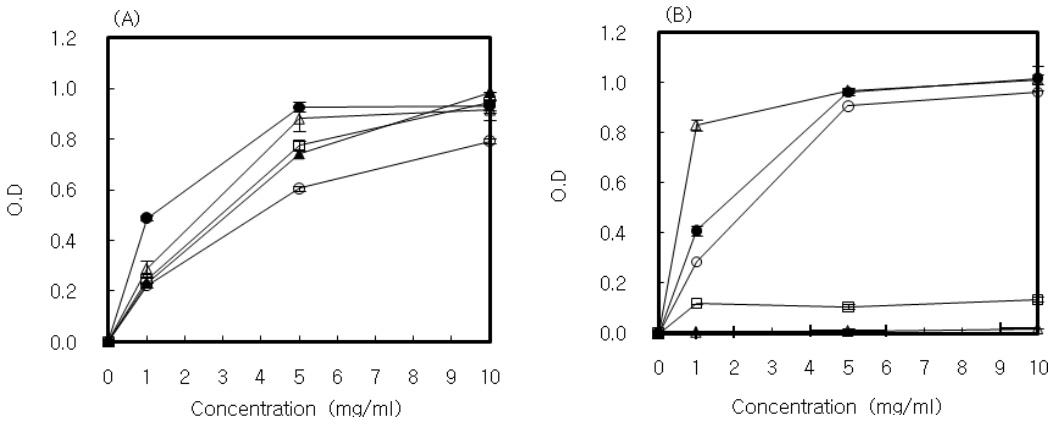


**Figure 2.** Reducing power of different parts of eggplant extracts. (A) 70% ethanol extracts, and (B) water extracts. ○, pulp; ●, peel; △, calyx; ▲, stem; and □, leaf.

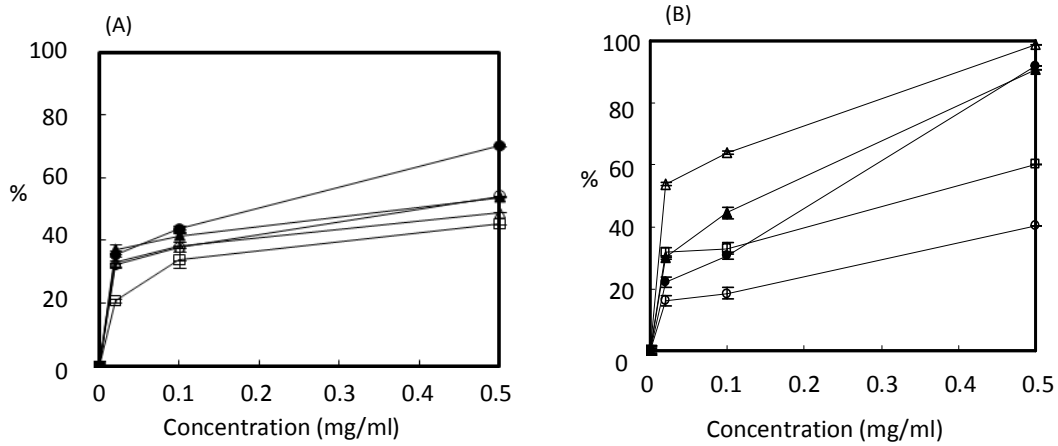


**Figure 3.** SOD-like activity of different parts of eggplant extracts. (A) 70% ethanol extracts, and (B) water extracts. ○, pulp; ●, peel; △, calyx; ▲, stem; and □, leaf.

were also summarized in Table 3.

## Relation between antioxidant components and antioxidant activity

In order to deduce a major contributing effect of antioxidant components to antioxidant activity of eggplant extracts, linear correlation studies were carried out with the results determined in this study. Since all antioxidant assays were determined at three different concentration of each eggplant extracts (1, 5, and 10 mg/ml in DPPH RSA and reducing power assays; 0.02, 0.1, and 0.5 mg/ml in SOD-like activity assay), correlation analysis was possible and necessary. As shown in Table 4, a strong correlation between TPC and DPPH RSA was found ($r^2$ = 0.7021, $P < 0.05$), and TPC was weakly correlated with reducing power ($r^2$ = 0.4102, $P < 0.05$). On the other hand, TPC did not show correlation with SOD-like activity ($r^2$ = 0.059, $P < 0.05$). Furthermore, TFC and anthocyanin contents were not highly correlated with any antioxidant activity (DPPH RSA, reducing power, and SOD-like activity) with $r^2 < 0.3$. Though antioxidant activity of anthocyanins in egg plant was well-known, the present study leads to the conclusion that TPC rather than anthocyanin are more correlated with antioxidant activity of eggplant. This fact was supported that Singh et al. (2009) isolated several phenolics and determined their antioxidant activity from eggplant pulp, where anthocyanin contents were very low.

## Conclusion

This study firstly reported that water extract of eggplant calyx has very strong antioxidant activity and high

**Table 4.** Correlation established between contents of antioxidant components and antioxidant activity.

| Content | Equation | | |
|---|---|---|---|
| | **DPPH RSA** | **Reducing power** | **SOD-like activity** |
| TPC | y = 0.2643x + 13.561<br>$r^2$ = 0.7021, P<0.05 | y = 0.0028x + 0.2552<br>$r^2$ = 0.4102, P<0.05 | y = -0.0594x + 52.257<br>$r^2$ = 0.059, P<0.05 |
| TFC | y = 0.5353x + 32.502<br>$r^2$ = 0.2965, P<0.05 | y = 0.0039x + 0.4919<br>$r^2$ = 0.0855, P<0.05 | y = -0.2459x + 51.001<br>$r^2$ = 0.104, P<0.05 |
| Anthocyanin contents | y = 0.2186x + 24.138<br>$r^2$ = 0.1658, P<0.05 | y = 0.001x + 0.4884<br>$r^2$ = 0.0189, P<0.05 | y = 0.1435x + 31.238<br>$r^2$ = 0.1188, P<0.05 |

amounts of phenolic contents, suggesting that the potential as food additives to increase antioxidant activity in foods.

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