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Role of polyphenols in antioxidant capacity of napiergrass from different growing seasons

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Abstract

Napiergrass, used in Taiwan as a new organic nourishment, showed fluctuating antioxidant capacities under different growth conditions. An index for selection of best quality material is therefore needed. Since polyphenols made the highest contribution to their antioxidant capacity, the individual effects of plant parts, growing seasons, and growth periods on both polyphenols and antioxidant activities were studied, using two varieties of napiergrass (purple, NBM and green, TLG2). Results demonstrated that leaves of NBM had a higher antioxidant capacity than their stems and all parts of the green varieties. Classification of napiergrass according to polyphenols revealed the effects of varieties and seasons on the anthocyanins and their close relationship to antioxidant capacity. Discriminant analysis confirmed that anthocyanins are the most effective component in classifying napiergrass. Therefore, red colour of anthocyanin might be used as a good index for the physiological activity of napiergrass.

Keywords: Napiergrass; Polyphenol; Antioxidant capacity; Growth condition

1. Introduction

Napiergrass (*Pennisetum purpureum*), rich in minerals and vitamins and easily grown in Taiwan, has become an important local plant. In recent years, due to its beneficial effects on human health and improved taste and texture, it prevails in Taiwan as an organic functional juice. However, its function and antioxidant compounds frequently vary when using materials from different sources. Therefore, a quality index for this material is necessary. In a previous paper, polyphenols were found to be the major compounds responsible for antioxidant capacity of this material (Wu et al., 2002). However, how the growing season or plant parts affect the compounds of interest and their antioxidant capacities remains unknown.

Phenolic compounds have long been regarded as the UV-B screen for plants (Schmitz-Hoerner & Weissenböck, 2003). Their significance as dietary antioxidants has also been highlighted in recent years and thought to account for the cholesterol lowering, anticancer, antinflamintory or other physiological activity (Cao, Sofic, & Prior, 1996; Prior et al., 1998; Velioglu, Mazza, Gao, & Oomah, 1998). Environmental and developmental factors have been reported to affect the accumulation of polyphenols. Chlorogenic acid and flavanoids, including anthocyanin, accumulated in apple skin showed a strong dependency on fruit position on tree, season or developmental stage (Awad, de Jager, van der Plas, & van der Krol, 2001). Changes of UV-B absorbing phenolic compounds accumulated in wheat seedling (Sharma, Anand, Sankhalkar, & Shetye, 1998), anthocyanin changes during growth of mango leaf (Nii, Watanabe, Yamaguchi, & Nishimura, 1995) or epidermal cells where the polyphenols were mainly accumulated (Rozema et al., 2002) have been reported. In addition, anthocyanins exhibit attractive red colour (Tsai & Huang, 2004) and higher antioxidant activity than phenolic acids (Rice-Evans, Miller, & Paganga, 1997). It seems that antioxidant capacity might vary in response to polyphenol changes due to various growing conditions.

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This study aimed to determine the most significant phenolic components responsible for the antioxidant capacity of napiergrass, and provide a simple index to evaluate the physiological function of this food material. Effects of varieties, plant parts, growing seasons and growth period on their antioxidant activity, as well as the relationship among colour, antioxidant capacity and growth factors, were investigated and subjected to statistic analysis.

2. Materials and methods

2.1. Materials

Leaves and stems from two varieties of napiergrass (NBM, TLG2) were harvested in the spring, summer, fall, or winter and growing for 6, 8 or 10 weeks at 4 plantations in Pingtung, Taiwan. All the samples were freeze dried and ground into powder, then stored at 0 °C.

2.2. Methods

Water extracts of napiergrass samples were evaluated to compare their antioxidant capacity in DPPH scavenging ability, reducing power, and inhibition of peroxidation. Phenolic compounds determined by HPLC were further used to run principal component analysis (PCA) and discriminant analysis in order to elucidate which phenolic compound is most responsible for their fluctuating antioxidant capacity. Finally, a simple index for monitoring the antioxidant capacity of various materials was provided.

2.2.1. Extraction

Two grams of dried powder samples were cooked with 100 ml boiling water for 30 min and then stored in fridge (4 °C) for 24 h. The extract was then filtered and concentrated in a rotary vacuum evaporator (40 °C) and adjusted to 5 ml with distilled water before analysis.

2.2.2. Assay of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The radical scavenging activity was measured according to the method of Tsai and Huang (2004). One millilitre of freshly prepared 1 mM DPPH solution was added to the sample (4 ml), and kept at 25 °C for 30 min. The absorbance was read at 517 nm relative to the control (as 100%) and the percentage of scavenging effect was expressed as $[1-(A_{517}$ of sample/A_{517} of control$)] \times 100$.

2.2.3. Ferric reducing ability of plasma (FRAP) assay

FRAP assay is a method of measuring the reductants (antioxidants) to reduce Fe^{+3}–Fe^{+2}. The formation of blue colored Fe^{2+}-TPTZ complex (Fe^{+2} tripyridyltriazine), will increase in absorbance at 593 nm. This was described by Tsai and Huang (2004). Freshly prepared FRAP reagent (including acetate buffer, TPTZ and $FeCl_3$) 1.2 ml was added with water 0.12 ml and sample extract 0.04 ml. The absorbance at 593 nm of the mixture was measured after 6 minutes of reaction. The reducing power (μmole/l) was calculated from the standard curve constructed using $FeSO_4$ solution.

2.2.4. Inhibition of peroxidation

The peroxidation measurement by ferric thiocyanate was measured according to the method described by Wu et al., 2002. Two millilitres sample extract were mixed with 2.5 ml linoleic acid emulsion and 0.2 M potassium phosphate buffer (pH 7.0), and kept in the dark at 37 °C. The extract mixture (0.1 ml) was then added with 75% ethanol (4.7 ml), ammonium thiocyanate (30%, 0.1 ml) and 0.02 M iron(II) chloride tetrahydrate (0.1 ml) and the absorbance at 500 nm was measured after 3 min of reaction. The inhibition of peroxidation was expressed as $[1-(A_{500}$ of sample/A_{500} of control$)] \times 100$.

2.3. HPLC analysis (Tsai & Huang, 2004)

Twenty microlitres of the water extract were subjected to HPLC after filtration through 0.45 μm PTFE (poly-tetrafluoroethylene) film (Whatman International Ltd., Maidstone, England) and compared with the standard phenolic compounds. In this study, phenolic compounds include flavanol (catechin, epicatechin), flavone (rutin), flavonol (quercetin), anthocyanin and phenolic acid (coumaric acid, chlorogenic acid, gallic acid, ferulic acid and caffeic acid) (Meyer, Yi, Pearson, Waterhouse, & Frankel, 1997). Among them, anthocyanin was determined by using delphinidin-3-sambubiose as standard. HPLC condition is:

Column: Hitachi #3056 C-18 id 250 mm × 4.6 mm (Hitachi High-Technologies Corporation. 24-14 Nishi-Shimbashi 1-chome, Minato-ku, Tokyo, 105-8717, Japan).

Detector: Hitachi UV–VIS detector (280 nm) (Hitachi high-technologies Corporation, Tokyo, Japan).

Mobile phase: Gradient combination of 5% acetic acid (A) and acetonitrile (B) with A 100% from the beginning, 90% and 60% after 10 and 40 min, and back to 100% after 45 min.

Flow rate: 1.0 ml/min.

2.3.1. Statistical analysis (Chen, Huang, Ho, & Tsai, 1998)

Statistical analysis, including principal component analysis (PCA, Factor) and discriminant analysis of the data were conducted using SAS statistical software (SAS, 1988). General linear model procedures were used to determine treatment effects, and Duncan's multiple range tests were used to compare means. In the discriminant analysis, linear discriminant analysis (LDA) was performed on principle components. All experiments were carried out in triplicate.

3. Results and discussion

3.1. Effects of variety, plant part, growing season and growth period on antioxidant activity

As shown in Table 1, leaves of purple napiergrass (NBM) possessed the highest antioxidant capacity when

Table 1
Comparison of DPPH scavenging ability, reducing power and peroxidation inhibition of 2 water extract from two varieties of napiergrass with different plant parts

Variety	Plant parts	DPPH scavenging ability (%)	FRAP (μmol/l)	Inhibition of peroxidation (%)
NBM	Leaves	88.69[a]	2272.05[a]	86.99[a]
	Stem	15.35[c]	754.15[c]	63.14[b]
TLG2	Leaves	60.06[b]	1901.99[b]	85.88[a]
	Stem	0.67[d]	715.79[d]	62.32[b]

[a–d] Values in columns with different letter indicate the significant difference ($p < 0.05$).
FRAP: ferric reducing ability of plasma.

compared with their stems and all green varieties. NBM variety, as an example, showed that the leaves when compared to their stem, exert 5 times more free radical scavenging ability, 3 times more reducing power, and 1.4 times more peroxidation inhibition. The plants harvested in spring and summer showed higher reducing power (FRAP) (Fig. 1), scavenging effect on DPPH radical, and inhibition of peroxidation (data not shown). According to Wu et al. (2002), one anthocyanin-like polymer or coumaric acid is the compound primarily responsible for the antioxidant capacity of NBM or TLG2. The phenolic contents of leaves from NBM and TLG2 with different growth period and seasons were further determined by HPLC and listed in Tables 2 and 3. The major difference between NBM and TLG2 is the high content of anthocyanin as well as other flavonoids in the purple variety. (Anthocyanin and total flavonoids were about 8.78% and 74.57% of total phenols in NBM and 0.1% and 49.02% in TLG2, respectively). In addition, extremely high content of catechin, chlorogenic acid or caffeic acid in NBM summer samples and high content of catechin and anthocyanin in TLG2 samples grown in spring were found. These results suggested that, individual phenolic compounds, affected by environmental factors (Dela et al., 2003; Nii et al., 1995; Rozema et al., 2002; Sharma et al., 1998), may play an important role in the antioxidant capacity of napiergrass from different growth season. This can be further explained by the principal component analysis (PCA) of their phenolic compounds, including anthocyanins.

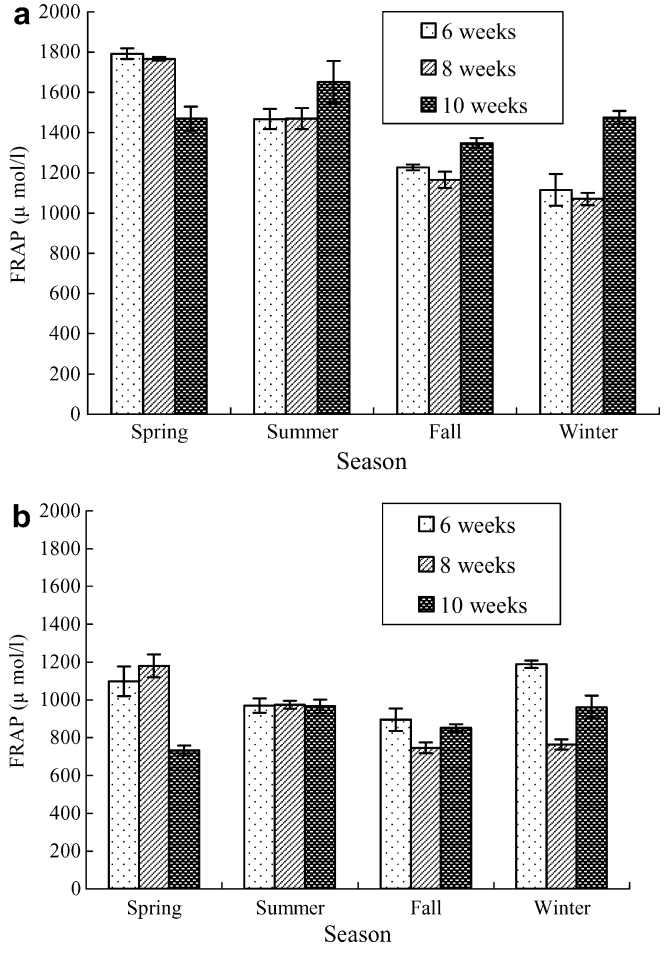

Fig. 1. Reducing power of water extract from (a) NBM and (b) TLG2 with different growing periods and seasons.

Table 2
Phenolic contents of leaves from NMB with different growth period and seasons

Phenolics	Weeks	Spring	Summer	Fall	Winter
Catechin[A]	6	1843.90^{by}	4968.23^{az}	1063.82^{cy}	897.56^{cy}
	8	1324.20^{bz}	6175.06^{ax}	1210.00^{bx}	1083.02^{bx}
	10	2371.99^{bx}	5856.29^{ay}	804.24^{cz}	769.73^{cz}
Epicatechin	6	3143.00^{by}	5830.09^{ax}	2158.62^{cy}	2119.43^{cx}
	8	2516.34^{bz}	4030.68^{ay}	1906.46^{cy}	1672.15^{cy}
	10	5667.35^{ax}	3937.78^{by}	3976.32^{bx}	912.61^{cz}
Rutin	6	7505.50^{ax}	7147.75^{az}	2564.75^{cy}	5776.75^{by}
	8	6506.14^{by}	7386.82^{ay}	2566.66^{dy}	4965.94^{cy}
	10	7389.32^{cx}	8454.73^{bx}	6102.75^{dx}	13139.60^{ax}
Quercetin	6	3011.38^{cx}	3247.05^{cy}	4514.93^{by}	5133.70^{ay}
	8	1000.94^{dz}	3946.75^{cx}	4738.74^{bxy}	5866.67^{ax}
	10	2295.44^{cy}	3762.61^{bx}	4796.59^{ax}	4354.32^{bz}
Anthocyanin	6	2157.60^{ax}	1196.88^{cy}	1518.52^{bx}	933.94^{dz}
	8	2271.43^{ax}	1168.21^{cy}	1337.48^{bz}	1156.48^{cy}
	10	1724.74^{by}	1784.55^{ax}	1452.09^{cy}	1763.73^{abx}
Coumaric acid	6	5549.25^{ay}	4602.89^{by}	3768.83^{cy}	3641.07^{cy}
	8	5797.71^{axy}	4796.08^{by}	3240.25^{dz}	3677.82^{cy}
	10	6103.28^{ax}	6040.00^{ax}	3584.98^{bx}	4204.52^{bx}
Chlorogenic acid	6	595.86^{cy}	1329.37^{ax}	398.49^{dx}	771.04^{bx}
	8	444.42^{bz}	1313.76^{ax}	308.13^{dy}	344.78^{cy}
	10	1038.98^{ax}	1102.29^{ay}	172.78^{cz}	151.89^{bz}
Caffeic acid	6	783.10^{bx}	883.16^{ay}	488.20^{cy}	494.32^{cx}
	8	255.53^{dy}	1204.62^{ax}	975.51^{bx}	391.50^{cy}
	10	209.00^{dz}	1087.63^{ax}	832.00^{bx}	336.10^{cz}

[a–d] Values in rows with different letter indicate the significant difference ($p < 0.05$).
[x–z] Values in columns with different letter indicate the significant difference ($p < 0.05$).
[A] μg/g dry weight.

Table 3
Phenolics contents of leaves from TLG2 with different growth period and seasons

Phenolics	Weeks	Spring	Summer	Fall	Winter
Catechin[A]	6	1240.25^{ay}	934.51^{bz}	943.91^{by}	1249.68^{ax}
	8	446.89^{dz}	1205.54^{bx}	1372.29^{ax}	755.07^{cy}
	10	4020.54^{ax}	1053.77^{by}	1185.26^{bx}	520.53^{cz}
Epicatechin	6	1223.97^{bz}	1774.18^{ax}	101.63^{cz}	91.60^{cz}
	8	2299.81^{ay}	805.84^{cy}	1190.83^{bx}	422.74dx
	10	2942.79^{ax}	296.58^{cz}	707.34^{by}	144.83^{dy}
Rutin	6	3814.42^{by}	4581.40^{ax}	3268.79^{cx}	2549.28dc
	8	2928.05^{bz}	2817.39b^{cy}	2713.25^{cy}	2727.75^{ax}
	10	4863.90^{ax}	2769.64^{dy}	3111.13^{cx}	3414.40^{by}
Quercetin	6	965.91b^{cx}	2088.39^{ax}	909.34^{cy}	1134.06^{by}
	8	895.21^{bx}	1474.00^{ay}	969.45^{bx}	1461.57^{ax}
	10	950.19^{ax}	1008.07^{az}	352.25^{bz}	428.90^{bz}
Anthocyanin	6	14.94^{az}	1.00^{dy}	2.16^{cx}	3.53^{by}
	8	18.34^{ax}	1.00^{cy}	1.00^{cy}	4.83^{bx}
	10	15.55^{ay}	2.08^{bx}	1.00^{cy}	1.00^{cz}
Coumaric acid	6	6154.29^{cx}	9852.49^{ax}	6658.31^{by}	6585.31^{by}
	8	4510.49^{dy}	8080.45^{by}	6825.83^{cy}	9130.60^{ax}
	10	3506.49^{dz}	7496.89^{cz}	8044.32^{bx}	8978.72^{ax}
Chlorogenic acid	6	783.97^{ax}	717.17^{ay}	255.08^{cx}	423.93^{by}
	8	386.07^{by}	361.04^{bz}	282.22^{cx}	731.46^{ax}
	10	307.09^{by}	1121.21^{ax}	152.40^{cy}	180.38^{cz}
Caffeic acid	6	610.03^{ax}	491.70^{by}	301.11^{cz}	314.05^{cz}
	8	261.11^{cz}	376.58^{cz}	949.63^{ax}	451.09^{bx}
	10	466.36^{cy}	996.58^{ax}	553.88^{by}	356.98^{dy}

$^{a–d}$ Values in rows with different letter indicate the significant difference ($p < 0.05$).
$^{x–z}$ Values in columns with different letter indicate the significant difference ($p < 0.05$).
[A] μg/g dry weight.

3.2. PCA for phenolic components in samples with different growth seasons and periods

All phenolic compounds were analyzed through PCA to provide better understanding of their variation. Each principal component or axis was a linear combination of the original variable. The first two principal components (eigen value greater than 1.0) account for about 66.66% of the total variance (data not shown). Fig. 2 shows a plot of the values for the first two principal components. Most phenolic compounds existed along the positive direction of the factor 1 axis except coumaric acid. Phenolic acids such as coumaric acid, chlorogenic acid and caffeic acid appeared along the positive direction of factor 2 axis, while flavanoids such as rutin, anthocyanin, quercetin and catechin relatively bend toward the negative direction. It is likely that the characteristic antioxidant capacity might come from a combination of flavonoids and phenolic acid. The polyphenols identified in the discrimination revealed that anthocyanin exhibited highest F value (172.04) and R square (0.95) followed by quercetin (F value and R square were 109.64 and 0.92) and catechin (F value and R square were 54.45 and 0.86). It leads to the conclusion that, anthocyanins might be the mostly effective compounds responsible for the high physiological activity of all napiergrass. The individual Trolox equivalent antioxidant activity (TEAC) value for cyanidin, quercetin, catechin and epicatechin were 4.4, 4.7, 2.4 and 2.4 mM, respectively, while TEAC for coumaric acid, ferulic acid, chlorogenic acid and caffeic acid were 2.2, 1.9, 1.3 and 1.3 mM, respectively (Rice-Evans et al., 1997), the high FRAP showed by spring sample might be explained by the high content of anthoycanin for NBM and catechin, epicatechin or anthocyanin for TLG2.

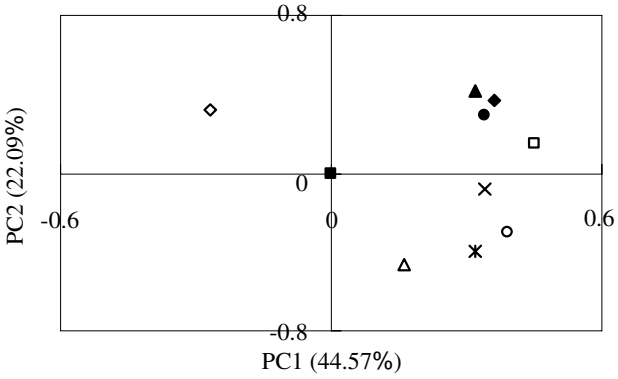

Fig. 2. Distribution of phenolic compounds from mixture of NBM and TLG2 in the canonical plot: ■ gallic acid, ♦ ferulic acid, ▲ chlorogenic acid, ● caffeic acid, □ epicatechin, ◇ coumaric acid, △ catechin, × rutin, ∗ quercetin, ○ anthocyanin.

3.3. Classification of napiergrass with different growth seasons according to polyphenols

As the canonical plots of projections of samples shown in Fig. 3, NBM and TLG2 samples are located on the opposite sides of the pc1 axis. This confirms the causative effect of anthocyanin in classifying the samples. Further

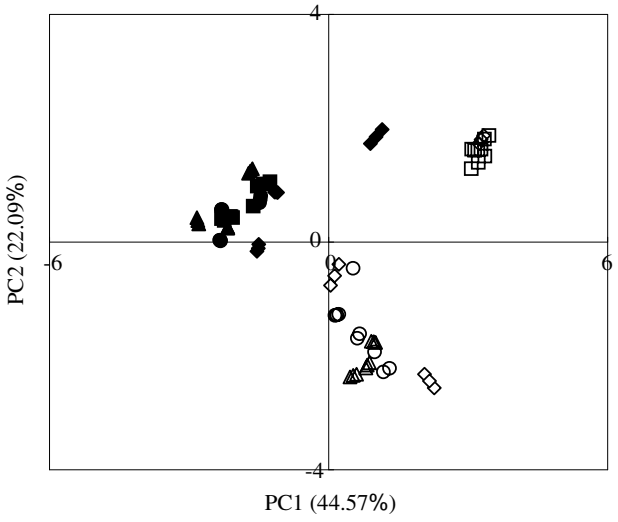

Fig. 3. Classification of napiergrass samples from different varieties (NBM and TLG2) and growing seasons on the basis of their phenolic compounds. ◇ NBM spring, □ NBM summer, ○ NBM fall, △ NBM winter ♦ TLG2 spring, ■ TLG2 summer, ● TLG2 fall, ▲ TLG2 winter.

comparison of summer sample of NBM or spring samples of TLG2 with samples gathered at other growth seasons showed the effect of growth seasons on the polyphenol content. This can be further explained by the significantly high content of chlorogenic acid and caffeic acid in NBM summer samples or high content of anthocyanin in TLG2 samples grown in spring. It appears that, different varieties as well as sunlight of different seasons may affect the accumulation of anthocyanins and phenolic compounds and consequently lead to the changes of antioxidant capacity (Awad et al., 2001). The lower content of anthoyocanin accumulated in summer samples of both NBM and TLG2 may be explained by heat stress (Dela et al., 2003) and is compatible with the lower FRAP values when compared with spring samples.

3.4. Correlation analysis

The correlation between the polyphenols and antioxidant capacity is summarized in Table 4. Anthocyanin, quercetin, rutin, coumaric acid and epicatechin showed significantly positive correlation with both DPPH scavenging ability and FRAP (correlation coefficient were 0.77, 0.58, 0.59, 0.73 and 0.65 for DPPH scavenging, while 0.87, 0.79, 0.66, 0.87 and 0.61 for FRAP). It revealed that anthocyanin and coumaric acid showed the highest relationship with FRAP among phenolic compounds, while quercetin was second. In addition, anthocyanin negatively correlated with coumaric acid (data not shown). It indicated that accumulation of the phenolic compounds influenced by the biosynthesis pathway (Awad et al., 2001) and the reducing power of napiergrass was surely affected by the anthocyanin and phenolic acids (Tsai, McIntosh, Pearce, Camden, & Jordan, 2002).

3.5. Anthocyanin and its colour intensity may be used as index of antioxidant capacity

Besides its role in antioxidant response of plants, anthocyanins are also important in visual attraction (Stintzing & Carle, 2004). It became possible to use the colour to estimate antioxidant capacity. Anthocyanins of napiergrass were further extracted and the effects of pH on their reducing activity were examined in association with color qualities. Results showed that, the Hunter a value, hue, A_{520} and FRAP decreased when pH varied from 2 to 8, but anthocyanin degradation index (DI) and DPPH scavenging ability increased (data not shown). It indicated that the reducing power decreased when red colour changed into brown. A close relationship between antioxidant capacity and A_{520} or Hunter a value was found (correlation coefficient 0.94 and 0.92, respectively). This is in agreement with the positive correlation between anthocyanin and FRAP in roselle (Tsai et al., 2002) or ORAC in blueberries (Kalt, McDonald, & Donner, 2000). It became practical to use the red color as a simple index to distinguish the antioxidant capacity of napiergrass plant from different varieties and seasons.

In conclusion, our results showed that the high antioxidant capacity of the leaves from NMB was mainly attributed to the exceptionally high content of anthocyanin, which resulted in very high colour intensity. Therefore, red colour of the material could be used as a tool to evaluate the antioxidant capacity of this food material. This finding could be practically applied to general consumers as well as food processors in choosing the best raw materials of napiergrass.

Table 4
The correlation among phenolic compounds and antioxidant capacities of napiergrass

	DPPH	FRAP	FTC
Ferulic acid	0.32**	0.37**	0.44
Chlorogenic acid	0.32**	0.21	0.26**
Caffeic acid	0.28*	0.25*	0.25*
Epicatechin	0.65**	0.61**	0.40**
Coumaric acid	0.73**	0.87**	0.28**
Catechin	0.36**	0.37**	0.60**
Rutin	0.59**	0.66**	0.63**
Quercetin	0.58**	0.79**	0.43**
Anthocyanin	0.77*	0.87**	0.48**

*,** Significant at 5% and 1% levels, respectively.
DPPH: DPPH scavenging ability; FRAP: ferric reducing ability of plasma; FTC: inhibition of peroxidation.

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