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The physico-chemical properties, toxicology and bioactivities characterization of mature *Basella alba* L. fruit-extract as a novel natural colorant

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Abstract

The mature *Basella alba* L. fruits are a rich source of natural colorant which deserves research interest and related products development. In this study, mature fresh fruits were blanched at 90°C for 2 min, followed by homogenization, filtration and freeze-drying to prepare *B. alba* colorant-extract powder (BACP). The yield is ca. 37 g BACP/kg fresh fruits. The BACP solution subjected to solid-phase extraction, semi-preparative HPLC isolation, the major red pigment was identified as gomphrenin I. In addition to gomphrenin I, betanidin-dihexose and isobetanidin-dihexose were also detected. BACP was easy to disperse in water and the maximum absorption wavelength at 540 nm. As had good recovering trait, and stability was smooth of BACP aqueous solutions (250 µg/mL) during pH 2 – 9. Except Fe²⁺ and Fe³⁺, other metal ions such as Na⁺, K⁺, Ca²⁺, Mg²⁺ had no influence. BACP aqueous solutions (1 mg/mL) to heat treatment at 90°C for 0 to 10 min, color intensity (absorbance at 540 nm) decreased from 0.56 to 0.36 and total phenolics contents changed in a range between 42.1 and 44.9 mg gallic acid/g BACP. All determined antioxidant activities, namely, trolox equivalent antioxidant capacity (TEAC), reducing power and DPPH free radical scavenging activities were stable against time of heat treatment. Altogether, BACP has good stability under certain conditions. The anti-inflammatory effects of accessed with a murine macrophage-like cell line (RAW 264.7) activated with 0.5 µg/mL lipopolysaccharide (LPS) and introduced with various concentrations of BACP for 24 h, cell viability was over 90% below 1000 µg/mL of BACP. Its significantly and dose-dependently suppresses the secretion of pro-inflammatory mediators and inhibited the production of NO (nitrite), PGE₂ (prostaglandin E₂), IL-12, IL-6, IL-1β and TNF-α. In toxicology and health-benefit assessment, 6-week-old ICR male mice were gavage-administered with various BACP doses of 0, 250, 500 and 1000 mg/kg B.W. for 28 days. After sacrificed and based on the results of serum biochemical analyses and organ histopathological examinations, there was no obvious toxicological hazard caused by BACP administration observed. As affected by doses of BACP administration, proliferation of the spleen lymphocytes was enhanced by an increase of dose administration. Expressions of IL-2 and IFN-γ of the Th1 cells increased while expressions of IL-4 and IL-5 of the Th2 cells after mediation with concanavalin A (Con A) decreased with an increase of dose administration. Enhancement of immunomodulatory activities of the test mice achieved by BACP administration was obvious.

Introduction

Basella alba L. is commonly grown for harvest of leaves as a green vegetable, however, use of its mature dark blue fruits with deep red-violet flesh has not been developed (Fig 1.). In this study, in an attempt to minimize the processes in preparation of *B. alba* colorant-extract powder (BACP) with warrant of toxicological acceptability, the mature *B. alba* fruits were blanched, homogenized, filtered and lyophilized. The aqueous BACP solutions were subjected to physico-chemical properties analysis for recovering trait, various pH value, metal ions, thermal stability characterization and also addressed on changes of antioxidant and free radical scavenging activities. The anti-inflammatory effects of accessed with a murine macrophage-like cell line (RAW 264.7) activated with 0.5 µg/mL lipopolysaccharide (LPS) and introduced with various concentrations of BACP. For toxicology and health-benefit assessment, ICR mice were gavage-administered with various doses of BACP for 28 days and followed by serum biochemical analyses, organ histopathological examinations and investigations of immunomodulatory activities.



Fig 1. Photograph of *Basella alba* leaves, flowers and fruits (A), blanching of *B. alba* colorant-extract powder (BACP) on a filter paper (B).

Basella alba L. colorant-extract powder (BACP) preparation:

The mature fresh fruits or defrosted fruits were water washed, drained, weighed and blanched with hot water at 90°C for 2 min. Then the fruits were cooled with tap water, drained and homogenized with a waring blender. The homogenate was filtered with a filtration cloth and the filtrate was centrifuged at 8050 g for 5 min. Then, the supernatant was lyophilized to prepare *B. alba* colorant-extract powder (BACP).

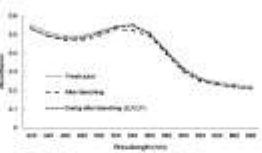
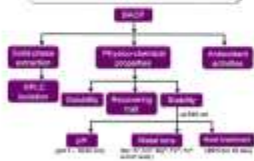
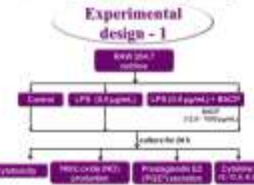


Fig 3. Recovering test of *B. alba* colorant-extract powder (BACP) from drying after mature fruits blanching. Each value represents mean ± SD (n = 3).

Experimental of physico-chemical properties



Experimental of toxicology and bioactivities characterization



Experimental design - 2



Fig 10. Histopathological examination (H-E stain) of the heart, liver, lung and kidney tissues of ICR mice after oral administration with different doses of *B. alba* colorant-extract powder (BACP) for 28 days. Control: 0.9 % normal saline; APL: 250 mg BACP/kg body weight; APM: 500 mg BACP/kg body weight; APH: 1000 mg BACP/kg body weight. Data are expressed as the mean ± SD (n = 5).

Fig 11. Viability of the spleen cells originated from ICR mice after oral administration with different doses of *B. alba* colorant-extract powder (BACP) for 28 days after mediation with lipopolysaccharide (LPS) and Con A. Each value represents mean ± SD (n = 5). Bars noted with different letters are significantly different (p < 0.05).

Fig 12. Proliferation of the spleen T lymphocytes originated from ICR mice after oral administration with different doses of *B. alba* colorant-extract powder (BACP) for 28 days after mediation with lipopolysaccharide (LPS) and Con A. Each value represents mean ± SD (n = 5). Bars noted with different letters are significantly different (p < 0.05).

Solvent	sol	insol	sol	sol	sol	sol	sol	sol	sol
Water	+	+	+	+	+	+	+	+	+
H ₂ O: A:EtOH	+	+	+	+	+	+	+	+	+
H ₂ O: A:EtOH: anhydrous ethanol	+	+	+	+	+	+	+	+	+
Acet. acetone, EtOAc, ethyl acetate, CH ₂ Cl ₂ , chloroform, Hex. hexane, Et ₂ O, ether, n-hexane, n-pentane	-	-	-	-	-	-	-	-	-

Sex	Maximum relative organ weights				
	Heart	Liver	Lung	Spleen	Kidney
Male	0.24 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	0.21 ± 0.01	0.22 ± 0.01
Female	0.23 ± 0.01	0.18 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	0.21 ± 0.01
Control	0.24 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	0.21 ± 0.01	0.22 ± 0.01
APL	0.24 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	0.21 ± 0.01	0.22 ± 0.01
APM	0.24 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	0.21 ± 0.01	0.22 ± 0.01
APH	0.24 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	0.21 ± 0.01	0.22 ± 0.01

Fig 9. Changes of the body weights of ICR mice during 28 day-experiment as affected by oral administration with different doses of *B. alba* colorant-extract powder (BACP): C: 0.9 % normal saline; APL: 250 mg BACP/kg body weight; APM: 500 mg BACP/kg body weight; APH: 1000 mg BACP/kg body weight. Data are expressed as the mean ± SD (n = 5).

Sex	Serum biochemical analysis				
	AST (U/L)	ALT (U/L)	ALP (U/L)	BUN (mg/dL)	Cr (mg/dL)
Male	45.0 ± 2.0	35.0 ± 2.0	120.0 ± 5.0	0.5 ± 0.05	0.2 ± 0.02
Female	44.0 ± 2.0	34.0 ± 2.0	115.0 ± 5.0	0.5 ± 0.05	0.2 ± 0.02
Control	45.0 ± 2.0	35.0 ± 2.0	120.0 ± 5.0	0.5 ± 0.05	0.2 ± 0.02
APL	45.0 ± 2.0	35.0 ± 2.0	120.0 ± 5.0	0.5 ± 0.05	0.2 ± 0.02
APM	45.0 ± 2.0	35.0 ± 2.0	120.0 ± 5.0	0.5 ± 0.05	0.2 ± 0.02
APH	45.0 ± 2.0	35.0 ± 2.0	120.0 ± 5.0	0.5 ± 0.05	0.2 ± 0.02

Fig 13. Th1/Th2 cytokine mRNA levels of the spleen T cells originated from ICR mice after oral administration with different doses of *B. alba* colorant-extract powder (BACP) for 28 days. Control: 0.9 % normal saline; APL: 250 mg BACP/kg body weight; APM: 500 mg BACP/kg body weight; APH: 1000 mg BACP/kg body weight. Each value represents mean ± SD (n = 5). Bars noted with different letters are significantly different (p < 0.05).

Conclusion

As generally observed, it is of merit that potent antioxidant and immunomodulatory activities of *Basella alba* colorant-extract powder (BACP) have been detected with no obvious toxicological health hazards and demonstrated as a novel source of natural colorant, and has value-added potential for use in development of foods conferring health benefits.



秋葵抗氧化能力及抑制醣解酵素活性之探討

Antioxidant activity and glycosidase inhibitory of okra (*Abelmoschus esculentus* (L.) Moench)

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摘要

秋葵 (*Abelmoschus esculentus* (L.) Moench), 又稱羊角豆, 為錦葵科 (Malvaceae) 秋葵屬 (*Abelmoschus*) 一年或多年生草本植物, 為常用食蔬。研究指出, 其具有抗菌、抗發炎、抗糖尿病及保護胃壁等功效。本研究以95% 乙醇萃取秋葵凍乾粉末, 探討秋葵乙醇萃取物抗氧化能力及抑制醣解酵素活性, 結果顯示, 秋葵乙醇萃取物之抗氧化能力呈劑量-反應效應, 於濃度 5 mg/mL 下, 其清除 DPPH 及 ABTS^{•+} 自由基能力分別為 89.94% 及 77.58%, IC₅₀ 為 1.55 mg/mL 及 1.97 mg/mL。總酚及類黃酮含量, 分別為 51.27 mg gallic acid equivalents/g dried weight 及 23.50 mg rutin equivalent/g dried weight。由抗氧化試驗結果顯示, 秋葵具良好之抗氧化能力, 故進一步測定其抑制醣解酵素活性能力。結果顯示, 秋葵抑制醣解酵素活性具劑量-反應效應, 於濃度 15 mg/mL 下, 其抑制 α-澱粉酶及 α-葡萄糖苷酶活性分別為 27.23% 及 54.18%, IC₅₀ 分別為 20.10 mg/mL 及 14.41 mg/mL。綜合上述, 秋葵乙醇萃取物具有良好之抗氧化活性及降血糖之潛力, 期能將其開發成保健產品, 以增加秋葵之產品多樣性及經濟價值。

關鍵字: 秋葵、抗氧化、醣解酵素、總酚、類黃酮

前言

秋葵 (okra) 原產於亞洲熱帶地區, 台灣每年 4-9 月為盛產期, 產地分佈於彰化、雲林、嘉義、高雄及屏東等地, 通常當做生菜食用。其具有特殊黏稠物質, 成分為由半乳糖、鼠李糖和半乳糖醛酸等組成之多醣體。文獻指出, 秋葵多醣可抑制胃黏膜幽門螺旋桿菌的附着力, 達到保護腸胃道的功效, 而其可溶性纖維透過調節腸道對葡萄糖之吸收速度, 而達穩定血糖之作用。有研究指出秋葵具有良好之抗氧化功效, 富含多種酚類化合物, 且文獻以證實植物中多酚物質具有抑制醣化酵素之能力, 故本研究探討秋葵之抗氧化活性及抑制醣化酵素之能力, 期能作為開發秋葵保健產品之依據。

實驗架構



結果

表一、秋葵乙醇萃取物之萃取率、總酚及類黃酮含量

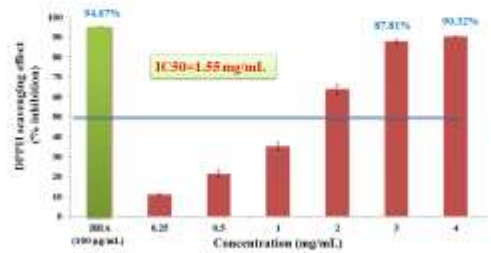
Table 1. Yield, total phenolics and flavonoids contents of ethanol extract from okra.

Yield (%)	Contents	
	Total phenolics (mg GAE/g)	Flavonoids (mg RE/g)
10.96±0.64	51.27±0.96	23.5±1.23

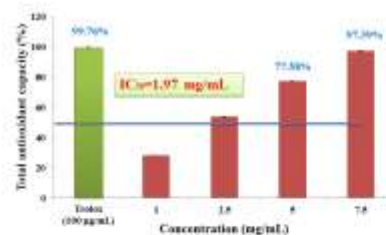
Each value is expressed as mean±S.D. (n=3).

Means with different letters within the same column differed significantly (p<0.05).

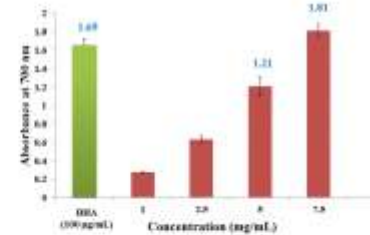
GAE: gallic acid equivalent; RE: rutin equivalent.



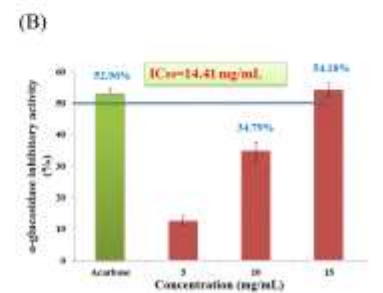
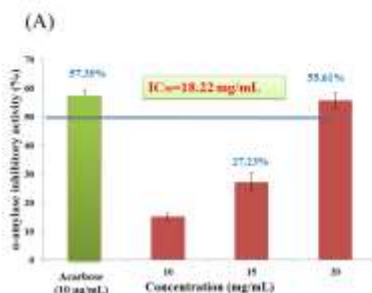
圖一、秋葵乙醇萃取物清除 DPPH 自由基之能力
Figure 1. Scavenging effect of okra ethanol extract on the DPPH radical. Each value is expressed as mean ± S.D. (n=3).



圖二、秋葵乙醇萃取物之總抗氧化能力
Figure 2. Total antioxidant activity of okra ethanol extract. Each value is expressed as mean ± S.D. (n=3).



圖三、秋葵乙醇萃取物之還原力
Figure 3. Reducing power of okra ethanol extract. Each value is expressed as mean ± S.D. (n=3).



圖四、秋葵乙醇萃取物對(A) α-amylase (B) α-glucosidase 抑制能力
Figure 4. α-amylase and α-glucosidase inhibitory activities of okra ethanol extract. Each value is expressed as mean ± S.D. (n=3).



結論

1. 秋葵乙醇萃取物之抗氧化能力呈劑量-反應效應, 於濃度 5 mg/mL 下, 其清除 DPPH 及 ABTS^{•+} 自由基能力分別為 89.94% 及 77.58%, IC₅₀ 為 1.55 mg/mL 及 1.97 mg/mL, 還原力達 1.21, 總酚及類黃酮含量, 分別為 51.27 mg/g 及 23.50 mg/g。

2. 醣化酵素抑制試驗結果顯示, 隨著秋葵乙醇萃取物濃度上升, 酵素抑制率也有上升之趨勢, 濃度呈劑量效應, 顯示其具有減緩餐後血糖急遽上升及維持血糖穩定之功效。

3. 綜合上述, 秋葵乙醇萃取物具有良好之抗氧化功效及降血糖之潛力, 期能開發保健產品, 增加秋葵之產品多樣性及經濟價值。



狐尾粟 (*Setaria italica* (L.) P. Beauv.) 一般組成成分及抗氧化活性之探討

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國立嘉義大學食品科學研究所

摘要

本研究主要是探討狐尾粟(俗稱小米),其一般組成成分、總酚、類黃酮含量及抗氧化活性。小米(Millet)是各種小規模栽培米穀類作物的通稱,其中台灣主要栽培品種為狐尾粟。將不同組別小米,包含穀殼、全穀、去殼、去殼蒸煮,粉碎後進行組成成分分析。結果顯示以穀殼之粗灰分、粗纖維、粗脂肪為最高,分別是6.26%、23.81%及10.97%;粗蛋白及無氮化合物含量以去殼後小米含量最高,分別為11.80%及73.10%;去殼蒸煮後小米水分含量13.3%為最高。不同組別小米經冷凍乾燥去除水分後粉碎,以70%丙酮萃取。結果產率以穀殼10.63%為最高,另外全穀為5.43%、去殼5.51%及去殼蒸煮1.84%。抗氧化成分分析中總酚及類黃酮含量皆以穀殼含量最高,其含量分別為183.4 GAE mg/g d.m.及36.37 CE mg/g d.m.。其中蒸煮過後的去殼小米在類黃酮含量高於未蒸煮之去殼小米,但總酚含量仍是去殼小米高於去殼蒸煮之小米。抗氧化活性分析結果以穀殼為最佳,其DPPH自由基清除能力為40.72%及總抗氧化能力為18.64%。

關鍵字: 小米、化學組成成分、總酚、類黃酮、抗氧化活性

前言

小米 (*Setaria italica* L. Beauv.) 為粟類 (Millet) 之一種, 英文名為狐尾粟 (Foxtail millet), 又稱義大利粟 (Italian millet), 主要分布於亞洲東南部及中亞, 為一年生禾本科草質植物。小米的外殼有白、紅、黃、黑、橙及紫各種顏色, 籽實卵圓狀, 粒小多而黃色, 小米有高利用價值且營養價值高, 更含有一般穀物缺乏的維生素A、B1、B2和E等, 亦含有少量硒素, 此外還含有多種有機酸類和纖維素等對人體健康之功能有極大的幫助。而對於小米抗氧化能力及成分的研究並不多, 因此本研究以不同組別(穀殼、全穀、去殼及去殼蒸煮)之小米, 探討其一般組成成分、抗氧化能力及成分之比較分析, 以期可提高小米應用之多樣性及推廣小米普遍性。

材料與方法

一、實驗材料

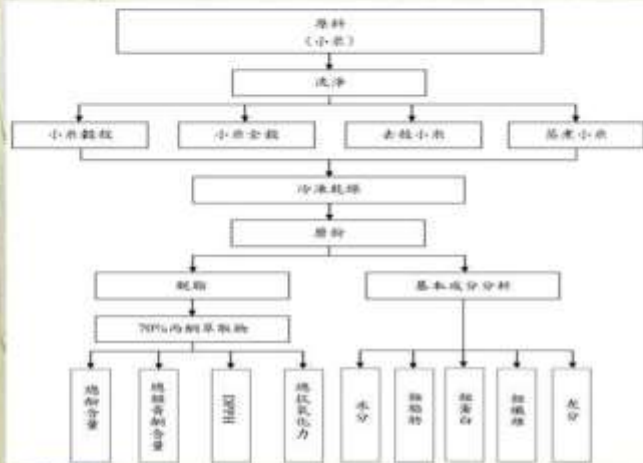
1. 實驗材料:

以台東拉勞蘭產銷班生產之台灣原生小米為材料, 分為四組: 穀殼、全穀、去殼及去殼蒸煮, 進行實驗。

2. 實驗藥品:

丙酮、碳酸鈉、亞硝酸鈉、氯化鋁、氫氧化鈉等, 購買自Merck公司; 沒食子酸、福林酚試劑、兒茶素、BHA、DPPH等, 購買自Sigma公司。

二、實驗方法



結果與討論

- 表一為不同組別小米組成成分分析, 結果顯示, 穀殼(Hull)及全穀(WG)皆因含有外殼, 故粗灰分及粗纖維所佔的比例較高, 其中穀殼含有豐富的脂溶性物質, 粗脂肪含量也與各組別中穀殼所占比例成正比。蒸煮小米(CG)其因加水蒸煮過, 使其水分含量偏高。
- 表二為不同組別小米經70%丙酮萃取後之產率, 小米樣品經粉碎、脫脂後於室溫下以70%丙酮萃取60分鐘再經濃縮, 凍乾後同樣以70%丙酮回溶進行後續試驗。其產率分別為穀殼10.63%、全穀5.42%、去殼5.51%及蒸煮1.84%, 以穀殼的產率為最高。
- 表三為不同組別小米其70%丙酮萃取物中所含之總酚及總類黃酮化合物之含量結果, 從結果顯示, 不同組別小米的總酚含量依序為: 穀殼>全穀>去殼>蒸煮小米; 不同組別小米的總類黃酮含量依序為: 穀殼>全穀>蒸煮>去殼。均以穀殼含量為最高, 其總酚含量為183.4 GAE mg/g d.m.及總類黃酮含量為36.37 CE mg/g d.m., 其中蒸煮後小米的總類黃酮含量高於去殼小米, 可能是因經過蒸煮後小米中抗氧化物質釋出造成。
- 圖一為不同組別小米其70%丙酮萃取物之DPPH自由基清除能力結果, 結果顯示50 ppm之BHA, 其DPPH自由基清除能力為82.69%; 小米部分則是以穀殼之DPPH自由基清除能力最高為40.72%, 去殼小米清除能力最低為3.64%, 蒸煮後小米其清除能力高於未蒸煮之去殼小米, 且與全穀的清除能力相近。
- 圖二為不同組別小米其70%丙酮萃取物之總抗氧化力結果, 結果顯示50 ppm之Trolox, 其總抗氧化力為9.71%; 小米部分則是以穀殼之總抗氧化力最高(18.64%), 且高於對照組, 去殼小米清除能力最低為7.45%。蒸煮後小米清除能力高於未蒸煮之去殼小米, 此結果與總類黃酮含量及DPPH自由基清除能力相似, 雖皆未高於穀殼及全穀, 但蒸煮有助於增加小米抗氧化能力的趨勢。

結論

綜合以上的結果得知, 不同組別小米其抗氧化能力及成分皆以穀殼組別含量為最高, 全穀次之, 上述兩組皆含有小米外殼, 由此可推論小米之抗氧化成分主要存在於外殼中, 蒸煮過後的去殼小米其抗氧化能力及成份高於未蒸煮過之去殼小米, 甚至在抗氧化成分比較中與全穀結果相近。因此小米應連殼一併蒸煮後食用, 可提高較多之抗氧化成分, 而小米穀殼可做為廢棄物再利用之材料, 以加工方式提升其產品價值, 以增進小米作物整體利用率。

表一、不同組別小米組成成分分析

Table 1. Proximate composition in different groups of foxtail millet

Groups	Composition (%)					
	Crude ash	Moisture	Crude fiber	Crude protein	Crude fat	Nitrogen-free-extract
Hull	6.26	10.04	23.81	9.97	10.97	38.95
WG ¹	2.74	11.96	8.05	9.86	5.12	62.27
DG	1.05	10.97	0.22	10.72	3.94	73.10
CG	1.09	13.35	0.38	10.95	3.68	70.55

1. Abbreviations: WG, whole grain; DG, dehull grain; CG, cooked grain.

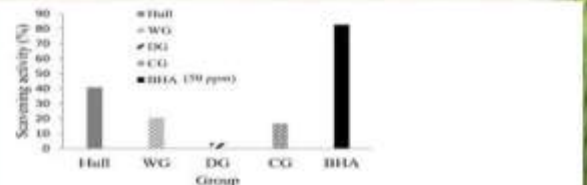
表二、不同組別小米其 70%丙酮萃取物中所含之總酚、總類黃酮化合物之含量及產率
Table 2. Total phenolics, flavonoids contents and yield of 70% acetone extract obtained from different groups of foxtail millet

Groups	Yield (%)	Content	
		Total phenolics ¹	Flavonoids ²
Hull	10.63	183.4	36.37
WG ²	5.42	111.4	17.48
DG	5.51	70.07	11.93
CG	1.84	60.73	12.59

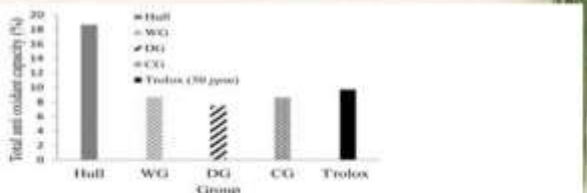
1. Total phenolics was expressed as milligram of gallic acid equivalent per gram of dry matter (GAE mg/g d.m.).

2. Flavonoids was expressed as milligram of catechin equivalent per gram of dry matter (CE mg/g d.m.).

3. Abbreviations: WG, whole grain; DG, dehull grain; CG, cooked grain.



圖一、不同組別小米其 70%丙酮萃取物 (1000 µg/ml) 之 DPPH 自由基清除能力
Fig. 1. The DPPH radical scavenging activity of 70% acetone extract (1000 µg/ml) obtained from different groups of foxtail millet.



圖二、不同組別小米其 70%丙酮萃取物 (1000 µg/ml) 之總抗氧化能力
Fig. 2. Total antioxidant capacity of 70% acetone extract (1000 µg/ml) obtained from different groups of foxtail millet.

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