SUPPLEMENTARY MATERIAL

Characterization of free and insoluble-bound phenolics of chia (Salvia hispanica L.) seeds

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Abstract

The content, antioxidant activity and composition of free and bound phenolics from chia seeds were investigated. The free phenolics were extracted by using ethanol and methanol of analytical purity, 70% v/v ethanol and by 70% v/v methanol. The bound phenolics were extracted from seeds after alkaline hydrolysis was done. The phenolics content obtained by aqueous alcoholic solutions was higher than the content obtained by using pure alcoholic solution (for 20.8% by 70% methanol and for 41.2% by 70% ethanol). The content of bound phenolics was 932 μ g g⁻¹. Among the identified phenolic compounds the apigenin 4'-*O*-glucoside was the most abundant, and among the acids, a rosmarinic was the most abundant in free phenolic extract and caffeic acid in bound. The correlation coefficients indicated the DPPH method and reducing power were more suitable for assessing the antioxidant activity than FRAP method.

Keywords: Salvia hispanica L.; seeds; phenolic compounds; antioxidant activity

Table S1. The content, SC_{50} and EC_{50} values and FRAP value for various alcoholic extracts

Solvents	70% Ethanol	Ethanol	70% Methanol	Methanol	Insoluble-bound phenolics
Phenolics content [*]	1221.94±43.24 ^a	718.84±24.03 ^b	1593.33±55.32 ^c	1262.63±42.54 ^a	931.89±28.65 ^b
The polarity index of solvent, P'	6.07	4.30	6.63	5.10	6.63
Extract dry residue content**	7.36±0.95 ^a	10.56±1.18 ^b	7.03±0.86 ^a	9.23±1.43 ^b	5.15±1.13 ^a
SC ₅₀ ^{**} , DPPH test	0.43±0.16 ^a	1.27±0.42 ^b	0.39±0.17 ^a	1.05±0.72 ^b	0.33±0.19 ^a
Phenolics content in dry matter of SC ₅₀ value for DPPH test ^{***}	14.28±5.32	17.29±4.36	17.6±7.71	28.73±3.28	17.92±2.10
EC ₅₀ **, Reducing power test	$12.14{\pm}1.16^{a}$	$26.75{\pm}2.74^b$	11.43±0.95 ^a	23.14±1.59 ^b	$0.88 \pm 0.47^{\circ}$
Phenolics content in dry matter of EC ₅₀ value for reducing power ^{***}	401.28±38.52	363.51±37.30	516.76±43.06	631.99±43.51	47.78±5.25
FRAP value ^{*****}	4.16±0.97 ^a	0.83±0.11 ^b	6.19±1.15 ^a	1.61 ± 0.26^{b}	3.18 ± 0.86^{a}
BHA equivalent ^{***}	86.32±5.63	16.96±1.03	128.61±14.57	33.21±4.76	65.63±8.11

of free phenolics and insoluble-bound phenolics from chia seeds

Within rows, values subscribed by the same letters are not significantly different at p > 0.05 * µg gallic acid equivalent per g of dry chia seeds ** mg dry residue per mL of extract *** µg per mL **** mmol Fe²⁺ per L of extract

Component/Content (µg g ⁻¹) [*]	Free phenolics	Bound phenolics
Gallic	69.88±8.56 ^a	8.48 ± 1.75^{b}
Chlorogenic	61.78±10.65 ^a	14.59±1.05 ^b
Caffeic	14.01 ± 1.27^{a}	28.26±3.54 ^b
<i>p</i> -Coumaric	ND	1.01±0.84
trans –Ferulic	6.28±2.23 ^a	1.76 ± 0.92^{b}
Syringic	$2.82{\pm}0.89^{a}$	4.71±0.93 ^b
Ellagic	ND	12.06±1.66
Rosmarinic acid	351.30±46.39	ND
Myricetin	$75.47{\pm}14.98^{a}$	30.27±4.52 ^b
NID 1 (7.98 min)**	322.99±67.89	ND
Kaempferol 3-O-glucoside-7-O-rhamnoside***	18.25±2.37	ND
Kaempferol 3-O-7-O-diglucoside***	ND	5.29±0.87
Kaempferol 3-O-rutinoside-7-O-glucoside***	ND	45.35±6.58
Protogenkwanin-4-O-glucoside***	ND	11.22±1.41
Apigenin 4- <i>O</i> -glucoside ^{***}	102.63±23.82 ^a	87.65±16.74 ^a
NID 2 (RT 64.81 min)***	ND	64.31±9.84
Total Kaempferol	18.25±2.37 ^a	50.64±6.64 ^b
Total identified	702.69±56.07	250.65±19.19
Total	1025.41±88.05	314.96±21.57

Table S2. The composition of free and bound phenolics from chia seeds

Values are mean of three measurements±standard deviation

Values with same superscript in the same row do not differ significantly (p > 0.05) * - expressed as µg acid or myrycetin per g on dry seed basis *** - expressed as µg coumaric acid per g on dry seed basis **** - expressed as µg quercetin equivalent per g on dry seed basis NID - nonidentified component

ND - non detected



Figure S1. The correlation between the content of free phenolics extracted by using various solvents and DPPH SC (a) reducing power (b) and FRAP value (c).