

Supplementary Materials

Table S1. Radical Scavenging activities of each component of MOTILIPERM on ABTS⁺ DPPH, deoxyribose degradation, and 2'-deoxyguanosine hydroxylation assays.

Medicinal plants	Scavenging activity (SC ₅₀ , µg/mL)			
	ABTS	DPPH	deoxyribose degradation ^a	2'-deoxyguanosine hydroxylation ^b
<i>Morinda officinalis</i> roots	> 500	> 200	16.73 ± 1.35	497.24
<i>Allium cepa</i> outer scales	4.51 ± 0.22	3.61 ± 0.39	30.48 ± 0.47	1.65
<i>Cuscuta chinensis</i> seeds	58.30 ± 0.23	32.06 ± 1.68	17.68 ± 0.99	> 1000

^a mannitol was used as a positive control (SC₅₀ value was 91.63 mM).

^b mannitol was used as a positive control (SC₅₀ value was 558.95 µM)

ABTS assay

The ABTS⁺ radical cation method [1] was modified to evaluate the free radical scavenging effect of each medicinal plant component of MOTILIPERM (*Morinda officinalis* roots, *Allium cepa* outer scales, and *Cuscuta chinensis* seeds). The ABTS reagent was prepared by mixing 5 mL of 7 mM ABTS with 88 µL of 140 mM potassium persulfate. After the mixture was kept in the dark at room temperature for 16 h to allow for the completion of radical generation, it was diluted with ethanol (1:44, v/v). To determine the scavenging activity, 190 µL of ABTS reagent was mixed with 10 µL of samples in a 96-well microplate and incubated at room temperature for 6 min. The final concentration of samples was prepared range from 0 (blank) to 100 µg/mL. After incubation, the absorbance was measured at 734 nm using a Bio-Rad Benchmark Plus microplate reader, and 75% ethanol was used as a control. The ABTS⁺ scavenging effect was calculated as: $(A_{\text{control}} - A_{\text{sample}}) / (A_{\text{control}}) \times 100\%$. The SC₅₀ values were obtained through extrapolation from the regression analysis. They denoted the sample concentration required to scavenge 50% of ABTS radicals. The antioxidant activities were evaluated using an SC₅₀ value.

DPPH assay

The DPPH assay was performed as described [1]. The reaction mixture, containing an ethanolic solution of 200 µM DPPH (100 µL) and two-fold serial dilutions of the sample (dissolved in 100 µL ethanol, with sample amounts ranging from 0.001 to 10 mg/mL), was placed in a 96-well microplate and incubated at 37°C for 30 min. After incubation, the absorbance was read at 517 nm and the scavenging activity was determined by the following equation: $\% \text{ scavenging activity} = (A_{\text{control}} - A_{\text{sample}}) / (A_{\text{control}}) \times 100$. The SC₅₀ values were obtained using the same method as for the ABTS⁺ radical scavenging assay.

Deoxyribose degradation assay

Deoxyribose degradation by hydroxyl radical ($\bullet\text{OH}$) was carried out with slight modification

as described [2]. Samples were dissolved in absolute ethanol (0.001 – 10 mg/ml). An aliquot of sample solution was brought to 800 µl in phosphate buffer (25 mM, pH 7.2). Then, 50 µl of deoxyribose (3 mM), 100 µl of Na₂EDTA (1 mM), 100 µl of FeCl₃ (3.2 mM) and 100 µl of H₂O₂ (1 mM) were added. The reaction was initiated by mixing 100 µl of ascorbic acid (100 µM) and the total volume of the reaction mixture was adjusted to 800 µl with buffer. After incubation at 37 °C for 60 min, the reaction was terminated by 250 µl of trichloroacetic acid (2.8%, w/w). The color was then developed by addition of 125 µl of TBA (1%, in 50 mM NaOH aqueous solution) and heating in an oven at 100 °C for 15 min. The mixture was cooled and absorbance was measured at 532 nm (Bio-Rad Benchmark Plus microplate reader) against the buffer (as blank). The hydroxyl radical-scavenging activity was expressed as: $(A_{\text{control}} - A_{\text{sample}}) / (A_{\text{control}}) \times 100$.

2'-Deoxyguanosine hydroxylation assay

The production of 8-hydroxy-2'-deoxyguanosine (8-OHdG) was performed as described [2]. The experimental conditions for the reaction of 2'-deoxyguanosine with •OH radicals were set as follows: E-tubes with a 1 l final volume, containing phosphate buffer pH 7.2 (25 mM), 2'-deoxyguanosine (150 µM), samples concentrations ranging from 0 (absence) to 10 mg/mL, FeCl₃ (100 µM), EDTA (100 µM), and ascorbic acid (200 µM)). To initiate the reaction, H₂O₂ (1 mM) was added and the samples incubated at 37°C for 60 min. Assays were also carried out with ethanol and mannitol as radical scavengers. Assays were performed in triplicate, and after incubation the samples were frozen until injection into HPLC the same day. The SC₅₀ values were calculated by non-linear regression of the percentage of inhibition of 8-OHdG versus the concentration of samples

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2. Herraiz, T.; Galisteo, J. Hydroxyl radical reactions and the radical scavenging activity of β-carboline alkaloids. *Food Chem.* **2015**, *172*, 640-649.