更多期刊、圖書與影音講座，請至【元照網路書店】www.angle.com.tw

Journal of Food and Drug Analysis, Vol. 17, No. 5, 2009, Pages 333-341 藥物食品分析 第十七卷 第五期

Determination of Antioxidative Properties of *Morinda citrifolia* Using Near Supercritical Fluid Extraction

CHIN-HUI CHEN[1,2], TSUEY-PIN LIN[3], YU-LING CHUNG[4], CHING-KUO LEE[1], DONG-BOR YEH[4] AND SHIH-YING CHEN[3,5]*

[1.] *Graduate Institute of Pharmacognosy, Taipei Medical University, Taipei, Taiwan, R.O.C.*
[2.] *Department of Medical Technology, Yuanpei University of Science and Technology, Hsin-Chu, Taiwan, R.O.C.*
[3.] *Department of Health and Nutrition, Chia-Nan University of Pharmacy and Science, Tainan, Taiwan, R.O.C.*
[4.] *Department of Biotechnology, Chia-Nan University of Pharmacy and Science, Tainan, Taiwan, R.O.C.*
[5.] *Department of Applied Life Science and Health, Chia-Nan University of Pharmacy and Science, Tainan, Taiwan, R.O.C.*

(Received: September 17, 2008 ; Accepted: August 3, 2009)

ABSTRACT

Limited knowledge is available regarding the hydrophobic antioxidants present in the *Morinda citrifolia* L (Noni). Moreover, supercritical fluid carbon dioxide (SF-CO_2) extraction technique is well known with many superior extraction properties. The purpose of this study was to investigate the antioxidative properties of crude extracts from the leaves, green and brown stems, and fruit of Noni plant obtained by near SF-CO_2 extraction. Extracts from brown stems by near SF-CO_2 extraction under high pressure and low temperature conditions afforded the highest total phenolic content (23.14 ± 1.83 mg/g) and the best reducing power (59.82 ± 6.28 ascorbic acid equivalents mg/g). It also possessed the greatest antioxidative capacities, including Trolox equivalent antioxidant capacity assay (564.52 ± 41.58 μmol/g), scavenging of 2,2-diphenyl-1-picrylhydrazyl (effective concentration : EC_{50} = 6.87 ± 0.14 mg/L), and superoxide radical scavenging activity (505.12 ± 26.68 SOD unit/g), which indicated that the antioxidative activity is not only due to phenolic compounds but also due to non-polar antioxidants. Extracts from the brown stems of *M. citrifolia* could be a significant source of antioxidants when isolated by near SF-CO_2 extraction.

Key words: *Morinda citrifolia*; Noni, antioxidative activity, supercritical fluid carbon dioxide, phenolic compounds, reactive oxygen species

INTRODUCTION

Naturally occurring chemicals from plants have been consumed by people for many years and have recently begun to receive much attention for their medicinal use as safe antioxidants(1,2). *Morinda citrifolia* (Rubiaceae), commonly known as Noni, is a plant typically found in Hawaii, Tahiti, tropical Asia, and southern Taiwan. The bark, stems, roots, leaves, and fruit of this plant have been used traditionally as folk remedies for many diseases including diabetes, hypertension, and cancer(3-6). Noni has been reported to have a broad range of therapeutic actions including anti-bacterial, anti-viral, anti-fungal, anti-tumor, analgesic, anti-helmintic, anti-hypertensive, anti-inflammatory, and immune enhancing effects(3,4,6-9). Despite the large number of medical claims that have been made for Noni's efficacy, little is known about its true pharmacological potential or mechanisms of action.

Recently, increasing attention has been paid to the association between reactive oxygen species (ROS) and a wide range of degenerative processes and diseases including aging, cancer, diabetes, and cardiovascular disease(10). Normally, cells and tissues possess antioxidant defense mechanisms to ensure the removal of ROS. Some of these mechanisms are controlled endogenously (e.g. superoxide dismutase) while others are provided by exogenous sources such as food or dietary supplements(11). Understandably, there has been an increasing demand to evaluate the antioxidant properties of chemicals from plant origin rather than to look at synthetic options(12-15). Flavonol glycosides from the Noni leaf have a scavenging activity for 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals(21). In addition, the work of Zin *et al.*(33) demonstrated that a methanol extract from the Noni root and ethyl acetate extracts from the

* Author for correspondence. Tel: +886 0933083420; Fax: +886-6-2667097; E-mail: shihying@mail.chna.edu.tw; osyc@ms48.hinet.net

更多期刊、圖書與影音講座，請至【元照網路書店】www.angle.com.tw

Noni leaf, fruit, or root exhibit significant antioxidant activity as determined by the ferric thiocyanate method or thiobarbituric acid test[16]. Subsequently, Zin *et al.*[34] reported that crude extracts of the Noni root, leaf, and fruit fractionated on a Sephadex LH-20 column with an ethanol eluate exhibit high antioxidant activity[17].

Traditional extraction methods, such as solvent extraction, have been used to isolate the naturally occurring bioactive hydrophilic compounds from plant materials; however, there are few adjustable parameters in these methods that can be used to optimize the selectivity of the extraction processes. Further, organic solvents have disadvantageous properties including flammability, toxicity, higher reactivity, and pollution of the environment. Therefore, it is desirable for researchers to develop alternate extraction techniques with better selectivity and efficiency than solvent extraction methods[18]. Approximately 200 compounds have already been identified from Noni extracted by traditional methods, such as anthraquinones, flavonoids, glycosides, iridoids, lignans and triterpenoids[19]. Limited knowledge is available regarding the hydrophobic antioxidants present in the Noni plant. Supercritical fluid carbon dioxide (SF-CO_2) is an excellent non-polar solvent that has been widely used in the processing of fats and oils for extraction purposes[20]. SF-CO_2 extraction technology has been chosen for a number of beneficial reasons such as its non-flammability, non-toxicity, lower reactivity, affordability, and allows for minimal environmental impact[21]. One of the major advantages offered by SF-CO_2 extraction is the ability to market the extracts of natural products without organic solvent residue. Thus, from a toxicological point of view, and taking into consideration of the polarity of antioxidants in extracts, SF-CO_2 is safer and better suited for the food industry than solvents such as methanol, acetone, or hexane.

The purpose of this study was to assess the antioxidant capabilities of crude extracts from the Noni plant's leaf, green stem, brown stem, and fruit by near SF-CO_2 at various pressure and temperature conditions via the Trolox equivalent antioxidant capacity (TEAC) assay, bleaching of DPPH free radicals, reducing power, the ability to scavenge reactive oxygen species (superoxide ions, hydroxyl radicals and hydrogen peroxides), and ferrous ion chelating activity. The methods and procedures used in this study will be useful for the development of a reproducible extraction and test for Noni products for clinical applicability.

MATERIALS AND METHODS

I. *Chemical Reagents*

The chemicals and reagents used in this study were purchased from Sigma Co. Ltd. (St. Louis, MO, USA), Mallinckrodt Baker Inc. (Paris, KY, USA), Wako Co. Ltd. (Osaka, Japan), and Nacalai Tesque Inc. (Kyoto, Japan).

II. *Plant Materials*

Fresh Noni leaves, green stems and brown stems, and fruits were obtained from the Jen-An Farm harvesting garden, Tainan District, Taiwan. Samples were washed with running tap water, separated before being chopped into pieces and oven-dried at 37°C for 5 days and subsequently ground into powder that was passed through a sieve with mesh size of 20. All samples were tested for water content, which was < 2% in all cases. The powders were separately sealed in plastic bottles and stored at -80°C until analysis to avoid moisture or deterioration.

III. *Near Supercritical Fluid Carbon Dioxide Extraction*

The SF-CO_2 extraction instrumentation was purchased from the High Pressure Equipment Company (Erie, PA, USA) and assembled by Ivorist International Co., Ltd. (Taipei, Taiwan). The extraction chamber is a 50 mL stainless steel vessel. Near supercritical fluid extractions were conducted at pressures of 1,500 or 3,500 psi and temperatures of 35°C or 50°C for 3 hr of static, followed by 1 hr of dynamic extraction based on the protocol of Pan *et al.*[22] The 50 mL extraction vessel was loaded with 10 g dry ground Noni powder and the extracted analytes were collected in 5 mL absolute ethanol in a 20 mL volumetric flask. To improve the collection efficiency, the 20 mL volumetric flask was placed in an ice bath during the dynamic extraction stage. The extracted samples (resuspended in absolute ethanol) were subsequently concentrated, dried at room temperature and stored at -80°C. The yields of extracts were calculated by the constant weight methods, i.e., weight of sample after extraction/the weight of the original material (10 g). Prior to analysis of antioxidant activities, samples were resuspended with absolute ethanol and centrifuged at 8,000 g for 10 min at 4°C. The supernatants were collected and serially diluted for antioxidant activity assay under final concentration of ethanol was less than 1%.

IV. *Assays for Antioxidant Activity and Total Phenolics*

The TEAC was determined based on the method of Re *et al.*[23] and results were expressed as the μmol of Trolox with the equivalent antioxidant activity as 1 g of extract. The free radical scavenging activity of Noni extracts was assayed by the method of Tadolini *et al.*[24] based on scavenging of the stable radical DPPH. The EC_{50} was calculated as the effective concentration (mg/L) of crude extract producing 50% of the DPPH radical scavenging effect. The reducing power and hydrogen peroxide scavenging activity of the extracts were determined according to the method of Yen *et al.*[25], hydroxyl

更多期刊、圖書與影音講座，請至【元照網路書店】www.angle.com.tw

radical scavenging activity assay was performed as described by Chang *et al.*[26], and the superoxide anion scavenging activity was determined using a RANDOX RANSOD kit according to the manufacturer's instructions (RANDOX Laboratories, Antrim, UK). Metal (ferrous) ion chelating activity was determined by the method of Aruoma *et al.*[27] and the EC_{50} was calculated as the effective concentration of crude extract (g/L) producing 50% of the ferrous ion chelating effect. Finally, total phenolic contents of the extracts were determined according to the method of Germano *et al.*[28]. Gallic acid was used as the standard for the calibration curve, and total phenolics were expressed as milligrams of gallic acid equivalents (GAE) per gram of extract.

V. *Statistical Analysis*

A three-way (4×2×2) analysis of variance (ANOVA) that consisted of plant material, pressure, and temperature was used to test the interactions between any two factors and among these three factors. Subsequently, the two temperatures and two pressures were combined into four extraction methods, including 3500 psi at 35°C, 3500 psi at 50°C, 1500 psi at 35°C, and 1500 psi at 50°C. Assay results were determined in triplicate and the continuous variables were expressed as the mean ± standard deviation (SD). Mixed models were established to test the differences among the four extraction methods for various parts of the Noni plant. The partial correlation (γ_s), which adjusted for different plant parts and different extraction methods, was calculated to describe the correlation between the different assays. All statistics were calculated using SAS version 9.1.3 software (SAS Inc., Cary, NC, USA). Because multiple comparisons between each of the methods were implemented, the significant level was defined as 0.01 ($p < 0.01$).

RESULTS AND DISCUSSION

Considering the widespread use of *M. citrifolia* (Noni), presently and historically, and the lack of knowledge regarding mechanisms of action in hydrophobic compounds of this plant, this study was aimed to evaluate the antioxidative activity of various parts of the Noni plant extracted under various conditions by near SF-CO_2.

I. *Extraction Yields and Content of Total Phenolics*

Table 1 lists the percent yields extracted under various conditions from the four parts (leaf, green stem, brown stem, and fruit) of the Noni plant. The high pressure (3500 psi) extraction conditions resulted in higher yields than lower pressure (1500 psi). Further, the fruit extraction yields were markedly higher than those from any other plant part. Interestingly, the brown stems had a higher yield than green stems. Overall, the yields were greater when extracted at 35°C than at 50°C, except for the leaf and brown stem which had better yields at the higher temperature under high pressure.

As described in Table 2, extraction at high pressure and high temperature resulted in the extraction of the highest levels of phenolic compounds compared to any other extraction conditions for both the leaves and green stems. For the brown stems, highest phenolic levels were extracted at 3500 psi and 35°C whereas less total phenolics were extracted at 1500 psi and 50°C. Overall, the highest levels of total phenolics were extracted from the fruit of the Noni plant at 1500 psi and 50°C and the lowest from the fruit at 1500 psi and 35°C (Table 2).

While the fruit resulted in the greatest percent yield by near SF-CO_2 extraction method (by all four extraction conditions, but most impressively at higher pressure), the highest amount of total phenolics was identified in the brown stem extract obtained at most extraction conditions (particularly 3500 psi and 35℃;23.14 mg/g), and the lowest amount of total phenolics in the fruit extracted at low pressure and low temperature. Therefore, a high yield by near SF-CO_2 extraction does not necessarily result in a high yield of total phenolics content. In addition, Noni brown and green stem extracts under high pressure (3500 psi) had a more total phenolics content than extracts obtained at low pressures (1500 psi). The results also indicate that higher total phenolics content is dependent on the optimal near SF-CO_2 extraction condition.

Table 1. Extraction yields of the four parts of the Noni plant using various near supercritical CO_2 extraction conditions

	Extraction Conditions			
Noni plant part	3500 psi; 35°C	3500 psi; 50°C	1500 psi; 35°C	1500 psi; 50°C
Leaf	0.62*	0.73	0.42	0.30
Green stem	0.48	0.40	0.35	0.22
Brown stem	0.60	0.75	0.40	0.33
Fruit	1.97	1.77	0.50	0.47

*: extraction yields expressed as % dry weight.

更多期刊、圖書與影音講座，請至【元照網路書店】www.angle.com.tw

Table 2. Total phenolics content and antioxidant activity of extracts from various parts of the Noni plant using four different extraction conditions

	Extraction Conditions				Comparison
	3500 psi; 35°C (Method A)	3500 psi; 50°C (Method B)	1500 psi; 35°C (Method C)	1500 psi; 50°C (Method D)	
Total phenolic content (mg/g)					
Leaf	3.74 ± 0.09	10.15 ± 0.87[a]	4.61 ± 0.08[b]	7.55 ± 0.17[a,b,c]	A = C < D < B
Green stem	9.09 ± 0.59	9.12 ± 0.14	6.57 ± 0.15[a,b]	6.84 ± 0.40[a,b]	C = D < A = B
Brown stem	23.14 ± 1.83	9.40 ± 0.66[a]	13.69 ± 0.32[a,b]	3.86 ± 0.06[a,b,c]	D < B < C < A
Fruit	2.59 ± 0.08	6.14 ± 0.41[a]	0.49 ± 0.004[a,b]	14.82 ± 0.83[a,b,c]	C < A < B < D
Antioxidative activities (EC_{50})					
DPPH radical scavenging activity (mg/L)					
Leaf	120.56 ± 5.09	36.25 ± 1.44[a]	145.53 ± 5.13[a,b]	112.84 ± 3.59[b,c]	C < A = D < B
Green stem	86.58 ± 7.76	111.39 ± 1.88[a]	127.13 ± 7.33[a]	171.72 ± 8.85[a,b,c]	D < B = C < A
Brown stem	6.87 ± 0.14	135.49 ± 4.56[a]	73.67 ± 5.22[a,b]	195.85 ± 10.62[a,b,c]	D < B < C < A
Fruit	157.07 ± 3.26	373.97 ± 6.59[a]	597.26 ± 24.43[a,b]	651.81 ± 24.03[a,b,c]	D < C < B < A
Hydroxyl radical scavenging activity (mg/L)					
Leaf	UD	UD	UD	UD	-
Green stem	UD	UD	UD	UD	-
Brown stem	27.29 ± 2.84	40.71 ± 0.38	UD	UD	-
Fruit	UD	UD	UD	UD	-
Hydrogen peroxide scavenging activity (g/L)					
Leaf	UD	UD	0.76 ± 0.07	1.20 ± 0.01	-
Green stem	UD	UD	UD	UD	-
Brown stem	UD	UD	UD	UD	-
Fruit	UD	2.73 ± 0.65	1.03 ± 0.05	UD	-
Ferrous ions chelating activity (g/L)					
Leaf	UD	0.59 ± 0.04	UD	0.25 ± 0.01	-
Green stem	UD	UD	UD	UD	-
Brown stem	UD	2.37 ± 0.01	UD	UD	-
Fruit	UD	UD	UD	UD	-

All data are expressed as mean ± standard deviation (SD). UD: undefined, indicate very low/no scavenging activity because the EC_{50} value was too large to detect. EC_{50} = effective concentration to induce a 50% response. [a] Significantly different between the present method and method A; [b]Significantly different between the present method and method B; [c]Significantly different between the present method and method C.

II. *Effect of Extraction Conditions on Antioxidative Activity of Leaves, Stems, and Fruit from the Noni Plant*

Also summarized in Table 2 are the antioxidative activities in the various extracts under the four different extraction conditions as determined by the DPPH radical scavenging activity, hydroxyl radical scavenging activity, hydrogen peroxide scavenging activity, and metal ion (iron) chelating activity. Figures 1 through 3 inclusively illustrated the antioxidant activities of the leaves, stems, and fruit of Noni under the various extraction conditions as determined using TEAC, superoxide radical scavenging activity, and reducing power assays.

更多期刊、圖書與影音講座，請至【元照網路書店】www.angle.com.tw

(I) *Antioxidative Activity of Leaf Extracts*

Extracts obtained at high pressure and temperature (3500 psi and 50°C) resulted in the best (i.e. the lowest EC_{50} value) DPPH scavenging activity (EC_{50} = 36.25 ± 1.44 mg/L), TEAC (173.11 ± 11.61 μmol/g), reducing power (40.02 ± 3.57 mg/g), and superoxide radical scavenging activity (141.72 ± 21.60 SOD unit/g). Hydroxyl radical scavenging activity was not detected in the Noni leaf extracts, regardless of the extraction conditions.

(II) *Antioxidative Activity of Green Stem Extracts*

The best DPPH scavenging activity (EC_{50} = 86.58 ± 7.76 mg/L), TEAC (91.06 ± 4.82 μmol/g), reducing power (37.47 ± 0.09 mg/g), and superoxide radical scavenging activity (509.05 ± 50.50 SOD unit/g) were observed when extracted at 3500 psi and 35°C. For hydroxyl radical scavenging activity, hydrogen peroxide scavenging activity, and ferrous ion chelating activity, the EC_{50}values were too large to detect.

(III) *Antioxidative Activity of Brown Stem Extracts*

The brown stem extracted at 3500 psi and 35°C resulted in a DPPH scavenging activity of 6.87 ± 0.14 mg/L (EC_{50}), a TEAC of 564.52 ± 41.58 μmol/g, the highest reducing power (59.8 ± 6.28 mg/g), and the highest superoxide radical scavenging activity (505.12 ± 6.28 SOD unit/g). Notably, the brown stem extracts obtained at 3500 psi with either 35°C or 50°C were the only extracts to obtain measurable EC_{50} values for hydroxyl radical scavenging activity; however, Noni brown stem extracts, regardless of the conditions, did not possess any hydrogen peroxide scavenging activity.

(IV) *Antioxidative Activity of Fruit Extracts*

For the best DPPH scavenging activity (EC_{50} = 157.07 ± 3.26 mg/L), TEAC (214.09 ± 0.63 μmol/g), and superoxide radical scavenging activity (76.46 ± 2.79 SOD unit/g) were obtained when extract at 3500 psi and 35°C (all $p < 0.01$) The highest reducing power (20.23 ± 1.05

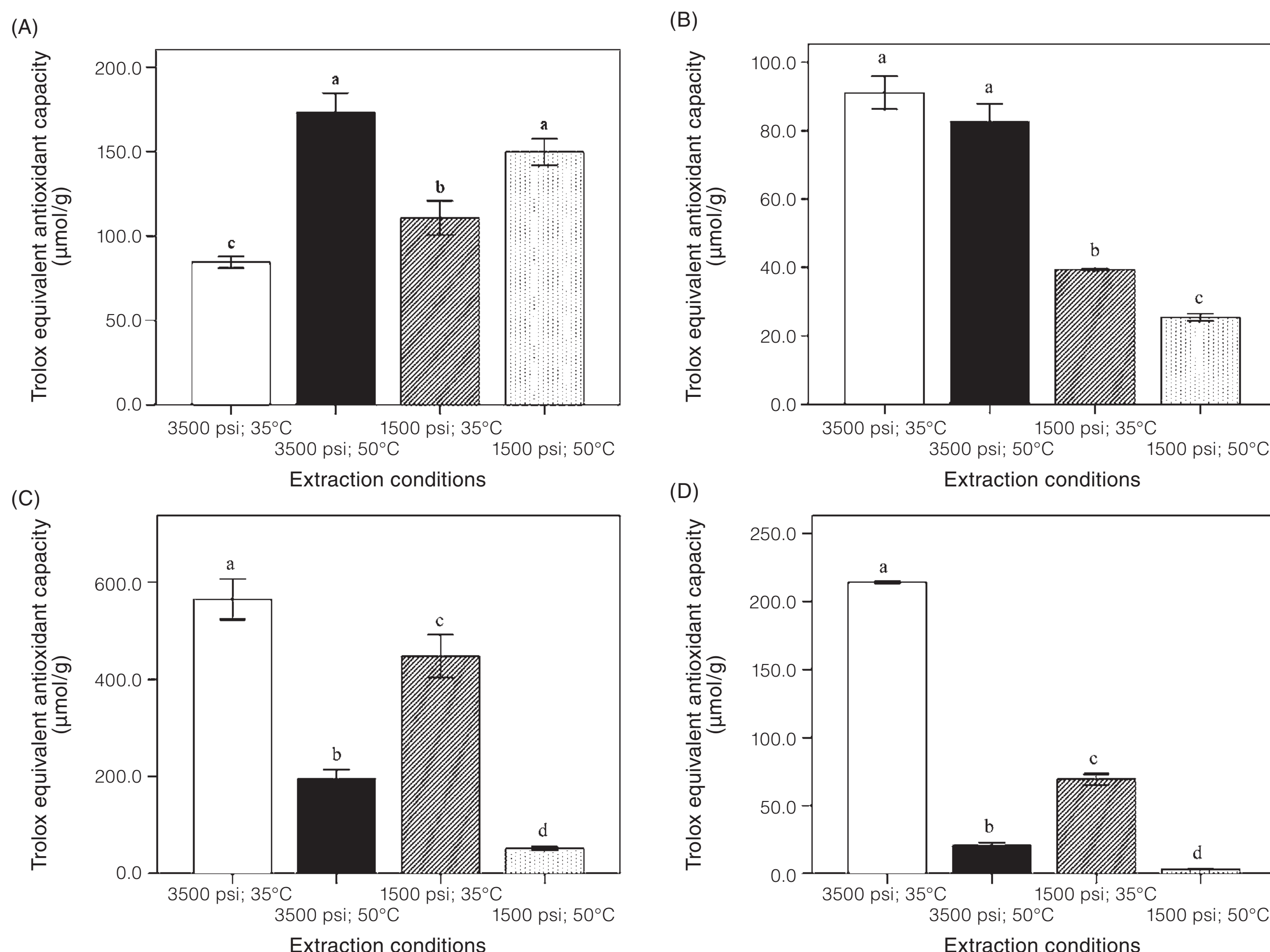

Figure 1. Trolox equivalent antioxidant capacity (TECA) of extracts from various parts of the Noni plant using various extraction conditions. (A) leaf , (B) green stem, (C) brown stem and (D) fruit. Data were presented as mean ± standard deviation. Different letters for extracts from the same part indicate a significant difference among various methods ($p < 0.01$). A mixed model was respectively established for each part.

更多期刊、圖書與影音講座，請至【元照網路書店】www.angle.com.tw

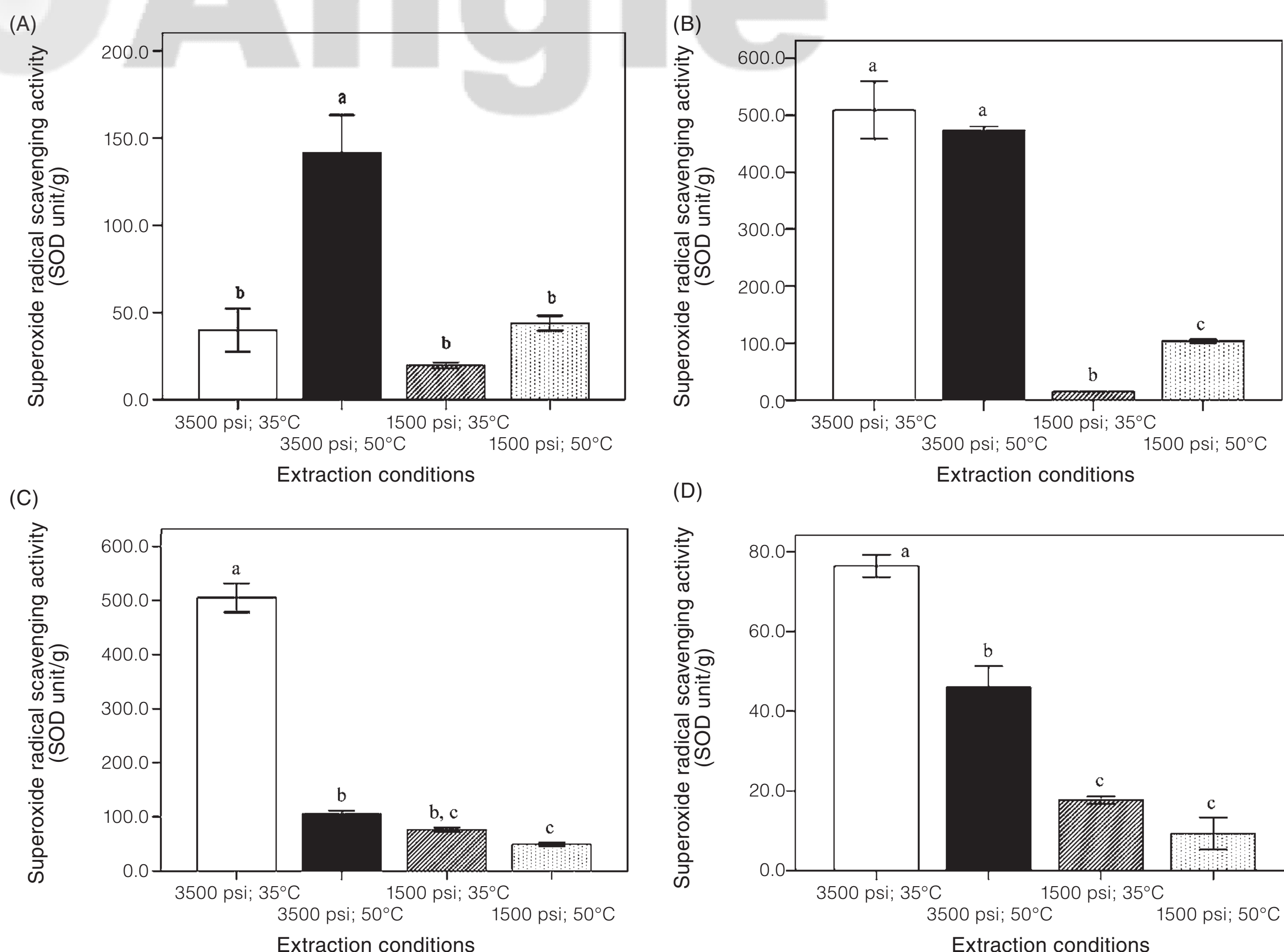

Figure 2. Superoxide radical scavenging activity of extracts from various parts of the Noni plant using various extraction conditions. (A) leaf, (B) green stem, (C) brown stem and (D) fruit. Data were presented as mean ± standard deviation. Different letters for extracts from the same part indicate a significant difference among various methods ($p < 0.01$). A mixed model was respectively established for each part.

mg/g) was detected in extracts obtained at 1500 psi and 50°C. Hydroxyl radical scavenging activity and ferrous ion chelating activity were not detected in fruit extracts, regardless of extraction conditions (Table 2).

III. *Correlation Analysis between Phenolic Content and Antioxidant Assays*

In this study, a correlation analysis between phenolic content and antioxidant assays was also performed. The partial correlation between total phenolics, DPPH radical scavenging ability, TEAC, reducing power, and superoxide radical scavenging activity were calculated. Significant partial correlations were identified between total phenolics, the EC_{50} of DPPH radical scavenging activity (γ_s = -0.71, $p < 0.01$), TEAC (γ_s = 0.30, p = 0.04), reducing power (γ_s = 0.55, $p < 0.01$), and superoxide radical scavenging activity (γ_s = 0.48, $p < 0.01$). In addition, the DPPH radical scavenging activity was positively correlated with the EC_{50} of TEAC (γ_s = -0.66, $p < 0.01$), reducing power (γ_s = -0.53, $p < 0.01$) and superoxide radical scavenging activity (γ_s = -0.55, $p < 0.01$). TEAC was correlated with superoxide radical scavenging activity (γ_s = 0.34, p = 0.02), but not with reducing power (γ_s = -0.01, p = 0.97). Reducing power increased as the superoxide radical scavenging activity increased (γ_s = 0.52, $p < 0.01$). It has been reported that DPPH radical scavenging ability is associated with reducing power[29], however, in the study of Siddhuraju *et al.*[30], DPPH radical scavenging ability did not parallel reducing power. In our analysis of the correlation among TEAC, DPPH radical scavenging ability, and reducing power, DPPH radical scavenging ability was associated with the reducing power when different near SF-CO_2 Noni extracts were compared (γ_s = -0.53, $p < 0.01$) but no correlation between TEAC and reducing power was observed (γ_s = -0.01, p = 0.97). Nevertheless, only the correlations between the DPPH radical scavenging activity and either total phenolic content or TEAC were strong. These results indicate that each of the above

更多期刊、圖書與影音講座，請至【元照網路書店】www.angle.com.tw

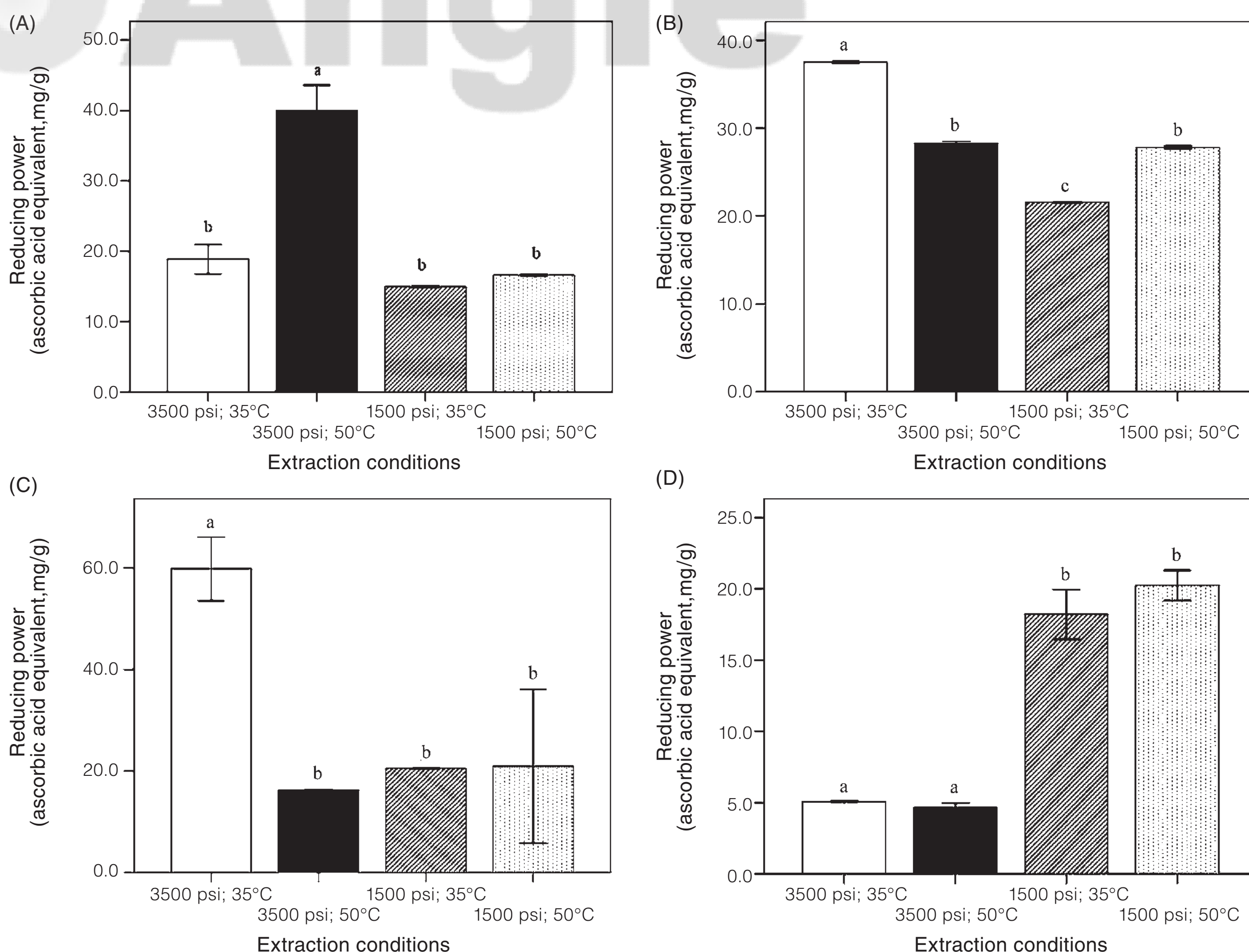

Figure 3. Reducing power of extracts from various parts of the Noni plant using various extraction conditions. (A) leaf, (B) green stem, (C) brown stem and (D) fruit. Data were presented as mean ± standard deviation. Different letters for extracts from the same part indicate a significant difference among various methods ($p < 0.01$). A mixed model was respectively established for each part.

assays has unique properties for detecting antioxidative activity and can not be substituted for each other. This finding is consistent with Collins[31] and suggests that the antioxidant properties of natural compounds should be examined by more than a single assay *in vitro* if their full therapeutic potential needs to be reflected *in vivo*.

It is well known that polyphenolic compounds contribute directly to antioxidative activity[32,33]. However, our experiments indicate that the antioxidative activity in the near SF-CO_2 Noni extracts might not only be attributed to the presence of phenolic compounds in the extract, but may also to other non-polar components isolated by the near SF-CO_2 extraction protocol.

Comparisons between different conditions of near SF-CO_2 extraction demonstrated that extractions performed under high pressure resulted in higher total phenolic contents, TEAC, DPPH radical scavenging ability, reducing power, and superoxide radical scavenging activity. The same results were seen in low temperature near SF-CO_2 extractions, apart from results for total phenolic contents and reducing power ($p > 0.01$). When compared to other plant parts, the brown stems of the Noni plant mostly had the higher total phenolic content, TEAC, DPPH radical scavenging ability, and reducing power ($p < 0.01$). Brown stems also showed a high superoxide radical scavenging activity. In general, hydroxyl radical scavenging ability is hard to detect for near SF-CO_2 extracted samples as compared to samples processed by common absolute ethanol extraction method[34]. In this study, we investigated different near SF-CO_2 extraction methods and found that the hydroxyl radical scavenging abilities of the brown stems at 3500 psi and either 35°C or 50°C were retained at reasonably high strength (3.3 and 2.5 times stronger than Trolox (EC_{50} = 100.86 mg/L)), respectively. Moreover, the hydroxyl radical scavenging activities of the brown stem did not interfere with ferrous ions chelating abilities (γ_s = 0.50, p = 0.67), especially in the extracts obtained at 3500 psi and 35°C due to the lack of ferrous ion chelating activity.

更多期刊、圖書與影音講座，請至【元照網路書店】www.angle.com.tw

Brown stems extracted at 3500 psi and 35°C also exhibited a higher DPPH scavenging activity, TEAC, reducing power, superoxide radical scavenging activity (more than 500 SOD unit/g), than the extracts from the different parts of the plant or by different extraction methods. These results indicate that the brown stem extract under high pressure and low temperature conditions provides a good source and method for isolating extracts possessing antioxidant activity.

Noni fruit juice has been reported to have great potential to scavenge reactive oxygen free radicals and protect tissues from consequent lipid peroxidation[5,6]. Noni fruit juice may also protect the liver from extrinsic carcinogenic CCl_4 exposure (a liver carcinogen and lipid peroxidation inducer)[8]. Interestingly, Noni fruit extracts, regardless of the near SF-CO_2 extraction method used, consistently demonstrated very poor antioxidant activity when comparing to the stem and leaf extracts in our study. This indicates that the extraction methods significantly affect the isolated antioxidant species even though the materials come from the same source.

It is encouraging to note that most natural antioxidant compounds often act synergistically with each other to produce a broad range of antioxidative activities and an effective defense system against free radical attack[16]. It has also been reported that phenolic compounds can directly scavenge hydroxyl radicals and are good inhibitors of lipid peroxidation *in vitro*[32]. The near SF-CO_2 brown stem extract obtained under high pressure and low temperature condition possessed a higher TEAC as well as a higher scavenging activity for DPPH, superoxide, and hydroxyl radicals. This extract also exhibits a greater reducing power and possesses relatively high amounts of phenolic compounds. These characteristics of the Noni brown stem extract are probably due to the presence of different phenolics in the samples, with different antioxidative activities involved in various mechanisms of oxidation inhibition. The study described herein suggests that the antioxidative activity exhibited in near SF-CO_2 Noni extracts was not only attributed to the phenolic compounds but also due to other non-polar components isolated by the near SF-CO_2 extraction. Further research on the purification and identification of these active compounds would be worthwhile.

CONCLUSIONS

Significant differences in the antioxidant activity of the fruit, stems, and leaves of the Noni plant extracted under various extraction conditions were observed as assessed by seven different assays of antioxidative activity ($p < 0.01$). This data suggests that several compounds possessing differential polarity may contribute to the antioxidative activity of the Noni leaf, green stem, brown stem, and fruit extracts. Extracts from the brown stem obtained under high pressure and low temperature conditions (3500 psi and 35°C) might provide a significant source of exogenous antioxidants, crucial in combating the oxidative stress. The major mechanisms underlying the antioxidant effects of the Noni brown stem extract may be due to a strong hydrogen-donating ability and good superoxide and hydroxyl radical scavenging abilities. Further research is thus warranted.

ACKNOWLEDGMENTS

Authors are grateful to Ms. Ching-Hsin Chiang for her assistance in sample preparation and lab analyses. All of the contributing authors claim no conflict of interest in regard to the publication of these results. This study was supported by the National Science Council, Taiwan, Republic of China, under Grant NSC91-2626-B-041-003.

REFERENCES

1. Abbott, I. A. and Shimazu, C. 1985. The geographic origin of the plants most commonly used for medicine by Hawaiians. J. Ethnopharmacol. 14: 213-222.
2. Vimala, S. and Adenan, M. I. 1999. Malaysian tropical forest medicinal plants: a source of natural antioxidants. J. Trop. Forest. Prod. 5: 32-38.
3. Hirazumi, A., Furusawa, E., Chou, S. C. and Hokama, Y. 1994. Anticancer activity of *Morinda citrifolia* (noni) on intraperitoneally implanted Lewis lung carcinoma in syngeneic mice. Proc. West. Pharmacol. Soc. 37: 145-146.
4. Hirazumi, A., Furusawa, E., Chou, S. C. and Hokama, Y. 1996. Immunomodulation contributes to the anticancer activity of *Morinda citrifolia* (noni) fruit juice. Proc. West . Pharmacol. Soc. 39: 7-9.
5. Wang, M. Y. and Su, C. 2001. Cancer preventive effect of *Morinda citrifolia* (Noni). Ann. N. Y. Acad. Sci. 952: 161-168.
6. Yanine, C. B., Vaillantb, F., Perezb, A. M., Reynesc, M., Brillouetc, J. M. and Bratc, P. 2006. The noni fruit (*Morinda citrifolia* L.): a review of agricultural research. J. Food Compost. Anal. 19: 645-654.
7. Hirazumi, A. and Furusawa, E. 1999. An immunomodulatory polysaccharide-rich substance from the fruit juice of *Morinda citrifolia* (noni) with antitumour activity. Phytother. Res. 13: 380-387.
8. Wang, M. Y., West, B. K., Jensen, C. J., Nowicki, D., Chen, S. U., Palu, A. K. and Anderson, G. 2002. *Morinda citrifolia* (Noni): a literature review and recent advances in Noni research. Acta Pharmacol. Sin. 23: 1127-1141.
9. Sang, S., Liu, G., He, K., Zhu, N., Dong, Z., Zheng, Q., Rosen, R. T. and Ho, C. T. 2003. New unusual iridoids from the leaves of noni (*Morinda citrifolia* L.) show inhibitory effect on ultraviolet B-induced transcriptional activator protein-1 (AP-1) activity. Bioorg. Med.

更多期刊、圖書與影音講座，請至【元照網路書店】www.angle.com.tw

Chem. 11: 2499-2502.
10. Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M. and Telser, J. 2007. Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol. 39: 44-84.
11. Halliwell, B. and Gutteridge, J. M. C. 1999. Free Radicals in Biology and Medicine. 3rd ed. pp. 105-245. Oxford University Press. London, U. K.
12. Halliwell, B. 1996. Oxidative stress, nutrition and health. Experimental strategies for optimization of nutritional antioxidant intake in humans. Free Radic. Res. 25: 57-74.
13. McClements, J. and Decker, E. A. 2000. Lipid oxidation in oil-water emulsions: impact of molecular environment on chemical reactions in heterogeneous food system. J. Food Sci. 65: 1270-1282.
14. Kaplan, M., Mutlu, E. A., Benson, M., Fields, J. Z., Banan, A. and Keshavarzian, A. 2007. Use of herbal preparations in the treatment of oxidant-mediated inflammatory disorders. Complement. Ther. Med. 15: 207-216.
15. Sang, S., Cheng, X., Zhu, N., Stark, R. E., Badmaev, V., Ghai, G., Rosen, R. T. and Ho, C. T. 2001. Flavonol glycosides and novel iridoid glycoside from the leaves of *Morinda citrifolia.* J. Agric. Food Chem. 49: 4478-4481.
16. Zin, Z. M., Hamid, A. A. and Osman, A. 2002. Antioxidative activity of extracts from Mengkudu (*Morinda citrifolia* L.) root, fruit and leaf. Food Chem. 78: 227-231.
17. Zin, Z. M., Hamid, A. A., Osman, A. and Saari, N. 2006. Antioxidative activities of chromatographic fractions obtained from root, fruit and leaf of Mengkudu (*Morinda citrifolia* L.). Food Chem. 94: 169-178.
18. Liu, B., Li, W., Chang, Y., Dong, W. and Ni, L. 2006. Extraction of berberine from rhizome of Coptis chinensis Franch using supercritical fluid extraction. J. Pharm. Biomed. Anal. 41: 1056-1060.
19. Pawlus, A. D. and Kinghorn, A. D. 2007. Review of the ethnobotany, chemistry, biological activity and safety of the botanical dietary supplement *Morinda citrifolia* (noni). J. Pharm. Pharmacol. 59: 1587-1609.
20. Fattori, M., Bulley, N. R. and Meisen, A. 1988. Carbon dioxide extraction of canola seed: oil solubility and effect of seed treatment. J. Am. Oil Chem. Soc. 65: 968-974.
21. Güclü-Űstündağ, O. and Temelli, F. 2005. Solubility behavior of ternary systems of lipids, cosolvents and supercritical carbon dioxide and processing aspects. J. Supercrit. Fluids 36: 1-15.
22. Pan, W. J., Liou, G. K., Joe, L. I., Shia, B. F. and Li, M. S. 1994. Comparison of essential oil extracted by supercritical fluid carbon dioxide and steam distillation from cinnamonium. J. Chin. Med. 5: 199-207.
23. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. A. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26: 1231-1237.
24. Tadolini, B., Juliano, C., Piu, L., Franconi, F. and Cabrini, L. 2000. Resveratrol inhibition of lipid peroxidation. Free Radic. Res. 33: 105-114.
25. Yen, G. C., Duh, P. D. and Tsai, H. L. 2002. Antioxidant and pro-oxidant properties of ascorbic acid and gallic acid. Food Chem. 79: 307-313.
26. Chang, L. W., Yen, W. J., Huang, S. C. and Duh, P. D. 2002. Antioxidant activity of sesame coat. Food Chem. 78: 347-354.
27. Aruoma, O. I. and Haillwell, B. 1988. The iron-binding and hydroxyl radical scavenging action of anti-inflammatory drugs. Xenobiotica 18: 459-470.
28. Germano, M. P., De Pasquale, R., D'Angelo, V., Catania, S., Silvari, V. and Costa, C. 2002. Evaluation of extracts and isolated fraction from *Capparis spinosa* L. buds as an antioxidant source. J. Agric. Food Chem. 50: 1168-1171.
29. Yen, G. C. and Duh, P. D. 1993. Antioxidant properties of methanolic extracts from peanut hull. J. Am. Oil Chem. Soc. 70: 383-386.
30. Siddhuraju, P., Mohan, P. S. and Becker, K. 2002. Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): a preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp. Food Chem. 79: 61-67.
31. Collins, A. R. 2005. Assays for oxidative stress and antioxidant status: applications to research into the biological effectiveness of polyphenols. Am. J. Clin. Nutr. 81: 261S-267S.
32. Rice-Evans, C. A., Miller, N. J. and Paganga, G. 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic. Biol. Med. 20: 933-956.
33. Yang, J. H., Lin, H. C. and Mau, J. L. 2002. Antioxidant properties of several commercial mushrooms. Food Chem. 77: 229-235.
34. Chung, Y. L., Wang, J. S. and Chen, S. Y. 2004. Comparison of antioxidative activity of crude extracts from leaves, stems and fruits of *Morinda citrifolia* (Noni). pp. 76-77. Master Thesis, Chia-Nan University of Pharmacy and Science. Tainan, Taiwan.