Check for updates

Original Article

Journal of Evidence-Based Complementary & Alternative Medicine
2017, Vol. 22(4) 573-579
© The Author(s) 2017
Reprints and permission:
sagepub.com/journalsPermissions.nav
DOI: 10.1177/2156587216686689
journals.sagepub.com/home/cam
SAGE

Health Promotion Potential of Vegetables Cultivated in Northern Thailand: A Preliminary Screening of Tannin and Flavonoid Contents, 5α-Reductase Inhibition, Astringent Activity, and Antioxidant Activities

Naphatsorn Kumar, PhD[1,2], and Chaiyavat Chaiyasut, PhD[3]

Abstract

Many of scientific evidences suggest that regular consumption of fruits and vegetables can prevent chronic diseases. In Northern Thailand, there are many vegetables that are usually consumed by local people. In this study, 17 local vegetables were selected and extracted with 95% ethanol. The extracts were screened for 5α-reductase inhibitory activity, total tannin and flavonoid contents, astringent and antioxidant activities. The results showed that *Spondias pinnata* and *Ocimum americanum* had the highest tannin and flavonoid content, respectively. *Neptunia oleracea* was the most potent 5α-reductase inhibitor. For antioxidant activities, *S pinnata* was the most potent ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenger and the most potent ferric reducer. *Polygonatum odoratum* was the most potent hydrogen peroxide scavenger and *Leucaena leucocephala* was the most potent DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenger among the tested samples. This suggests that local vegetables in Northern Thailand have a health promotion potential, which can be further developed as nutritional supplements, preventive medicines, and topical products for cosmetic purposes.

Keywords

antioxidant, health promotion, tannin, vegetable, 5α-reductase

Received August 12, 2016. Received revised November 14, 2016. Accepted for publication December 4, 2016.

There are many pieces of evidences to suggest that consumption of vegetables has a positive impact on health promotion and chronic diseases prevention. It was reported that high content of vegetables and fruits in daily meal could reduce the incidence of chronic diseases such as various types of cancer, cardiovascular diseases, type 2 diabetes, Alzheimer's disease, and age-related functional decline.[1-3] In addition, it was reported that people with multimorbidity (the presence of 2 or more chronic diseases/conditions in an individual)[4] who consumed more of fruits, vegetables, and grain products exhibited healthier stages.[5] Phytochemical antioxidants may play a significant role in the protective and health promoting effects of vegetables.[6] Furthermore, it was suggested that additive and synergistic effects of various phytochemicals in plants accounted for better health effect than single phytochemical or dietary supplement.[2] Therefore, the effect of antioxidants on disease prevention and good health status maintenance has been extensively investigated.

Oxygen can be either friend or foe because the vital oxygen can damage important key organs of the living systems, both plants and animals, including humans. Reactive oxygen species (ROS) can be spontaneously formed in the body during normal physiological processes. Although the human body has many protective mechanisms to neutralize those ROS, an imbalance of ROS and protective mechanisms may occur and lead to many diseases. External sources of antioxidants are needed to maintain a healthy status.[7]

[1] School of Cosmetic Science, Mae Fah Luang University, Chiang Rai, Thailand
[2] Phytocosmetics and Cosmeceuticals Research Group, Mae Fah Luang University, Chiang Rai, Thailand
[3] Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand

Corresponding Author:
Chaiyavat Chaiyasut, PhD, Faculty of Pharmacy, Chiang Mai University, Suthep Road, Suthep District, Chiang Mai, 50100, Thailand.
Email: chaiyavat@gmail.com

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 3.0 License (http://www.creativecommons.org/licenses/by-nc/3.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (https://us.sagepub.com/en-us/nam/open-access-at-sage).

Astringent is the term that is used to describe acerbity taste with dry and puckering feeling in the mouth. Astringency is usually related to the tannins, which are a type of plant polyphenols. The astringency of tannin caused by the binding of a tannin and protein in the taste buds.[8] Tannins exhibit numerous benefits on the diseases prevention and treatments, including wound-healing properties. Tannins are also useful in the treatment and prevention of allergies, cancers, and some cardiovascular disorders. Moreover, most of the tannin-containing plants are considered as a health promoting food, such as tea, wine, pine bark, emblica, and so on.[9,10]

5α-reductase or Δ^4-3-oxo-steroid 5α-oxidoreductase (5αR, EC 1.3.99.5) is a microsomal enzyme that plays a major role in the reduction of various Δ^4-3-oxo-steroids, such as testosterone, progesterone, and corticosterone. In humans, 2 isoforms of 5αR, type 1 5αR and type 2 5αR are present. Type 1 isoform is usually expressed in the brain, liver, and skin while type 2 isoform is typically expressed only in androgen-dependent tissues such as the prostate and genital tissue.[11] The important function of 5αR in humans is to catalyze the metabolism of testosterone to a more potent androgen, dihydrotestosterone (DHT). DHT is necessary for normal male growth. However, excessive production of DHT can cause androgen-dependent disorders, for example, benign prostate hyperplasia, prostate cancer, androgenic alopecia, acne, and hirsutism.[12] Although many medicines can be used to treat these androgen-dependent disorders, most of them have negative and unacceptable side effects. Finasteride, a synthetic 5αR inhibitor, can cause erectile dysfunction and abnormal sexual function in some individual. In previous studies, Kumar et al[13,14] demonstrated that many of vegetables and fruits found in Thailand, including orange, mangosteen, basil, black peppers, phakkaawtong, galangal, ginger, gourd, lemongrass, emblica, and many plants were high potency enzyme inhibitors. In Thailand, vegetables are cheap and can be accessed by people regardless of social status and income. Therefore, it was the aim of this study to focus on the preliminary screening of antioxidant, astringent, and 5α-reductase inhibitory activities of 17 vegetables cultivated in Northern Thailand, in order to utilize local vegetables as a functional food or further development as a dietary supplement, nutraceutical, alternative medicine, and topical cosmeceutical products.

Materials and Methods

Plant Materials

Seventeen popular local vegetables, as listed in Table 1, were purchased from local markets in Chiang Mai, Thailand. They were then identified by the botanist at the Faculty of Pharmacy Herbarium, Chiang Mai University. After that, the plant samples were dried at 45°C. They were ground by using an electric grinder. Then the prepared plants were further extracted by maceration with 95% ethyl alcohol. The extracts were concentrated under controlled pressure and temperature using a rotary evaporator (Eyela, Tokyo, Japan).

Table 1. Vegetable Common Name, Scientific Name, and the Part Used in This Experiment.

Common Name	Scientific Name	Part(s) Used
Ivy gourd	*Coccinia grandis* (L) Voigt	Whole plant
Chinese kale	*Brassica albograbra* LH Bailey	Leaf and stem
Water mimosa	*Neptunia oleracea* Lour	Whole plant
Water morning glory	*Ipomoea aquatica* Forssk	Whole plant
White popinac	*Leucaena leucocephala* (Lam) de Wit	Leaf
Sweet potatoes	*Ipomoea batatas* (L) Lam	Leaf
Hog plum	*Spondias pinnata* (Lf) Kurz	Fruit
Chinese radish	*Raphanus sativus* L	Leaf and stem
Phak khut	*Diplazium esculentum* (Retz) Sw	Leaf and stem
Bai ya nang	*Tiliacora triandra* (Colebr) Diels	Leaf
Phak phai	*Polygonum odoratum* Lour	Leaf and stem
Phak siao	*Bauhinia purpurea* L	Leaf
Wing bean	*Psophocarpus tetragonolobus* (L) DC	Pod
Cowpea	*Vigna unguiculata* (L) Walp subsp *unguiculata*	Pod
Cabbage	*Brassica oleracea* L var *capitata* L	Leaf
Chinese cabbage	*Brassica chinensis* L var *chinensis*	Leaf
Hoary basil	*Ocimum americanum* L	Leaf

Chemicals

Dithiothreitol, sucrose, tannic acid, and NADPH were purchased from Sigma Chemical (St Louis, MO) in the highest quality grade. Methanol and dichloromethane were purchased from Fisher Scientific (Fair Lawn, NJ) in high-performance liquid chromatography (HPLC) grade. Ninety-five percent ethyl alcohol was purchased from Liquor Distillery Organization Excise Department (Chachoengsao, Thailand). Other chemicals were purchased from Tokyo Chemical Industry (Tokyo, Japan) in the highest quality grade.

Animals

Three 6-week-old male Sprague-Dawley rats were obtained from the National Laboratory Animal Center, Bangkok, Thailand, and housed under a 12-hour light/dark cycle with free access to food and water. The protocol of this study was approved by the Animal Research Ethics Committee of the Faculty of Pharmacy, Ubon Ratchathani University, Ubon Ratchathani, Thailand.

Total Tannin Content

Total tannin content (TTC) was determined by using Folin-Ciocalteu phenol reagent with tannic acid as a standard, adapted from the method reported by Son et al.[16] Two hundred microliters of diluted plant extracts was added to 1.0 mL of 0.2 N Folin-Ciocalteu phenol reagent in a test tube and incubated for 4 minutes at room temperature. Then, 800 μL of 7.5% sodium carbonate solution was added. Reactions were incubated in a dark chamber at room temperature for 2 hours. The absorbance was measured at a wavelength of 725 nm. TTC of each sample was expressed as milligrams tannic acid equivalent per gram fresh plants (mg TAE/g FP).

Total Flavonoid Content

Total flavonoid content (TFC) was analyzed by aluminum chloride colorimetry followed by the modified method of Ardestani and

Yazdanparast.[17] One hundred and fifty microliters of 15% w/w sodium nitrite ($NaNO_2$) solution was added to 1 mL of diluted plant extracts and incubated for 6 minutes at room temperature. Then, 150 μL of 15% w/w aluminum chloride solution was added to the mixture and further incubated for 6 minutes at room temperature. Afterward, 700 μL of 8% w/w sodium hydroxide solution was added. The reactions were incubated in a dark chamber at room temperature for 15 minutes. The absorbance was measured at 510 nm. TFC of each sample was expressed as milligrams quercetin equivalent per gram fresh plants (mg QE/ g FP).

Evaluation of 5α-Reductase Inhibitory Activity

Rat microsomal suspension was prepared using the method described previously.[14] Excised Sprague-Dawley rat livers were minced with scissors and homogenized with 0.02 M phosphate buffer (pH 6.5) containing 0.32 M sucrose and 1 mM dithiothreitol. The liver homogenate was centrifuged twice at 4500 × *g* at 0°C for 30 minutes each time. All the supernatants were collected. The resulting supernatants containing microsomal particles were tested for soluble protein by using the Lowry method[15] and kept at −50°C until use as an enzyme source.

5α-Reductase inhibition assay was performed by using the previously reported method by our research group.[14] The mixture of reaction solution containing 200 μL of plant extracts in 95% ethanol, 1.3 mL of 0.02 M phosphate buffer (pH 6.5), 300 μL of 500 ppm testosterone in methanol, and 0.7 mL of rat microsomal suspension was prepared. Reactions were initiated by the addition of 500 μL of 0.77 mg/mL NADPH in phosphate buffer saline pH 6.5, followed by incubation in a water bath at 37°C for 30 minutes. The reactions were stopped by addition of 5.0 mL dichloromethane. Then, 0.5 mL of 100 ppm propyl *p*-hydroxybenzoate in methanol was spiked as an internal standard for HPLC. Four milliliters of the organic phase were decanted and evaporated to dryness under controlled pressure. The residues were redissolved in 5.0 mL of methanol. An aliquot of 10 μL was injected into the HPLC system (Agilent 1100 series, using a Shodex C18-4E column, 250 × 4.6 mm, 5 μM). The mobile phase was a mixture of methanol and deionized water (65:35) with a flow rate of 1.0 mL/min. An ultraviolet detector at 242 nm was utilized. All the results were expressed as milligrams finasteride equivalents per gram fresh plants (mg FE/ g FP).

Astringent Activity

The astringent activity of the extracts was analyzed according to the modified method reported by Son et al.[16] One milliliter of 1 mg/mL of swine hemoglobin in deionized water was mixed with 1000 μL of 1 mg/mL solution of various plant extract in deionized water by using vortex mixer. The mixture was further centrifuged at 1000 × *g* at 25°C for 10 minutes. A supernatant was collected and measured for ultraviolet/visible absorbance at 407 nm. Tannic acid (1 mg/mL) was used as a positive control and deionized water was used as a negative control. Astringent activity was calculated by the following equation:

$$\text{Astringent activity } (\%) = [(A_{ctrl} \times A_{sample})/A_{ctrl}] \times 100$$

Antioxidant Activities

ABTS Radical Scavenging Activity. ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging activity was analyzed by the modified method reported by Re et al.[18] ABTS radical was prepared by reacting 7 mM of ABTS with 2.45 mM of potassium persulfate at a ratio of 2:1. Reacted solution was kept in a dark chamber for at least 6 hours prior to the experiment. The prepared ABTS radical solution was further diluted to an appropriate concentration. For the determination of radical scavenging activity, 1.0 mL of diluted plant extract was mixed with 1.0 mL of diluted ABTS radical solution and then incubated in a dark chamber for 3 minutes. The absorbance was measured at 734 nm. Ascorbic acid was used as a standard. ABTS radical scavenging activity of each sample was expressed as milligrams ascorbic acid (vitamin C) equivalent per gram fresh plants (mg VCE/ g FP).

DPPH Radical Scavenging Activity. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was determined by the modified method of Ardestani and Yazdanparast.[17] One milliliter of diluted plant extracts in 95% ethanol was mixed with 1 mL of 0.2 mM DPPH in 95% ethanol. The reactions were incubated in a dark chamber at room temperature for 30 minutes. The absorbance was measured at 517 nm. Ascorbic acid was used as a standard. DPPH radical scavenging activity of each sample was expressed as milligrams ascorbic acid (vitamin C) equivalent per gram fresh plants (mg VCE/ g FP).

Hydrogen Peroxide Scavenging Activity. Hydrogen peroxide scavenging activity was determined by method described previously with some modifications.[19] One milliliter of diluted plant extract in deionized water was mixed with 1.0 mL of 0.15% w/v hydrogen peroxide solution. The absorbance was measured at 230 nm. Ascorbic acid was used as a standard and deionized water was used as a control. Hydrogen peroxide scavenging activity of each sample was expressed as milligrams ascorbic acid (vitamin C) equivalent per gram fresh plants (mg VCE/ g FP).

Ferric Reducing Antioxidant Power (FRAP) Assay. FRAP assay was analyzed by the method reported by Benzie et al.[20] FRAP reagent was first prepared by mixing 20 mM $FeCl_3$ in deionized water with 10 mM TPTZ (2,4,6-tri[2-pyridyl]-s-triazine) in 40 mM HCl solution and 0.1 M acetate buffer pH 3.6 in a ratio of 1:1:10 by volume. For FRAP assay, 1.0 mL of diluted plant extract was mixed with 1.0 mL of FRAP reagent. The reactions were incubated in a dark chamber at room temperature for 4 minutes. The absorbance was measured at 593 nm. Ascorbic acid was used as a standard. FRAP value of each sample was expressed as milligrams ascorbic acid equivalent per gram fresh plants (mg VCE/ g FP).

Results and Discussion

Seventeen vegetables that are usually consumed by the local people in Northern Thailand were selected for the study. After botanical identification step, the plants were extracted with 95% ethyl alcohol. Since ethyl alcohol is a middle polar solvent, one can obtain a wide range of phytochemicals from low polarity to high polarity. Furthermore, ethyl alcohol is considered as a safe and environment-friendly solvent so that the extract could be used as nutritional supplements, medicines, or even cosmetic ingredients. The selected plants and parts used are shown in Table 1.

The extracts were first screened for total tannin and flavonoid contents. In the evaluation of total tannin content, Folin-Ciocalteu reagent was used. The reaction mechanism

Table 2. Total Tannin and Total Flavonoid Contents, ABTS and DPPH Radicals Scavenging, Hydrogen Peroxide Scavenging Activities, and FRAP Assay of Selected Vegetables.*

Vegetable Extract	Total Tannin (mg TAE/g FP)	Total Flavonoid (mg QE/g FP)	ABTS Scavenging (mg VCE/g FP)	DPPH Scavenging (mg VCE/g FP)	H_2O_2 Scavenging (mg VCE/g FP)	FRAP Assay (mg VCE/g FP)
Hog plum	8.69 ± 0.68^{a}	10.64 ± 0.31^{c}	23658.13 ± 476.03^{a}	464.83 ± 9.29^{b}	10.60 ± 0.03^{p}	7686.56 ± 574.85^{a}
White popinac	7.49 ± 0.36^{b}	10.06 ± 0.26^{d}	19915.38 ± 714.57^{b}	565.46 ± 4.36^{a}	12.37 ± 0.04^{o}	5592.76 ± 343.44^{b}
Phak phai	6.32 ± 0.16^{c}	16.50 ± 0.36^{b}	5640.42 ± 96.20^{c}	41.18 ± 3.41^{d}	44.01 ± 0.06^{a}	231.03 ± 4.89^{e}
Hoary basil	4.30 ± 0.13^{d}	20.41 ± 0.26^{a}	2955.98 ± 71.98^{e}	44.58 ± 1.66^{d}	30.34 ± 0.14^{d}	1001.18 ± 56.19^{c}
Bai ya nang	2.94 ± 0.04^{e}	9.82 ± 0.32^{d}	2210.31 ± 37.49^{f}	27.57 ± 3.23^{e}	27.14 ± 0.15^{f}	101.91 ± 2.31^{e}
Sweet potatoes	1.23 ± 0.05^{f}	4.48 ± 0.06^{e}	3808.89 ± 53.74^{d}	46.70 ± 2.84^{d}	20.62 ± 0.15^{g}	990.22 ± 12.80^{c}
Phak siao	1.19 ± 0.19^{f}	3.80 ± 0.03^{f}	2978.28 ± 178.33^{e}	19.73 ± 5.01^{e}	32.85 ± 0.03^{c}	907.29 ± 18.73^{cd}
Ivy gourd	1.04 ± 0.12^{g}	3.02 ± 0.01^{g}	1855.69 ± 191.84^{f}	49.68 ± 1.77^{cd}	28.28 ± 0.04^{e}	757.79 ± 16.44^{cd}
Water mimosa	0.63 ± 0.03^{h}	0.97 ± 0.01^{i}	2583.29 ± 94.85^{ef}	44.96 ± 0.81^{d}	16.86 ± 0.01^{l}	464.97 ± 28.69^{de}
Chinese radish	0.53 ± 0.06^{i}	1.13 ± 0.04^{i}	658.68 ± 33.76^{g}	59.44 ± 3.42^{c}	19.50 ± 0.04^{i}	292.85 ± 7.23^{de}
Wing bean	0.53 ± 0.01^{i}	0.87 ± 0.02^{ij}	755.34 ± 14.72^{g}	58.41 ± 0.66^{c}	18.55 ± 0.01^{k}	394.14 ± 10.85^{de}
Chinese kale	0.52 ± 0.01^{i}	0.75 ± 0.01^{ij}	523.00 ± 39.50^{g}	43.55 ± 1.62^{d}	37.26 ± 0.04^{b}	374.46 ± 6.54^{de}
Phak khut	0.48 ± 0.03^{ij}	2.29 ± 0.05^{h}	503.24 ± 23.34^{g}	6.19 ± 1.03^{f}	19.04 ± 0.03^{j}	424.55 ± 15.49^{de}
Water morning glory	0.39 ± 0.01^{jk}	0.79 ± 0.00^{ij}	562.33 ± 41.32^{g}	42.64 ± 2.58^{d}	15.77 ± 0.16^{m}	216.21 ± 4.46^{e}
Cowpea	0.37 ± 0.01^{k}	0.45 ± 0.02^{jk}	609.95 ± 22.35^{g}	53.49 ± 0.89^{cd}	13.29 ± 0.11^{n}	159.47 ± 11.70^{e}
Cabbage	0.21 ± 0.00^{l}	0.16 ± 0.00^{k}	332.27 ± 9.74^{g}	57.96 ± 0.29^{c}	19.96 ± 0.04^{h}	119.19 ± 1.94^{e}
Chinese cabbage	0.13 ± 0.00^{m}	0.12 ± 0.01^{k}	257.41 ± 10.23^{g}	57.60 ± 0.31^{c}	16.61 ± 0.10^{l}	99.19 ± 3.25^{e}

Abbreviations: ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; FP, fresh plant; TAE, tannic acid equivalent; QE, quercetin equivalent; VCE, vitamin C equivalent.
*Data are expressed as mean ± standard deviation of triplicate experiment. Data in column with different letters are significantly different ($P < .05$).

is based on a formation of blue complex between the reagent and polyphenols. Tannic acid was used for standard calibration line construction. As shown in Table 2, the plant extract that contained the highest tannin content is hog plum or *Spondias pinnata* extract with tannin content of 8.69 ± 0.68 mg TAE/g FP, followed by white popinac or *Leucaena leucocephala* and phak phai or *Polygonum odoratum* with tannin contents of 7.49 ± 0.36 and 6.32 ± 0.16 mg TAE/ g FP, respectively. The fruit of hog plum has acidic and astringency taste, and usually fermented with salt before served as a pickled fruit.[21] This was related to the previous studies by Manik et al[22] and Khatoon et al[21] who found the tannin in the exocarp of the fruit and fruit flesh by phytochemical screening with $FeCl_3$ reagent, respectively. The vegetable that had the lowest tannin content was Chinese cabbage or *Brassica chinensis*, which had tannin content of 0.13 ± 0.00 mg TAE/ g FP. For flavonoid content, aluminum chloride colorimetry technique was used, and the results were shown in Table 2. From Table 2, the plant extract exhibited the highest flavonoid content was hoary basil or *Ocimum americanum*, which had total flavonoid content of 20.41 ± 0.26 mg QE/g FP, followed by phak phai and hog plum, which had total flavonoid content of 16.50 ± 0.36 and 10.64 ± 0.31 mg QE/g FP, respectively. The result was in agreement with Vieira et al[23] who investigated the flavonoid profiling of *O americanum* cultivated in different areas. Previous reports also suggested that phak phai and hog plum also contain high level of flavonoid.[21,24]

5α-Reductase inhibitory activity of the plant extracts was further analyzed by using the microsome from rat as an enzyme source. Although many methods can be used to evaluate this activity, many of them require radioactive materials or sophisticated instrumental techniques.[25-29] In this experiment, simple isocratic HPLC analysis[13,14] was used to evaluate the 5α-reductase inhibitory activity of the vegetable extracts. The results are shown in Table 3. From Table 3, it is seen that water mimosa or *Neptunia oleracea* extract had the highest 5α-reductase inhibitory activity with 4.38 ±

Table 3. 5α-Reductase Inhibitory Activity of Selected Vegetables.*

Vegetable Extract	5α-Reductase Inhibitory Activity (mg FE/ g FP)
Water mimosa	4.38 ± 0.20^{a}
Ivy gourd	1.87 ± 0.01^{b}
Phak phai	1.59 ± 0.01^{c}
Water morning glory	1.49 ± 0.01^{c}
Hog plum	1.28 ± 0.05^{d}
Sweet potatoes	1.21 ± 0.01^{de}
Chinese kale	1.13 ± 0.01^{def}
Wing bean	1.12 ± 0.00^{ef}
Phak siao	1.49 ± 0.01^{fg}
Cabbage	0.99 ± 0.01^{gh}
Chinese cabbage	0.91 ± 0.01^{gh}
Chinese radish	0.88 ± 0.01^{gh}
Cowpea	0.86 ± 0.00^{gh}
White popinac	0.84 ± 0.01^{gh}
Bai ya nang	0.79 ± 0.01^{h}
Hoary basil	0.41 ± 0.00^{i}
Phak khut	0.02 ± 0.01^{j}

Abbreviations: FE, finasteride equivalent; FP, fresh plant.
*Data are expressed as mean ± standard deviation of triplicate experiment. Data in column with different letters are significantly different ($P < .05$).

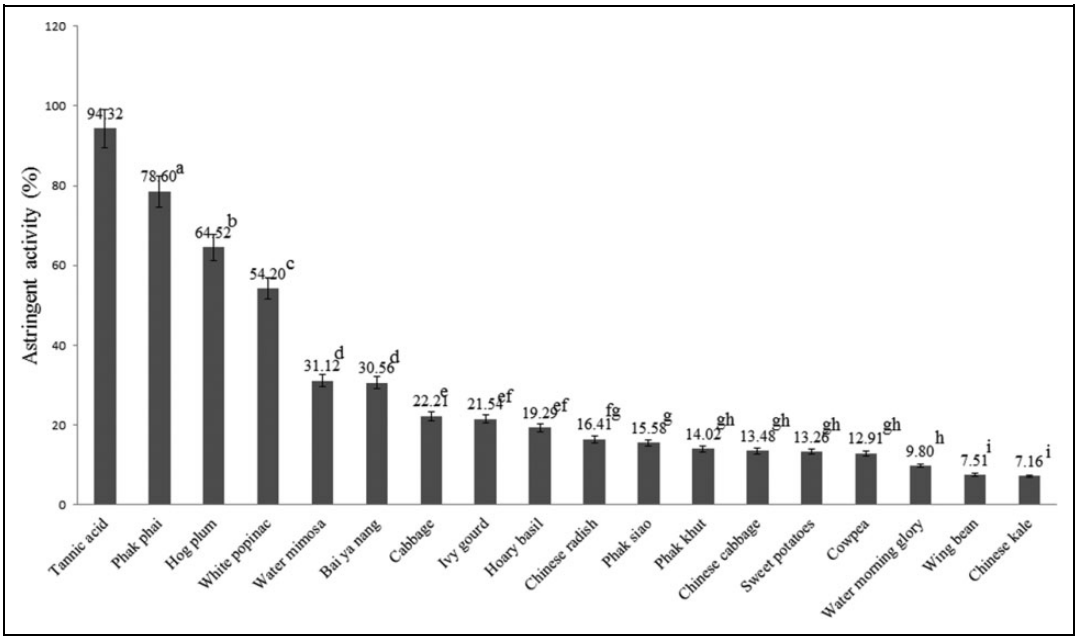


Figure 1. Astringent activities of tannic acid, a positive control, and vegetable extracts.

0.20 mg FE/ g FP, followed by ivy gourd or *Coccinia grandis* and phak phai extracts, which had 5α-reductase inhibitory activities equal to 1.87 ± 0.01 and 1.59 ± 0.01 mg FE/ g FP, respectively. Phak khut or *Diplazium esculentum* extract was nearly inactive against 5α-reductase enzyme (0.02 ± 0.01 mg FE/g FP). Previous research indicated that substances in polyphenol classes such as flavonoids, isoflavones, anthraquinones, phenolic acids,[27] and tannins[26] possessed an anti-5α-reductase activity.

In the next step, the astringent activity of the vegetable extracts was analyzed by using hemoglobin precipitating technique. Astringency is involved in the process of protein complex formation. Astringency is considered as an antinutrient factor. But it is useful as an active ingredient in topical care products, where tannin can bind to a protein in skin pore, resulting in a reduction of enlarged pore size and sebum secretion.[16] Astringent activity of tannic acid, a positive control, in this experiment was 94.32% ± 1.41%. This was comparable to the previous experiment of Son et al.[16] Astringent activities of other vegetable extracts are shown in Figure 1. It was found that phak phai exhibited the highest astringent activity with 78.60% ± 1.55%, followed by hog plum and white popinac, which had astringent activities of 64.52% ± 1.55% and 54.20% ± 0.58%, respectively. The lowest astringent activity was observed in Chinese kale or *Brassica albograbra*, with 7.16%. When compared with previous study of Son et al.[16] who studied the astringent activities of green apple rind extract extracted, phak phai had lower astringent activity than green apple rind extracted with 70% ethanol and fractionated with hexane (101.7%) but comparable to the extract that fractionated with chloroform (81.5%).

There was a strong correlation between astringent activity and total tannin content (Pearson's correlation coefficient, $r = 0.869$, $P < .001$) and between astringent activity and total flavonoid content (Pearson's correlation coefficient, $r = 0.639$, $P < .001$).

Antioxidant activities of vegetable extracts were screened by using 2 radical scavenging models, which were ABTS and DPPH radical scavenging assays, and 2 reduction models, which were hydrogen peroxide scavenging and FRAP assays. The results are shown in Table 2.

For radical scavenging assays, ABTS radical scavenging activity revealed that hog plum extract exhibited the highest ABTS radical scavenging activity with 23658.13 ± 476.03 mg VCE/g FP, followed by white popinac and phak phai with 19915.38 ± 714.57 and 5640.42 ± 96.20 mg VCE/g FP, respectively. The plant extract that had the lowest ABTS radical scavenging activity was Chinese cabbage with 257.41 ± 10.23 mg VCE/g FP. The results of DPPH radical scavenging activity revealed that white popinac was the most potent DPPH radical scavenger with 565.46 ± 4.36 mg VCE/g FP, followed by hog plum and Chinese radish or *Raphanus sativus* with 464.83 ± 9.29 and 59.44 ± 3.42 mg VCE/g FP. Phak phai, which was the third potent ABTS radical scavenger, was less active against DPPH radical. However, this conformed to the study of Nanasombat and Teckchuen[24] who reported that phak phai was the least potent DPPH radical scavenger but had the highest total phenolic content among the vegetables selected in their experiment. It may be assumed that phak phai acts as a potent radical scavenger in polar environment only.

There was strong correlation between total tannin content and ABTS scavenging activity (Pearson's correlation coefficient, $r = 0.886$, $P < .001$) and between total tannin content and DPPH scavenging activity (Pearson's correlation coefficient, $r = 0.772$, $P < .001$). There were also correlations between total flavonoid content and ABTS scavenging activity (Pearson's correlation coefficient, $r = 0.461$, $P < .001$) and

between total flavonoid content and DPPH scavenging activity (Pearson's correlation coefficient, $r = 0.297$, $P < .05$).

For reduction assays, the results of hydrogen peroxide scavenging activity showed that phak phai was the most potent hydrogen peroxide scavenger with 44.01 $\pm$ 0.06 mg VCE/g FP, followed by Chinese kale and phak siao or *Bauhinia pirpurea* with 37.26 $\pm$ 0.04 and 32.85 $\pm$ 0.03 mg VCE/g FP, respectively. Surprisingly, hog plum was the least potent hydrogen peroxide scavenger with 10.60 $\pm$ 0.03 mg VCE/g FP. However, in the FRAP assay, hog plum was the most powerful ferric reducer, with 7686.56 mg VCE/g FP, followed by white popinac and hoary basil with 5592.76 $\pm$ 343.44 and 1001.18 $\pm$ 56.19 mg VCE/g FP.

There was a strong correlation between total tannin content and FRAP assay (Pearson's correlation coefficient, $r = 0.800$, $P < .001$) and between total flavonoid content and FRAP assay (Pearson's correlation coefficient, $r = 0.360$, $P < .01$).

The results suggest that tannin content of the selected vegetable in this experiment had the strong correlation with ABTS and DPPH radical scavenging activities, ferric reduction, and astringent activity. These results were related to the previous studies about plant tannins and their biological activities.[9,10,30,31]

Since oxidative stress and radicals are involved with many chronic diseases, local vegetables may have a potential for further development into health promotion nutritional supplements, preventive medicines, and also topical products for cosmetic purposes.

Conclusions

In the current work, 17 common local vegetables in Northern Thailand were selected and extracted with 95% ethanol. The extracts were screened for 5α-reductase inhibitory activity, total tannin and flavonoid contents, astringent activity, and antioxidant activities. The results showed that *N mimosa* extract was the most potent 5α-reductase inhibitor. *S pinnata* had the highest total tannin content and was the most potent ABTS radical scavenger and the most potent ferric reducer. *O americanum* had the highest flavonoid content. *P odoratum* had the highest astringent activity and was the most potent hydrogen peroxide scavenger. *L leucocephala* was the most potent DPPH radical scavenger. There were strong correlations between total tannin content and astringent activity, ABTS and DPPH radical scavenging activities, and ferric reducing power. The results suggested that vegetables might have an impact on health promotion and vegetable extracts can be further developed into nutritional supplements, preventive medicines, or topical products for cosmetic purposes.

Acknowledgments

We would like to thank Miss Wannaree Charoensup, the botanist, for great help in identification of plant samples. We also would like to thank School of Cosmetic Science, Mae Fah Luang University and Faculty of Pharmacy, Chiang Mai University for providing the necessary facilities and support to conduct this research.

Author Contributions

NK performed the experiments, data collection and analysis, and drafted the manuscript. CC contributed in the research planning and guidance, manuscript writing, and critical review of the data.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This research was financial supported by Thailand Research Fund and Mae Fah Luang University under new researcher promotion program, contract number TRG5880142.

Ethical Approval

The protocol for the preparation of rat microsomal enzyme was approved by the Animal Research Ethics Committee of the Faculty of Pharmacy, Ubon Ratchathani University, Ubon Ratchathani, Thailand.

References

1. Dauchet L, Amouyel P, Hercberg S, Dallongeville J. Fruit and vegetable consumption and risk of coronary heart disease: a meta-analysis of cohort studies. *J Nutr*. 2006;136:2588-2593.
2. Liu RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *Am J Clin Nutr*. 2003;78(suppl):517S-520S.
3. Riboli E, Norat T. Epidermologic evidence of the protective effect of fruit and vegetables on cancer risk. *Am J Clin Nutr*. 2003; 78(suppl):559S-569S.
4. van den Akker M, Buntinx F, Knottnerus JA. Comorbidity or multimorbidity what's in a name? A review of literature. *Eur J Gen Pract*. 1996;2:65-70.
5. Ruel G, Shi Z, Zhen S, et al. Association between nutrition and the evolution of multimorbidity: the importance of fruits and vegetables and whole grain products. *Clin Nutr*. 2014;33:513-520.
6. Del Rio D, Rodrigues-Mateos A, Spencer JP, Tognolini M, Borges G, Crozier A. Dietary (poly)phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic disease. *Antioxid Redox Signal*. 2013;18:1818-1892.
7. Benzie IF. Evolution of dietary antioxidants. *Comp Biochem Physiol A*. 2003;136:113-126.
8. Ashok PK, Upadhyaya K. Tannins are astringent. *J Pharmacogn Phytochem*. 2012;1(3):45-50.
9. Ghosh D. Tannins from foods to combat diseases. *Int J Pharm Res Rev*. 2015;4(5):40-44.
10. Yang B, Liu P. Compositon and biological activities of hydrolysable tannins of fruits of *Phyllanthus emblica*. *J Agric Food Chem*. 2014;62:529-541.
11. Liu S, Yamauchi H. Different patterns of 5α-reductase expression, cellular distribution, and testosterone metabolism in human follicular dermal papilla cells. *Biochem Biophys Res Commun*. 2008;368:858-864.

12. Bruchovsky N, Wilson JD. The conversion of testosterone to 5-α-androstan-17-β-ol-3-one by rat prostate in vivo and in vitro. *J Biol Chem*. 1968;243:2012-2021.
13. Kumar T, Sirilun S, Rungseevijitprapa W, Suttajit M. Screening of steroid 5α-reductase inhibitory activity and total phenolic content of Thai plants. *J Med Plant Res*. 2011;5:1265-1271.
14. Kumar N, Rungseevijitprapa W, Narkkhong N-A, Suttajit M, Chaiyasut C. 5α-reductase inhibition and hair growth promotion of some Thai plants traditionally used for hair treatment. *J Ethnopharmacol*. 2012;139:765-771.
15. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem*. 1951;193:265-275.
16. Son DH, Nam MH, Hong CO, et al. 5α-Reductase inhibitory effect and astringent activity of green apple rind extract on human keratinocytes and fibroblast cells. *Biosci Biotechnol Biochem*. 2013;77:714-721.
17. Ardestani A, Yazdanparast R. Antioxidant and free radical scavenging potential of *Achillea santolina* extracts. *Food Chem*. 2007;104:21-29.
18. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decoloration assay. *Free Radic Biol Med*. 1999;26:1231-1237.
19. Gülçin İ, Alici HC, Cesur M. Determination of in vitro antioxidant and radical scavenging activities of propofol. *Chem Pharm Bull*. 2005;53:281-285.
20. Benzie IF, Strain JJ. The ferric reducing ability of plasma as a measure of "antioxidant power": the FRAP assay. *Anal Biochem*. 1996;239:70-76.
21. Khatoon S, Siddiqui HH, Hussain T, Firdous H. Botanical and phytochemical evaluation of fruits of *Spondias pinnata*. *Pharm Lett*. 2015;7:276-280.
22. Manik MK, Islam SMH, Wahid MA, et al. Investigation of in vitro antioxidant, antimicrobial and thrombolytic activity of the exocarp of *Spondias pinnata* (Anarcadiaceae). *Can Chem Trans*. 2013;1:191-201.
23. Vieira RF, Grayer RJ, Paton AJ. Chemical profiling of *Ocimum americanum* using external flavonoids. *Phytochemistry*. 2003;63:555-567.
24. Nanasombat S, Teckchuen N. Antimicrobial, antioxidant and anticancer activities of Thai local vegetables. *J Med Plant Res*. 2009;3:443-449.
25. Park JS, Yeom MY, Park WS, et al. Enzymatic hydrolysis of green tea seed extract and its activity of 5α-reductase inhibition. *Biosci Biotechnol Biochem*. 2006;70:387-394.
26. Liu J, Ando R, Shimizu K, et al. Steroid 5α-reductase inhibitory activity of condensed tannins from woody plants. *J Wood Sci*. 2008;54:68-75.
27. Hiipakka RA, Zhang HZ, Dai W, Dai Q, Liao S. Structure-activity relationships for inhibition of human 5α-reductases by polyphenols. *Biochem Pharmacol*. 2002;63:1165-1176.
28. Fujita R, Liu J, Shimizu K, et al. Anti-androgenic activities of *Ganoderma lucidum*. *J Ethnopharmacol*. 2005;102:107-112.
29. Shimizu K, Kondo R, Sakai K, Buabarn S, Dilokkunanant U. A geranylated chalcone with 5α-reductase inhibitory properties from *Artocarpus incisus*. *Phytochemistry*. 2000;54:737-739.
30. Deng YT, Liang G, Shi Y, et al. Condensed tannins from *Ficus altissima* leaves: structural, antioxidant, and antityrosinase properties. *Process Biochem*. 2016;51:1092-1099.
31. Zarin MA, Wan HY, Isha A, Armania N. Antioxidant, antimicrobial and cytotoxic potential of condensed tannins from *Leucaena leucocephala* hybrid-Rendang. *Food Sci Human Wellness*. 2016;5:65-75.