PHARMACOGNOSTIC SPECIFICATION OF *NARINGI CRENULATA* STEM WOOD

Mayuree Kanlayavattanakul[1,*], Ampai Phrutivorapongkul[2], Nattaya Lourith and Nijsiri Ruangrungsi[3,4]

[1]School of Cosmetic Science, Mae Fah Luang University, Chiang Rai, Thailand
[2]Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand
[3]Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand
[4]College of Public Health Sciences, Chulalongkorn University, Bangkok, Thailand

ABSTRACT: Pharmacognostic specification of *Naringi crenulata* stem wood, a traditional cosmetics in Southeast Asia, was done by studying on twelve wood samples from different sources. The powdered stem wood had a sweet natural fragrance but tasteless. Stem wood fibers were predominately found with large amount of longitudinal cells in addition to high lignin content in cell wall. Wood parenchyma contained starch granules and calcium oxalate crystals with oil globules thoroughly distributed. Alkaloids and coumarin tests were positive. Moisture content and loss on drying were 6.125% ± 0.653 and 7.564% ± 1.146, respectively. Total and acid insoluble ash contents were 1.198% ± 0.515 and 0.035% ± 0.077, respectively. Extractive values of 95% EtOH, EtOAc and H_2O were 0.165% ± 0.058, 0.036% ± 0.008 and 0.533% ± 0.117, respectively. HPLC chromatograms of twelve wood samples were similar in patterns but diverse in quantity. Arbutin content was 0.750% ± 0.414 of the crude extract weight.

Keywords: Arbutin, Botanical lightening agent, *Hesperethusa crenulata*, *Naringi crenulata,* Pharmacognostic specification

INTRODUCTION: Natural cosmetics including herbal and botanical cosmetics are of cosmetic consumer interest according to their safety compare to cosmetics containing the synthetic ingredients or animal raw materials[1)]. However, natural extracts' quality depends on several factors effecting the chemical constituents[2)]. Specification of plant is, therefore, a certify method to ensure plant quality prior to be supplied as cosmetics or medication raw materials.

Naringi crenulata (Roxb.) Nicolson (syn. *Hesperethusa crenulata* (Roxb.) Roem) (Rutaceae) widely distributes in Asia-tropical zones particularly Myanmar and Thailand where it is well-known for its use in traditional cosmetics. Application of moist powdered wood on face sustains in Burmese culture including some part of Northern Thailand, which obviously maintains its aesthetic efficiencies preventing acne and oily skin, provides soft and fresh skin texture[3)] in addition to skin lightening ability and sunscreen action preventing sunburn, which consequently causes wrinkle, freckle and dry skin condition incorporating into skin aging[4)]. Although *N. crenulata* has long been used in traditional cosmetics, this wood has never been specified on its standard of quality neither nor its monograph which currently absent. Thus, the wood from several sources in Thailand were pharmacognostically examined. HPLC fingerprint of each wood extract was done. A skin lightening agent, Arbutin, was used as a marker as it was previously found in an aerial part of *N. crenulata*[5)]. Quantification of Arbutin in each source was analyzed and compared.

MATERIALS AND METHODS:

Wood samples

Twelve stem woods of *N. crenulata* were collected from different sources during April–July 2007. The collected fresh woods were immediately air dried. The barks and stems were subsequently divided and the plant samples were further numbered based on source location. Voucher specimens were maintained at Mae Fah Luang University, Chiang Rai, Thailand.

Wood pharmacognosy

Macroscopic characters were examined on shape and size, color and external markship, fracture and internal color including odor. Microscopic characters were physiologically observed by means of microscope (Zeiss Axioskop, Germany) with safranin staining solution. Major

*To whom correspondence should be addressed.
E-mail: mayuree@mfu.ac.th,
Tel. +66 5391 6832, fax.+66 5391 6831

chemical constituents, contamination substance, moisture content, loss on drying, ash content and extractive values were determined by the literature methods[6]. Briefly, alkaloids were test with Mayer and Dragendroff reagents which positively observed in white and orange precipitates, respectively. Coumarins were detected by visualization of the extract's vapor reacted with NaOH (1 M) under 366 nm (fluorescence). Moisture content was determined by Azeotropic distillation with toluene whereas loss on drying was conducted at 105 °C of which the losing weight refers to contents of moisture and volatile compounds. Total ash content was done by burning wood powder (4 g) at 450 ºC to afford carbonless ash which was further weighted, HCl (2 M) was added into the remaining ash and boiled, neutralized by water, dried and further burn at 500 ºC until the constant weight was obtained for acid insoluble ash calculation. Extractive values were performed in 95% EtOH, EtOAc and water, separately. The wood powder (5 g) was macerated in 100 ml of solvent in sealed vessel for 24 hr with gently shake under ambient temperature, filtrated and adjusted to 100 ml with solvent. An aliquot (20 ml) was transferred into evaporating dish, weighted, evaporated to dryness and further dried in oven at 105 ºC until the constant weight was obtained.

HPLC analysis

HPLC chromatograms were carried out on a Waters 2695 Alliance equipped with a Waters 2996 photodiode array detector (measured at 280 nm) using a reversed phase column (Alltech, Prevail C18 5μm, 250 × 4.6 mm, stainless steel with Alltech, Prevail all-guard cartridge C18 5μm, 7.5 × 4.6 mm). The HPLC components were controlled through Waters Empower II software. Compounds were successively separated at a flow rate of 1 ml/min using the following linear gradient solvent system; acetonitrile (AcCN)/3% acetic acid (AcOH) in H_2O (v/v); at 0 min, 10:90; at 45 min, 55:45, held at 55:45.

Quantification of Arbutin was performed by HPLC of which the calibration curve was prepared from standard Arbutin (Aldrich, USA) conc. 0.1, 10.0, 50.0, 100.0 and 500.0 ppm, respectively, with the relative coefficient of more than 0.99 by using of the mentioned solvent system. All of the measurements were done in triplicate.

RESULTS AND DISCUSSION:

Five woods were collected from Northern Thailand which were sample no. 1, 3-6. Four samples were collected from the Northeast (sample no. 7, 8), East (sample no. 9) and West (sample no. 10), respectively. Three wood samples were purchased from folk drug stores located at the boarder of Northern Thailand and Myanmar which were sample no. 2, 11 and 12. The pharmacognostic characters of the fresh plant harvesting in Thailand and the selling wood were then compared as the purchased woods was claimed cultivated in Myanmar where this traditional cosmetics is widely used.

Macroscopic characters of *N. crenulata* appearance were in a cylindrical form in 2 to 6 inches diameter and 20 inches long with the externally brown and internally pale yellow color. The trunk contained fracture fibrous of which the surface embedded with a narrow cortex separated from a central cylinder. Microscopic characters were examined both in transverse section and powder. The powdered stem woods were in pale yellow with a sweet natural fragrance but tasteless. Transverse section showed parenchyma, phloem, xylem and parenchyma containing starch gain and calcium oxalate crystals as shown in Fig. 1 with their shape and size illustrated in Fig. 2. Bordered pitted vessel parenchyma was found with a thick cell wall and pore canal. Starch granules obviously distributed thoroughly with a prism shape of calcium oxalate crystals. Sclereid cells were often found together with high lignin content in the thick cell wall. Tissues were mostly condensed wood fibers and wood pith compounded of rounded shape cell with a thin cell wall of which lager cell arranged into pith. Occasionally, parenchyma of pith containing oil globule and prism crystal of calcium oxalate were found.

None of any contaminants were found because most of them were collected from the fresh plants and the stem woods were separated from barks after air dried prior to grind into powder for further observation.

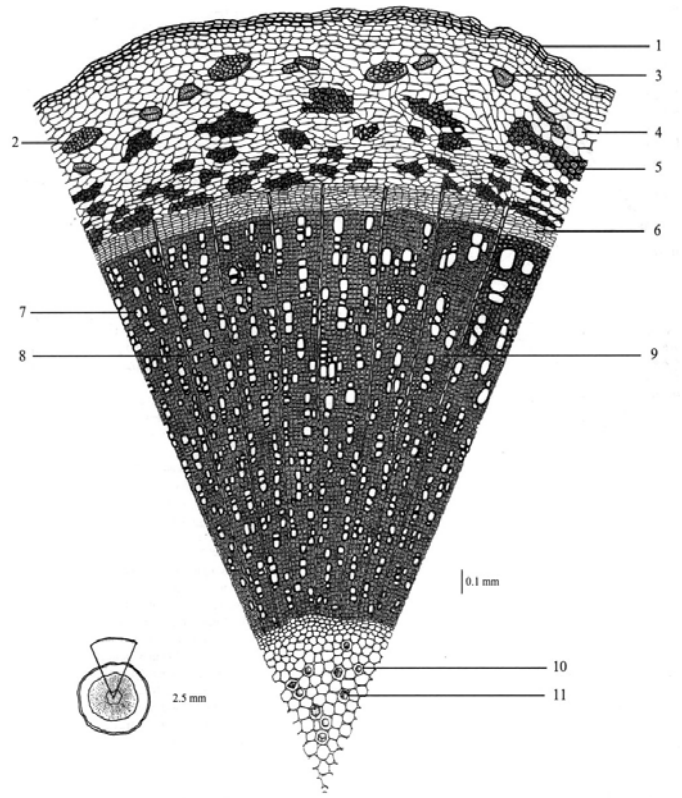


Figure 1 Transverse section of *N. crenulata* (Roxb.) Nicolson stem :
1. epidermis layer, 2. starch granules, 3. sclereid, 4. parenchyma of cortex, 5. cortical fiber, 6. phloem tissue, 7. xylem vessel, 8. xylem ray, 9. xylem fiber, 10. parenchyma of pith containing oil globule, 11. parenchyma containing prism crystal

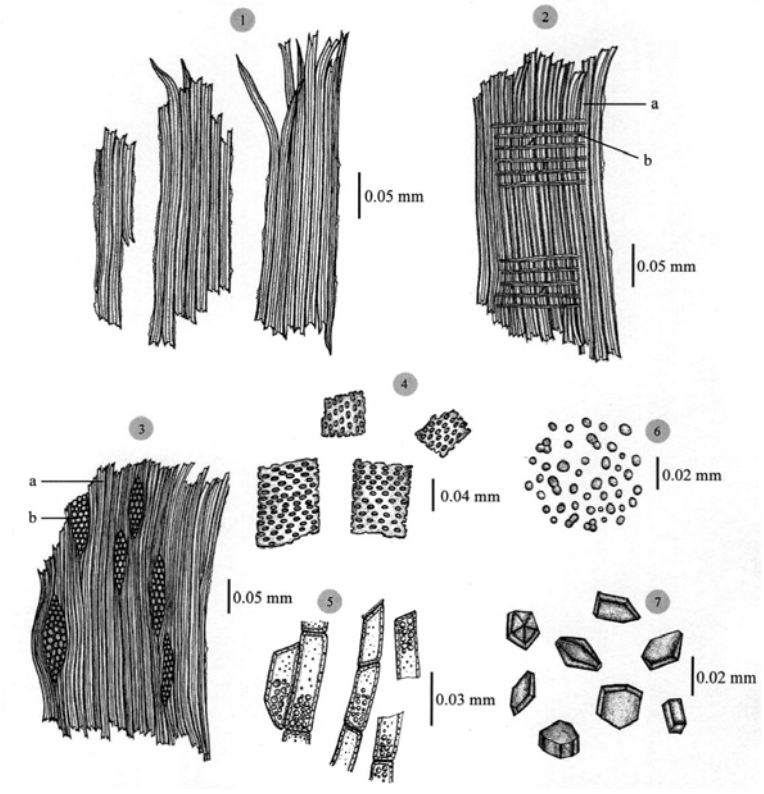


Figure 2 Powdered N. crenulata (Roxb.) Nicolson:
1. fragment of wood fibers, 2. fragment of xylem ray in radial longitudinal view (2a. wood fiber, 2b. wood parenchyma), 3. fragment of xylem ray in tangential longitudinal view (3a. wood fiber, 3b. wood parenchyma), 4. fragment of bordered pitted vessel, 5. fragment of wood parenchyma containing starch grain in longitudinal view, 6. starch grain, 7. prism crystals of calcium oxalate

Table 1 Pharmacognostic characters (% by weight) of *N. crenulata* and its Arbutin content

No.	Moisture content	Loss on drying	Total ash content	Acid insoluble ash content	Extractive value			Arbutin content
					95% EtOH	EtOAc	H_2O	
1	6.167 ± 0.144	9.377 ± 0.006	1.193 ± 0.006	0.012 ± 0.002	0.124 ± 0.004	0.027 ± 0.003	0.539 ± 0.014	0.434 ± 0.009
2	6.667 ± 0.144	8.973 ± 0.006	0.716 ± 0.002	0.018 ± 0.002	0.111 ± 0.012	0.039 ± 0.005	0.405 ± 0.008	0.470 ± 0.174
3	6.250 ± 0.000	6.480 ± 0.344	2.378 ± 0.010	0.007 ± 0.002	0.234 ± 0.022	0.047 ± 0.002	0.732 ± 0.011	0.829 ± 0.059
4	6.147 ± 0.144	8.903 ± 0.006	0.977 ± 0.003	0.003 ± 0.000	0.128 ± 0.002	0.040 ± 0.004	0.500 ± 0.015	0.451 ± 0.179
5	6.000 ± 0.250	6.540 ± 0.010	0.658 ± 0.005	0.022 ± 0.002	0.137 ± 0.002	0.029 ± 0.001	0.381 ± 0.006	0.268 ± 0.015
6	5.667 ± 0.144	7.097 ± 0.006	1.189 ± 0.004	0.279 ± 0.018	0.193 ± 0.005	0.046 ± 0.005	0.593 ± 0.023	1.711 ± 0.120
7	5.583 ± 0.288	8.543 ± 0.006	0.824 ± 0.002	0.010 ± 0.000	0.266 ± 0.002	0.046 ± 0.007	0.641 ± 0.026	0.992 ± 0.034
8	5.750 ± 0.000	5.973 ± 0.031	0.822 ± 0.002	0.017 ± 0.006	0.252 ± 0.002	0.027 ± 0.003	0.614 ± 0.017	0.888 ± 0.075
9	4.833 ± 0.144	7.760 ± 0.000	1.601 ± 0.002	0.003 ± 0.000	0.177 ± 0.012	0.042 ± 0.002	0.521 ± 0.035	0.993 ± 0.039
10	6.083 ± 0.289	6.533 ± 0.060	1.136 ± 0.004	0.041 ± 0.002	0.123 ± 0.002	0.033 ± 0.006	0.395 ± 0.008	0.920 ± 0.094
11	6.833 ± 0.381	6.967 ± 0.064	1.844 ± 0.004	0.006 ± 0.002	0.123 ± 0.010	0.035 ± 0.007	0.659 ± 0.015	0.403 ± 0.013
12	7.333 ± 0.144	7.627 ± 0.015	0.997 ± 0.006	0.003 ± 0.000	0.105 ± 0.005	0.025 ± 0.001	0.412 ± 0.013	0.642 ± 0.054
mean ± S.D.	6.125 ± 0.653	7.564 ± 1.146	1.198 ± 0.515	0.035 ± 0.077	0.165 ± 0.058	0.036 ± 0.008	0.533 ± 0.117	0.750 ± 0.414
max - min	7.333 – 4.833	9.973 – 5.973	2.378 – 0.658	0.279 – 0.003	0.266 – 0.105	0.047 – 0.025	0.733 – 0.381	1.711 – 0.268

Figure 3 HPLC Chromatograms of twelve wood samples

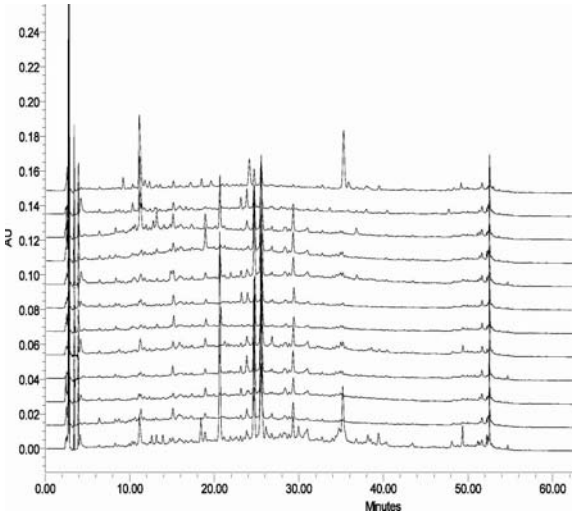
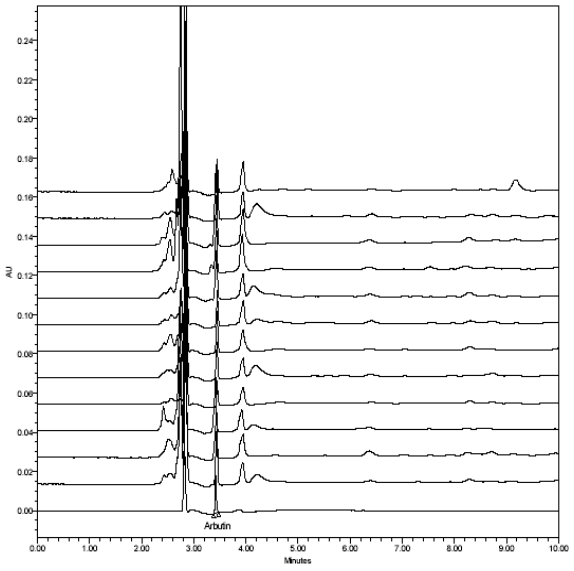


Figure 4 HPLC analysis of Arbutin in *N. crenulata* extracts from twelve sources (standard Arbutin was lied bellowed)



Moisture content and loss on drying were determined as shown in Table 1 as well as total ash and acid insoluble ash contents including extractive values of wood. Samples no. 11 and 12 showed high moisture content which may caused by a long time storage as they were purchased. Interestingly, the wood with the highest moisture content had the lowest acid insoluble ash content.

These results were explained by their geographic condition according to a lot of mineral content in soil as there are lignite and copper mines locate in sources number 6 and 10, respectively. The 95% EtOH crude extract of sample no. 7 had the highest percentage yield and the lowest was the wood with the highest moisture content meanwhile the highest extractive yield of EtOAc and H_2O was wood that had the highest total ash content. In addition to geographic condition where wood was cultivated, aged of plant and the period of harvesting influence on these pharmacognostic characters.

Alkaloids were detected in all sources, which were in accord with the literatures as well as coumarin[3, 7-9].

HPLC chromatograms of twelve wood samples were similar in patterns as shown in Fig. 3. However, quantity of their chemical compositions were varied. Plant identity would be confirmed by plant metabolites which similarly found in each source. Nevertheless, DNA finger print should ensure plant species.

Arbutin content was quantified in each source (Fig. 4). The skin lightening content was ranged from 0.268% - 1.711% as shown in Table 1 which higher than its content extracted from the whole plant (0.12 μg g^{-1})[5].Therefore, this presenting extraction method and HPLC analytical condition afforded more Arbutin content.

In addition, it was found that wood with the highest acid insoluble ash content had the highest Arbutin content whereas sample no. 5 that showed the lowest total ash content contained Arbutin in the lowest amount. Further study will compare Arbutin content in bark and wood of source no. 6 as *N. crenulata* was traditionally used only bark in some place.In addition, this source should be used in a natural skin lightening cosmetics development respecting to its Arbutin content.

N. crenulata grown in Thailand could be a potential source for natural cosmetics particularly a skin lightening cosmetics compared to those cultivated in Myanmar. In addition to *N. crenulata* pharmacognostic specification, microbial contamination will be further examined as well as stability of the extract for cosmetic preparation. Thus, this report represents pharmacognostic characters of *N. crenulata* available for standarization although it is not the fully monograph which remains absent.

ACKNOWLEDGEMENTS: This research was financially supported by the Office of the National Research Council of Thailand (NRCT).

REFERENCES:

1. Aburjai T, Natsheh FM. 2003. Plants used in cosmetics. Phytother. Res. 17: 987 – 1000.

2. Vollhardt J. 2001. Natural extracts. In: Barel AO, Paye M, Maibach HI, eds., Handbook of cosmetic science and technology New York: Marcel Dekker; p. 370 – 3.

3. Nayar MNS, Sutar CV, Bhan MK. 1971. Alkaloids of the stem bark of *Hesperethusa crenulata*. Phytochem. 10: 2843 – 4.

4. Cunningham WJ. 2001. Antiwrinkle products. In: Barel AO, Paye M, Maibach HI, eds., Handbook of cosmetic science and technology. New York: Marcel Dekker; p. 543 – 4.

5. Thongchai W, Liawruangrath B, Liawruangrath S. 2007. High-performance liquid chromategraphy determination of arbutin in skin-whitening creams and medicinal plant extracts. J. Cosmet. Sci. 58: 35-44.

6. Thai Pharmacopoeia Committee. 1995. Thai herbal pharmacopoeia V.1. Bangkok: Prachachon Press.

7. Dreyer DL, Pickering MV, Cohna P. 1972. Distribution of limonoids in the rutaceae. Phytochem. 11: 705 – 3.

8. Dreyer DL, Rigold JF, Basa SC, Mahanty P, Das DP. 1980. Chemotaxonomy of the rutaceae-XIII: The occurance of severine in *Atalantia monophylla* and *Hesperethusa crenulata*; A revised structure for severine. Tetrahedron 36: 827 – 9.

9. Nayar MNS, Bhan MK. 1972. Coumarins and other constituents of *Hesperethusa crenu lata*. Phytochem. 11: 3331 – 3