

AN EFFECTIVE METHOD FOR THE IDENTIFICATION OF STEM ADULTERATION IN BENCHA-LOGA-WICHIAN, A THAI TRADITIONAL PREPARATION

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ABSTRACT: Bencha-Loga-Wichian (BLW) is an official traditional Thai preparation for the treatment of fever that consists of five roots: *Capparis micracantha*, *Clerodendrum petasites*, *Ficus racemosa*, *Harrisonia perforata*, and *Tiliacora triandra*. Although the roots are recommended, the crude drugs sold in traditional drug stores are adulterated with stems, which might affect their efficacy and safety. Thus, the study aimed to establish a method for identifying stem adulteration in BLW using macroscopic, microscopic and TLC techniques. Adulterated stems of some crude drugs were easily identified by using only morphological characteristics; *Capparis micracantha* stems had grayish-brown, cracked and fissured bark while the root bark was light brown with longitudinal wrinkles, *Clerodendrum petasites* stems were hollow whereas the roots were solid, *Harrisonia perforata* stems had thorn-scarred bark and area of pith which were absent in the roots. We recommended anatomical characters for determining crude drugs of *Tiliacora triandra*. The stems had broad vascular bundles with narrow medullary rays in contrast with the roots. Stems and roots of *Ficus racemosa* were quite similar in morphology and anatomy, and needed TLC for identification. The established solvent system was dichloromethane: ethyl acetate: acetic acid (90:6:2), examined under UV 366 nm. The stem extracts of five plants and BLW demonstrated a fluorescent blue-green band at R_f of 38, which absented in the root extracts. These techniques were applied to determine the stem adulteration in commercial BLW products from five traditional drug stores and three hospitals. The results showed that most of investigated samples, crude drugs and capsules, were adulterated with stem.

Keywords: antipyretics, chemical fingerprint, pharmacognosy, microscopy, quality control

INTRODUCTION

In Thailand, herbal medicines have been included in the list of herbal medicinal products [1] as a part of the national list of essential medicines. The list of herbal medicinal products is composed of 21 single herbs and 50 preparations, including Bencha-Loga-Wichian (BLW), a preparation that is used as antipyretics. BLW consists of equal parts of the roots of *Capparis micracantha* DC. (Capparaceae), *Clerodendrum petasites* S. Moore (Lamiaceae), *Ficus racemosa* L. (Moraceae), *Harrisonia perforata* Merr. (Simaroubaceae), and *Tiliacora triandra* Diels (Menispermaceae).

Pharmacological studies have indicated that BLW possesses antipyretic and antinociceptive activities [2, 3], supporting its traditional use as derived from the roots of these plants. However, crude drugs sold in traditional drug stores are usually adulterated with stems [4], which might affect the efficacy and safety of the preparation. Indeed, different parts of

the same plant can possess different chemical constituents, exhibiting different activities [5-7]. Dissimilar chemical constituents have been reported for different organs of *F. racemosa* [8]. *H. perforata* wood contained chromones, coumarins, and phenylpropanoids [9], whereas its leaves contained limonoids [10], and the branches contained chromones, some of which were different from those in the wood [11]. Therefore, different BLW produced from the roots and stems of the five plants might contain different chemical constituents and possesses different activities.

Although a pharmacognostic study of the root parts of these plants in BLW has been conducted for authentication [4], techniques are also required for the stems, which are similar in appearance to the roots and commonly found as an adulterant in the preparation. Thus, the aim of this study was to investigate characteristics to be used to distinguish between the stems and roots of each plant in BLW using macroscopic, microscopic, and TLC techniques. The recommended differentiating characters from authentic samples can be applied for

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the identification of stem adulteration in commercial BLW products.

MATERIALS AND METHODS

Plant materials

Authentic samples: The stems and roots of *Capparis micracantha* DC. (PBM05097), *Clerodendrum petasites* S. Moore (PBM04786), *Ficus racemosa* L. (WHR00015), *Harrisonia perforata* Merr. (PBM05099) and *Tiliacora triandra* Diels (PBM05098) were collected from Surin province, Thailand. The voucher specimens were identified by Professor Wongsatit Chuakul, Mahidol University, and deposited at the Mahidol University Herbarium, Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University. All samples were cleaned, cut into small pieces and dried in a hot-air oven at 70 °C for 72 h to prepare crude drugs. A portion of each crude drug was ground, passed through a No. 40 sieve to produce powdered drugs and used in the examination.

Commercial samples: BLW products in crude form were purchased from five traditional drug stores in Bangkok, Thailand (ThanutchapornN_001 – ThanutchapornN_025). Capsules of BLW were obtained from two hospitals in Bangkok (ThanutchapornN_026 – ThanutchapornN_027) and another one in Surin Province (ThanutchapornN_028). All specimens were deposited at Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University.

Macroscopic and microscopic examinations

Macroscopic and microscopic examinations of the stems and roots of each plant were carried out according to standard procedures [12, 13]. Plant sections were cut by hand sectioning. The sections and powdered drugs were cleared with chloral hydrate solution, stained lignified cells with aniline sulfate solution and starch granules with iodine in potassium iodide [14]. The photomicrographs of the samples were taken with Olympus model CX 31 microscope. The macroscopical and microscopical characters of each plant were compared for distinguishing between the stem and root, and the differentiating characteristics were then used to identify stem adulteration in the commercial samples of the BLW.

Thin-layer chromatographic analysis

For individual plant extraction, 1.0 g of each powdered drug was extracted with 10.0 ml methanol. For BLW extraction, both authentic and commercial samples, 2.5 g of the mixed powdered drug (1:1:1:1:1) was extracted with 25.0 ml methanol. The extracts were heated on a water bath

for 15 min and filtered through filter paper. Solvent was again added to the residues, and the same procedure was repeated three times. The filtrates were combined and evaporated on a water bath, and the volume was adjusted to 5.0 ml [15].

An aliquot (10.0 µl) of each extract was applied to a TLC silica gel 60 F₂₅₄ precoated plate (Merck), developed in the solvent system of dichloromethane: ethyl acetate: acetic acid (90:6:2) and examined under UV light at 366 nm.

RESULTS

The characteristics used to differentiate between the stem and root crude drugs of BLW

The general appearances of stem and root crude drugs were similar. *Capparis micracantha*, *Ficus racemosa*, and *Harrisonia perforata* were chopped pieces of wood, which some attached to the bark and varied in size. *Clerodendrum petasites* and *Tiliacora triandra* were chopped as cylindrical pieces. However, the stems and roots of those plants had some different characteristics such as hollow stems of *Clerodendrum petasites* while the roots were solid. The anatomical characters of stems and roots of each plant clearly presented the differences such as the stems obviously presented lignified cells and area of pith, which was absent in the roots. Moreover, the powdered drugs characters of those stems and roots were almost the same that consisted of cork cells, sclereids, groups of vessels and fibers, xylem rays, non-lignified parenchyma, and starch granules. The specific characters of some cells, which only found in the stems were recorded and used for the identification.

The different morphological characters of those stem and root crude drugs, together with their anatomical and powdered characters were presented in Figure 1-5.

Thin-layer chromatographic analysis

After developing all of the methanolic extracts in the established solvent system, dichloromethane: ethyl acetate: acetic acid (90:6:2), only the stem extracts of the plant species and the preparations demonstrated a fluorescent blue-green band at an hRf of 38 when examined under 366 nm (Figure 6). Therefore, this was a clear and useful characteristic to distinguish the stems, and this band was used for determining stem adulteration in the commercial BLW.

Determination of stem adulteration in commercial crude drugs and finished products of BLW Commercial crude drugs of BLW

The crude drugs of BLW from five traditional drug stores were examined for stem adulteration using

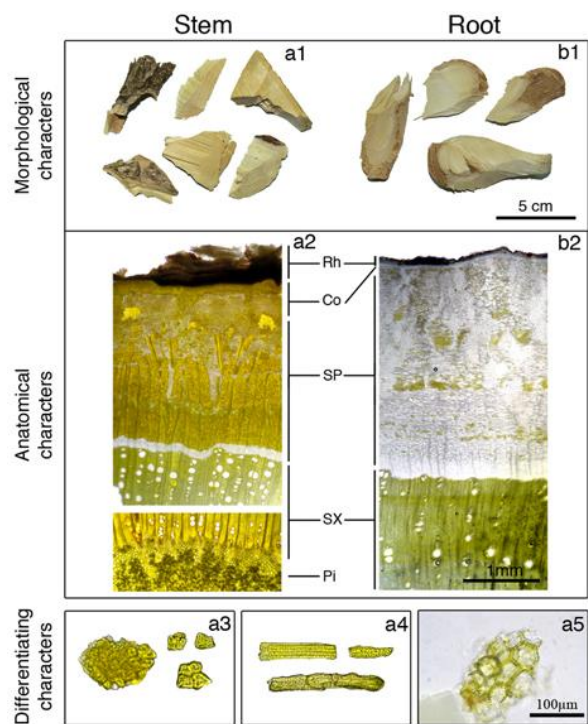


Figure 1 The differentiating characters between authentic stem (a) and root (b) of *Capparis micracantha* DC. a1, b1= crude drugs; a2, b2 = transverse sections of crude drugs; a3-a5 = differentiating characters from stem powder, a3 = small sclereids from Co, a4 = elongated sclereids from SP, and a5 = thick-walled and lignified cells from Pi
Abbreviation: Rh = Rhytidome, Co = Cortex, SP = Secondary phloem, SX = Secondary xylem, Pi = Pith

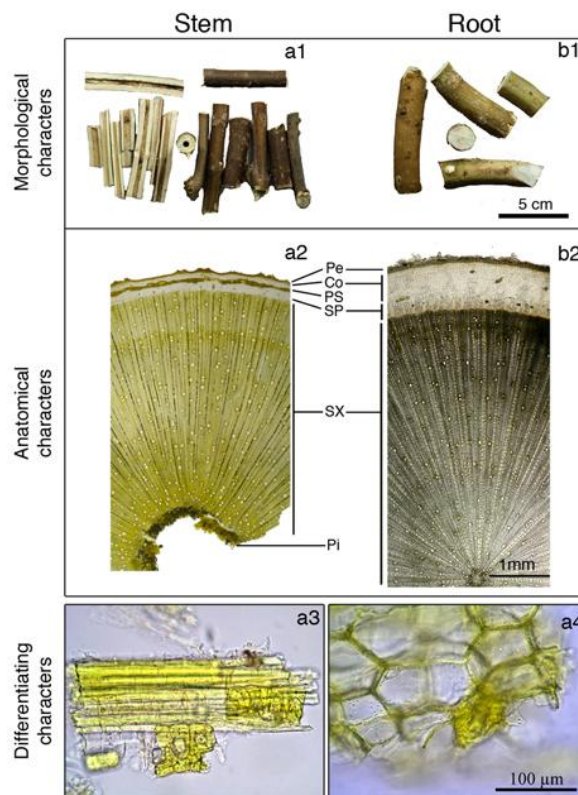


Figure 2 The differentiating characters between authentic stem (a) and root (b) of *Clerodendrum petasites* S. Moore. a1, b1= crude drugs; a2, b2 = transverse sections of crude drugs; a3-a4 = differentiating characters from stem powder, a3 = fragment of lignified fibers associated with sclereids from PS, a4 = lignified parenchyma with sclereids from Pi
Abbreviation: Pe = Periderm, Co = Cortex, PS = Pericyclic sclerenchyma, SP = Secondary phloem, SX = Secondary xylem, Pi = Pith

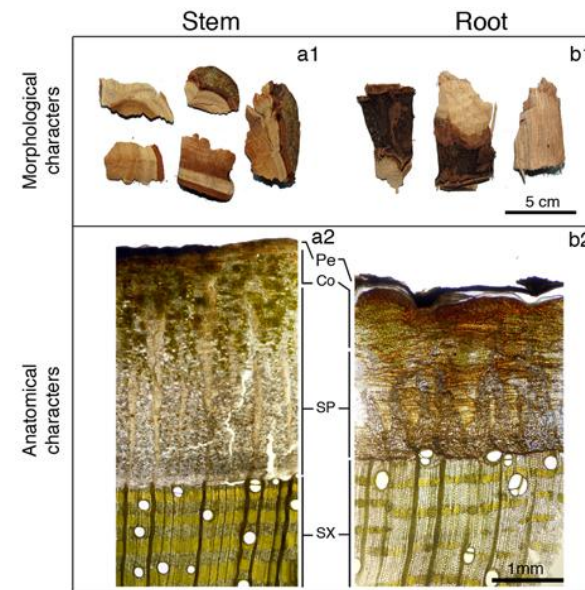


Figure 3 The differentiating characters between authentic stem (a) and root (b) of *Ficus racemosa* L. a1, b1= crude drugs; a2, b2 = transverse sections of crude drugs
Abbreviation: Pe = Periderm, Co = Cortex, SP = Secondary phloem, SX = Secondary xylem

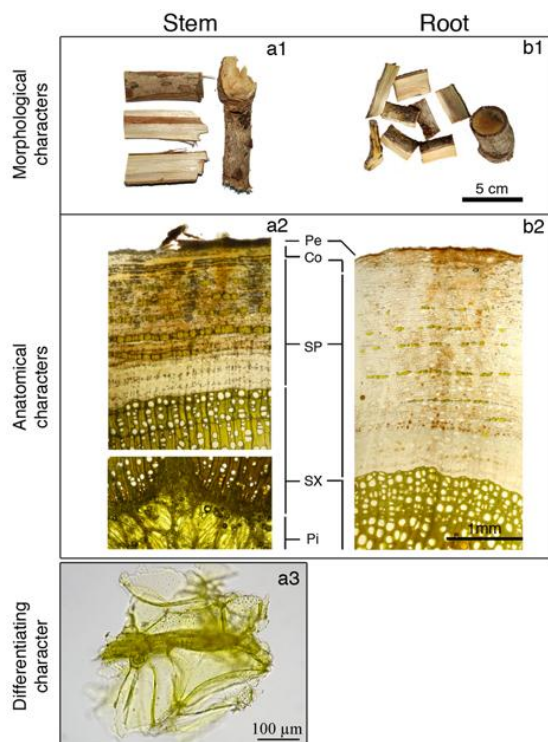


Figure 4: The differentiating characters between authentic stem (a) and root (b) of *Harrisonia perforata* Merr.

a1, b1= crude drugs; a2, b2 = transverse sections of crude drugs; a3 = fragment of flame-like cells from Pi

Abbreviation: Pe = Periderm, Co = Cortex, SP = Secondary phloem, SX = Secondary xylem, Pi = Pith

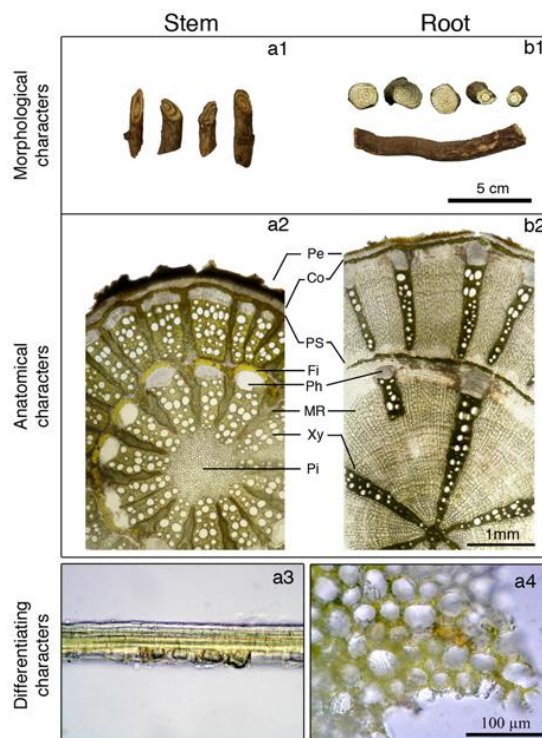


Figure 5 The differentiating characters between authentic stem (a) and root (b) of *Tiliacora triandra* Diels

a1, b1= crude drugs; a2, b2 = transverse sections of crude drugs; a3-a4 = differentiating characters from stem powder, a3 = fragment of lignified fibers associated with sclereids from PS, a4 = round, thick-walled and lignified parenchyma from Pi

Abbreviation: Pe = Periderm, Co = Cortex, PS = Pericyclic sclerenchyma of phloem, Fi = Phloem fiber, Ph = Phloem tissues, MR = Medullary rays, Xy = Xylem, Pi = Pith

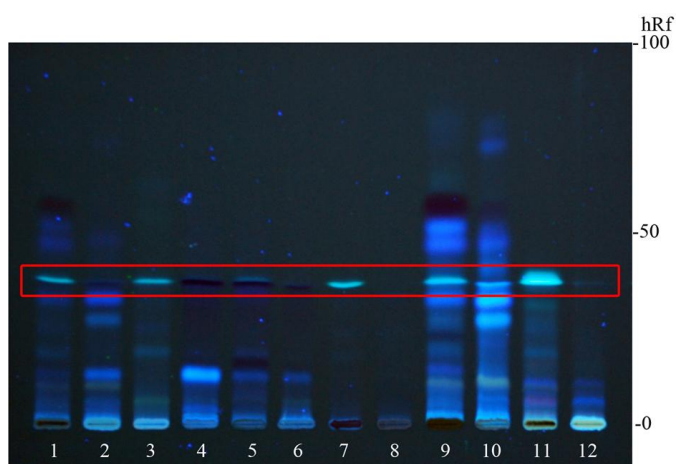


Figure 6 TLC fingerprint of methanolic extracts of mixed plants and individual plants in BLW.

Solvent system=dichloromethane:ethyl acetate:acetic acid (90:6:2), Detection=UV 366 nm.

Track 1 and 2 = mixed authentic stem (1) and mixed authentic root (2) extracts of a BLW preparation;

Track 3 and 4 = authentic stem (3) and root (4) extracts of *Capparis micracantha*;

Track 5 and 6 = authentic stem (5) and root (6) extracts of *Clerodendrum petasites*;

Track 7 and 8 = authentic stem (7) and root (8) extracts of *Ficus racemosa*;

Track 9 and 10 = authentic stem (9) and root (10) extracts of *Harrisonia perforata*;

Track 11 and 12 = authentic stem (11) and root (12) extracts of *Tiliacora triandra*.

Table 1 Identification of commercial crude drugs of BLW from five different traditional drug stores using morphological, anatomical and TLC characteristics

Plants	Part	Differentiating characteristics	Traditional drug stores					
			1	2	3	4	5	
<i>Capparis micracantha</i>	Stem	Morphology	Bark: grayish-brown to dark brown, rough, cracked and fissured	+	+	+	+	+
		Anatomy	Patches of densely packed sclereids in secondary phloem, and area of pith	+	+	+	+	+
		TLC	Fluorescent blue-green band at hRf of 38	+	+	+	+	+
	Root	Morphology	Bark: light brown with longitudinal wrinkle	-	-	-	-	-
		Anatomy	Few groups of sclereids scattered in secondary phloem, and no pith area	-	-	-	-	-
<i>Clerodendrum petasites</i>	Stem	Morphology	Hollow stem	+	+	+	+	+
		Anatomy	A pericyclic band of sclerenchyma beneath cortex	+	+	+	+	+
		TLC	Fluorescent blue-green band at hRf of 38	+	+	+	+	+
	Root	Morphology	Solid root	+	-	-	-	+
		Anatomy	No pericyclic band of sclerenchyma beneath cortex	+	-	-	-	+
<i>Ficus racemosa</i>	Stem	Morphology	Bark: greenish-brown to brown, smooth and thickness	+	-	+	+	+
		Anatomy	Broad bands of xylem fibers alternating with narrow bands of axial parenchyma	+	+	+	+	+
		TLC	Fluorescent blue-green band at hRf of 38	+	+	+	+	+
	Root	Morphology	Bark: reddish-brown, rough, and thickness	-	-	-	-	-
		Anatomy	Narrow bands of xylem fibers alternating with broad bands of axial parenchyma	-	-	-	-	-
<i>Harrisonia perforata</i>	Stem	Morphology	Thorn scars bark and found area of pith	+	-	+	+	+
		Anatomy	Area of pith	+	-	+	+	+
		TLC	Fluorescent blue-green band at hRf of 38	+	+	+	+	+
	Root	Morphology	Reddish-brown to brown bark, thickness, and no area of pith	-	-	-	-	-
		Anatomy	No pith area	-	-	-	-	-
<i>Tiliacora triandra</i>	Stem	Morphology	Internally brown with dark brown radiating bands and found central brown area of pith	+	+	+	+	+
		Anatomy	Broad vascular bundles with narrow medullary rays and found area of pith	+	+	+	+	+
		TLC	Fluorescent blue-green band at hRf of 38	+	+	+	+	+
	Root	Morphology	Internally off-white with brown radiating bands and no area of pith	-	-	-	+	-
		Anatomy	Narrow vascular bundles with markedly broad medullary rays	-	-	-	+	-

+ represents present character, -represents absent character

the established morphological and anatomical characteristics, and TLC fingerprints. The results showed that all of the commercial samples contained stem adulteration as shown in the Table 1 and Figure 7.

Commercial capsules of BLW

The commercial BLW capsules were evaluated for stem adulteration using the powdered drug and TLC characteristics. However, the powder in capsules was too fine to identify using the powdered drug characteristics. Thus, the powder was assessed for stem adulteration by TLC. The powder was extracted and developed in the established solvent system, dichloromethane: ethyl acetate: acetic acid

(90:6:2). The presence of a fluorescent blue-green band at an hRf of 38 under 366 nm (Figure 8) indicated that all of the commercial finished products were adulterated with stem.

DISCUSSION

The commercial crude drugs as well as finished products of BLW were easily determined by using the established differentiating characters. The stem adulteration of some crude drugs were identified by using only morphological characters such as *Clerodendrum petasites* stems were hollow in contrast to solid roots, *Capparis micracantha* were identified by using bark characters, grayish-brown

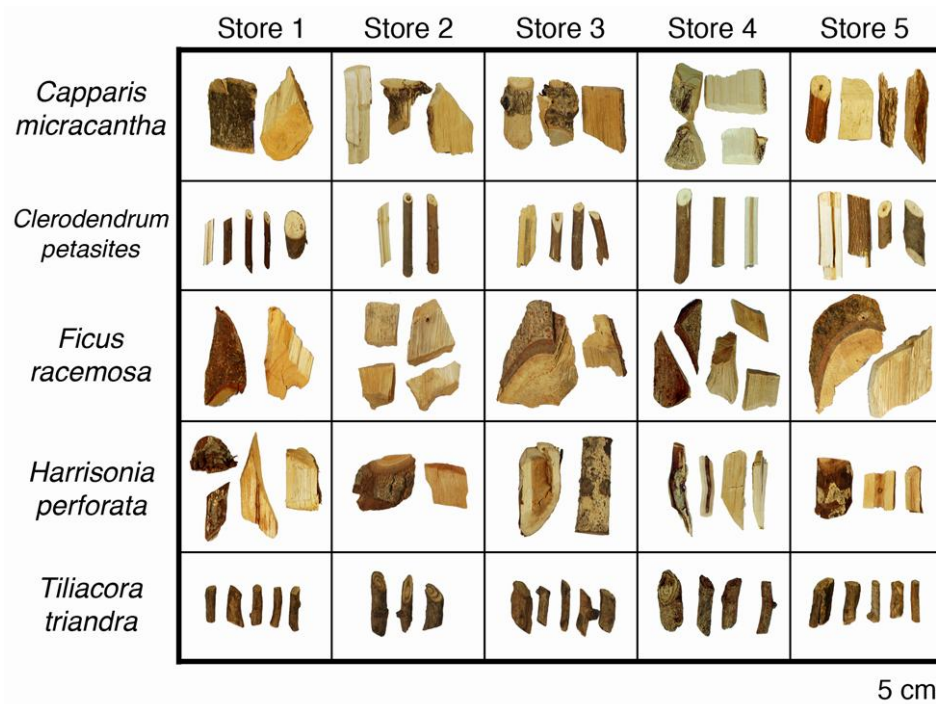


Figure 7 Commercial crude drugs of BLW from five different traditional drug stores

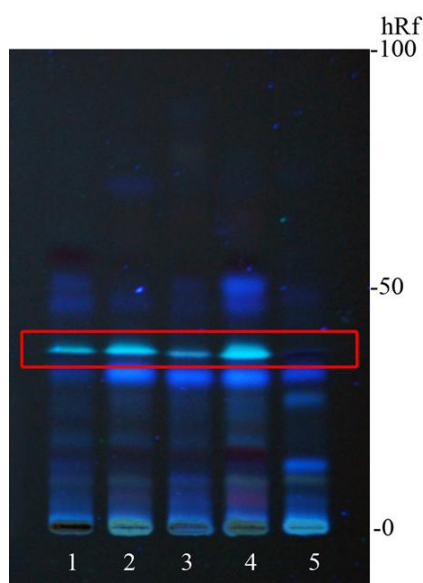


Figure 8 TLC fingerprint of the methanolic extracts of BLW from different sources

Solvent system=dichloromethane:ethyl acetate:acetic acid (90:6:2), Detection=UV 366 nm

Track 1 = Mixed authentic stems of BLW

Track 2 = Commercial capsule of BLW from Bangkok 1;

Track 3 = Commercial capsule of BLW from Surin;

Track 4 = Commercial capsule of BLW from Bangkok 2;

Track 5 = Mixed authentic roots of BLW

to dark brown, rough, cracked and fissured. However, because the bark can be detached, then the differentiating characteristics can be lost, we recommended to identify the stem adulteration using morphological characteristics together with anatomical characteristics and TLC fingerprinting. The selected anatomical characters for identifying stem were lignified cells and area of pith, which obviously presented and absented in some plants, while most of cells from the roots were non-lignified parenchyma. *Capparis micracantha* stems

had marked lignified sclereids, arranged in pericyclic band underneath rhytidome, groups in cortex and densely packed in secondary phloem. On the contrary, the roots had few groups of sclereids scattered in cortex and secondary phloem. *Clerodendrum petasites* stems had a pericyclic band of sclerenchyma, and lignified parenchyma and sclereids lining around hollow pith, which absented in the root. *Ficus racemosa* stem and root presented similarity in both cell types and arrangement. However, the stems had broad bands of xylem

fibers alternating with narrow bands of axial xylem parenchyma in contrast to the roots. *Harrisonia perforata* stems had tangential bands of lignified fibers arranged in secondary phloem and area of pith whereas the roots had small-scattered groups of lignified fibers in secondary phloem and absented pith area. *Tiliacora triandra* stems had broad vascular bundles alternating with narrow medullary rays, contrast to the roots, which had narrow vascular bundles alternating with broad medullary rays. Moreover, the stems presented sclereids beneath periderm, lignified fibers embedded in the pericyclic sclereids of inner ring, and a large area of pith, which was absent in the roots.

These identification methods, macroscopical, microscopical and TLC techniques, were simple, practical, effective and no expensive equipment was required. Moreover, such techniques have been used for distinguishing *Phyllanthus* from other species [16], and controlling the quality of medicinal plants in Jordan [17]. Singharachai and colleagues [4] also studied and established the pharmacognostic specification of the roots of five plant species in BLW. However, they reported only the root characters of those plants, which might not enough to determine the stem adulteration. According to our study, the stems and roots of each plant presented similar appearances, especially *Capparis micracantha* and *Ficus racemosa*, which were similar in both morphological and anatomical characters. Therefore, the established comparative stem and root characteristics in this study were helpful and convenient to distinguish between the stems and roots of each plant. The characteristics selected were clear and effective because they were only found in the stems. Moreover, we established a method for stem determination using TLC instead of HPLC because it is inexpensive and more practical.

The stem adulteration in the commercial BLW was determined in the crude drugs obtained from five traditional drug stores and the finished products obtained from three hospitals. The results demonstrated that all of the samples were adulterated with stems, which may affect the quality and efficacy of the preparation. Dhawan *et al.* found that the aerial part of *Passiflora incarnata*, which is traditionally used as an anxiolytic, possessed a high activity, whereas the underground parts showed no the activity [5]. Ibrahim *et al.* compared the analgesic activity of different parts of *Carissa edulis* and found that the fruits and seeds showed higher activities than the leaves, root bark and stem bark [7].

We established methods, including macroscopic, microscopic and TLC techniques, for detecting stem adulteration in commercial BLW preparations and found that stem adulteration was common, which might reflect the shortage of plant materials, especially the root parts of perennial woody trees. Therefore, further study on the activity and toxicity of the stems compared with the roots should be performed for further recommendation of stem substitution, which may be beneficial for sustainable harvesting and for protecting the plants from extinction.

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