

Botanical Origin and Identification of Krai-Krue Herbal Plant

Sawanee Sathornviriyapong^{1*}, Chayan Picheansoonthon^{2,3}, Rawat Tiasakul²,
Suppachai Tiyaworantan² and Vichai Reutrakul¹

ABSTRACT

Krai-krue is a crude drug commonly used in Thai traditional herbal remedies as an antipyretic, anti-inflammatory and muscle relaxant. This plant drug is a hard root with a characteristic smell and bitter taste. Despite its availability in Thai herbal drugstores, the botanical identity remains unknown. To reveal its botanical origin, the plant drug was traced back from herbal dispensaries in Bangkok to its natural habitats. Its identity was confirmed with the locals and traditional healers in the implemented areas. The collecting sites were revisited several times to collect complete specimens for botanical identification. The herbarium specimens were identified as *Aristolochia pierrei* Lec. and *A. tagala* Cham. (Aristolochiaceae). The roots were collected for comparative macroscopic and microscopic studies, with the samples purchased from three different herbal drugstores in Bangkok. The microscopic investigations employed different methods, including a technique specifically modified for cutting microscopic cross section of extremely hard root samples. All of the samples are morphologically and histologically alike, indicating that they may be all originated from the same *Aristolochia* genera. Statistical analysis the measured cells determined by ANOVA, however, appeared to be significantly different in corks and xylem vessels among the roots of *A. pierrei* Lec. and *A. tagala* Cham. ($p<0.05$). Thin-layered chromatographic data of these crude drugs also supported the botanical origin of Krai-krue to be *Aristolochia pierrei* Lec. as shown in the phylogenetic grouping based on their similarity coefficients ($0.94 \leq r \leq 0.98$) but relatively different from *A. tagala* Cham. ($r=0.64$).

Key words: Krai-krue, botanical origin, *Aristolochia pierrei* Lec., *A. tagala* Cham.

INTRODUCTION

The quality of crude drugs employed in Thai herbal product is considered questionable. It is a crucial obstacle for the research and development in Thai herbal remedies to meet an international standard and to ensure the claimed

therapeutic efficacy. There are several requirements needed for the specification of herbal raw materials and products (Department of Medical Science Ministry of Public Health, 1995; 2000). The correct botanical sources of the crude drugs are certainly among the most important ones.

Presently, several crude drugs used in

¹ Department of Botany, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.

² Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Science, Khon Kaen University, Khon Kaen 40002, Thailand.

³ The Royal Institute, Grand Palace, Bangkok 10200, Thailand.

⁴ Department of Chemistry, Faculty of Science, Mahidol University, Bangkok 10400, Thailand.

* Corresponding author.

Thai traditional medical formulas remain uncertain, or even unknown (Picheansoonthon *et al.*, 2001), Krai-krue, Plu-gae, Sa-khan, to mention a few. Moreover, several plant drugs, especially those imported, are previously misidentified. Recent studies, however, have disclosed the true botanical origins of some crude drugs, for example, Kushta-so (Picheansoonthon *et al.*, 2002), Kushtakakra (Picheansoonthon and Jirawongse, 2003), Tian-yaowapanee (Picheansoonthon and Jirawongse, 2003), and Chan-daeng (Picheansoonthon and Vichia, 2004).

Krai-krue, is a crude drug frequently employed in Thai traditional herbal remedy. It is a dried and cut root with a characteristic odor and a bitter taste, known to exhibit sedative, muscle relaxant, antipyretic and anti-inflammatory activities (Pongboonrod, 1979; Sintusarn, 1999; Picheansoonthon *et al.*, 2001). Although this crude drug is available in most herbal dispensaries throughout Thailand, its botanical origin remains unknown/uncertain. Only three recently published Thai herbal literatures have described the botanical origin of Krai-krue, either as a dried root of an unknown species of the genus *Jasminum* in Oleaceae. (Picheansoonthon *et al.*, 2001), or of *Raphistemma pulchellum* (Roxb.) Wall. in Asclepiadaceae (Vuthitham-mavech, 1997; Sintusarn, 1999). The latter species, however, does not have a long root with characteristic features of Krai-krue. The true botanical origin and the identity of Krai-krue is, therefore, awaited to be unmasked.

MATERIALS AND METHODS

Plant materials

Three 100-200 g samples of Krai-krue were purchased from three different herbal dispensaries in Samphanthawong district of Bangkok (Tai-hua-jan drugstore, Jao-grom-per herbal store, and Vechapong Co., Ltd). The suppliers were contacted to trace back the

collecting localities in the deep forests in Uttaradit, Phrae, Lampang, Pitsanulok, Sukhothai, Loei, and Sakon Nakhon provinces of Thailand. These areas were revisited several times to observe the harvesting activities and to collect complete herbarium specimens in Sakon Nakhon and Loei provinces for botanical identification and root samples for macroscopic and microscopic observations. The identity of the plant drugs was re-checked with local harvesters and traditional healers. The herbarium samples were compared with specimens deposited in the Bangkok Forest Herbarium(BKF).

Histological analysis

The five Krai-krue samples, three of which were purchased and two were collected from the forest, were submitted for histological studies. The transverse sections were prepared using rotary microtome, following the technique described by Berlyn and Micksche (1976) with some modifications. The dried and hard crude drug samples bought from the herbal dispensaries were first soaked in a softening agent (40% hydrofluoric acid) for a few days prior to the cutting process. The microscopic sections and powdered drugs of these specimens were stained with Fast Green FCF and Safranin O. Permanent slides were investigated under bi-ocular microscope and the findings were compared. All crude drug samples were prepared for powdered drug studies following the method described in the Thai Herbal Pharmacopoeia (Department of Medical Science, Ministry of Public Health 1995) employing three different maceration techniques: the modified chloral hydrate techniques for an overview study, the modified Franklin's method for an investigation of secondary tissues, and the glycerin/water technique for a study of starch granules (Berlyn and Micksche, 1976).

Chemical analysis

Approximately 50 g of the three coarsely

ground Krai-krue samples and two air-dried roots (from natural habitats) were macerated in 300 ml 99 % methanol. The methanolic extracts were filtered and evaporated to dryness. Small portions of the dried crude extracts were dissolved in 99% methanol (and filtered, if necessary) for thin layer chromatography (TLC)

TLC was performed using pre-coated TLC aluminium sheets of silica gel 60 PF₂₅₄ and mixtures of five mobile phases, i.e., dichloromethane:methanol (90:10 v/v), ethylacetate:methanol (92:8 v/v), diethylether: methanol (99:1 v/v), benzene:acetone (55:45v/v), and benzene:methanol (99:1 v/v). Solvent fronts of the mobile phases were allowed to ascend 5 cm above the line of sample application. The loaded 6 samples were a standard mixture of aristolochic acid I and aristolochic acid II, three extracts of Krai-krue from Jao-grom-per herbal store, Tai-hua-jan drugstore, Vechapong Co., Ltd., and two dried roots from natural habitats. The chromatograms were observed under short (254 nm) and long (366 nm) ultraviolet wavelengths, and also stained with chromium sulfate reagent. The hR_f values were calculated, using the following equation.

$$hR_f = \frac{\text{distance of the band separated}}{\text{distance of solvent front}} \times 100$$

Statistical and phylogenetic analysis

Under microscopic observations, the sizes of starch grains, parenchyma cells, sclereids, corks, xylem fibres and xylem vessels, were measured. The data were recorded as the mean \pm SE and compared by one-way ANOVA (Jacob, 1988; Bolton, 1997; Wanitbancha, 2002). Significant ANOVA was followed by *post hoc* Scheffle multiple comparisons procedure. A p-value of <0.05 was considered significant. Moreover, the amount of cork cell layers and the compared thickness values of barks were also documented.

Distinct characters from macroscopic,

microscopic and chemical studies of all samples were analyzed. Phylogenetic tree was constructed based on the coefficient value (r) as indicated by the degree of similarity.

RESULTS AND DISCUSSIONS

Taxonomic identification

The herbarium specimens from the Sakon Nakhon and Loei forest were identified as *Aristolochia pierrei* Lec. and *Aristolochia tagala* Cham. in Aristolochiaceae (Figure 1), based on taxonomic keys and by comparing with voucher specimens from the BKF. The following plant description with the cited taxonomic literature was compiled based on all specimens investigated.

Specimens of A. pierrei Lec. (Figure 1 Left): BKF 101900, BKF 126964, BKF 99091, BKF 115043, BKF 02059, BKF 97615, BKF 52395, BKF 47024, BKF 05416, BKF 54447, BKF 55916, BKF 090688, BKF 091782, BKF 58365, BKF 095104, BKF 122386, BKF 114460, BKF 113773, 102868 and BKF 091781, Tiasakul 014-45, 016-45, 017-45 and 022-45, deposited at the Medicinal Plant Herbarium of Khon Kaen University.

Specimens of A. tagala Cham. (Figure 1 Right): BKF 121250, BKF 117176, BKF 98148, BKF 11245, BKF 22545, BKF 119240, BKF 103308, BKF 39952, BKF 105887, BKF 97617, BKF 97616, BKF 58229, BKF 125638, BKF 125640, BKF 19974, BKF 085431, BKF 41127, BKF 52830, and BKF 083597, including Tiasakul 006-45, 007-45, and 013-45, deposited at the Medicinal Plant Herbatrium of Khon Kaen University.

Histological analysis

:Macroscopic studies

The three Krai-krue specimens purchased from three different herbal dispensaries in Bangkok (Figure 2A-C), the dried root of *Aristolochia pierrei* Lec. (Figure 2D), and *A.*

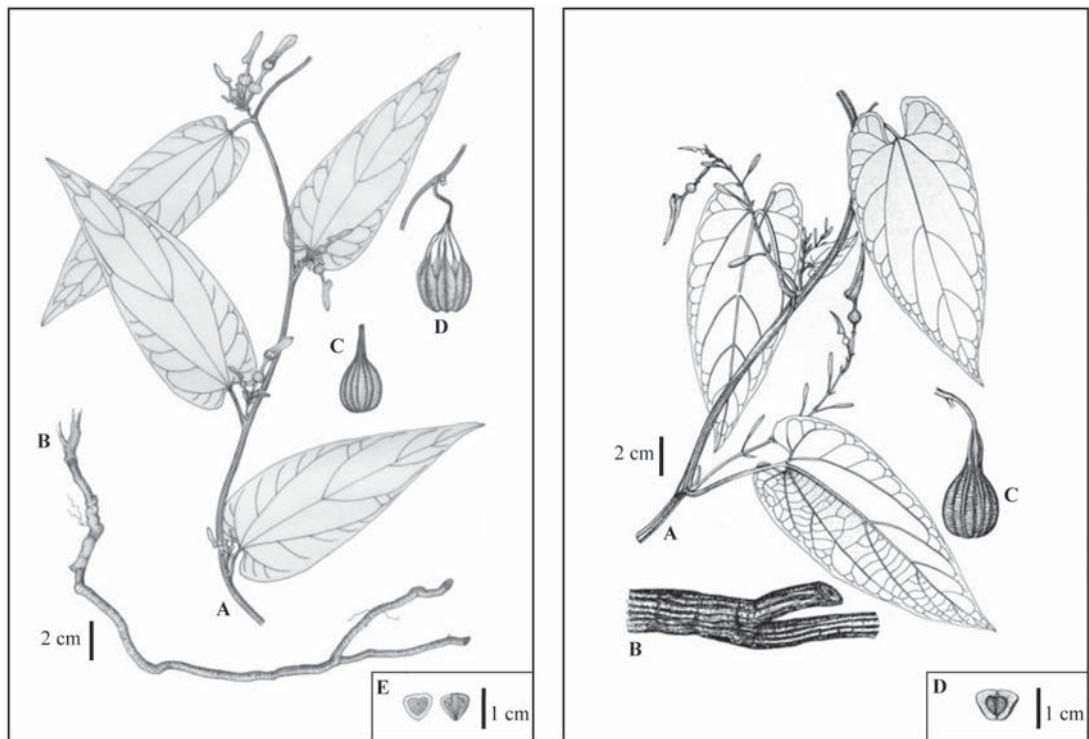


Figure 1 Left: Line drawing of *Aristolochia pierrei* Lec. (from Tiasakul 0015-45 and 024-45). A. a twig with leaves and inflorescences, B. part of a root, C-D. fruit, and E. seeds. Right: Line drawing of *Aristolochia tagala* Cham. (from Tiasakul 006-45 and 013-45). A. a twig with leaves and inflorescences, B. a part of the root, C. fruit; and D. seed.

tagala Cham. collected from Loei province of Thailand (Figure 2E), were morphologically alike (Table 1). All crude drugs were cylindrical or compressed-cylindrical, slightly tortuous, 3-10 cm long, 0.3-3.1 cm in diameter. Externally, they were yellowish brown to dark brown, rough and uneven, exhibiting longitudinal wrinkles and rootlet scars. The root texture was heavy and compact, but brittle. The surface fractured of sections dried roots were uneven with yellowish to light brown bark, broad wood, radial-arranged of medullary rays, and distinct yellowish-brown cambium rings. The fragrant smell and bitter taste are characteristic.

Macroscopic features, as shown in Table 1, indicated clear morphological similarities for all studied crude drugs. Medullary rays could be

seen in the segmented surface view of five samples. The numbers and morphology of medullary rays were different ranging from 4 to more than 10. Medullary rays of the roots of *A. tagala* Cham. formed a larger band compared with other samples. However, the number of radial-arranged of medullary rays and the colour were varied possibly due to the age of the root collected, and the position (along the root) cut, and also to the post-harvesting process, drying and storage. Moreover, pores of all samples are also different in numbers, scattering patterns, and sizes. The pores of *A. pierrei* Lec., *A. tagala* Cham. and Krai-krue roots purchased from Jao-grom-per herbal store were evenly scattered and abundant.

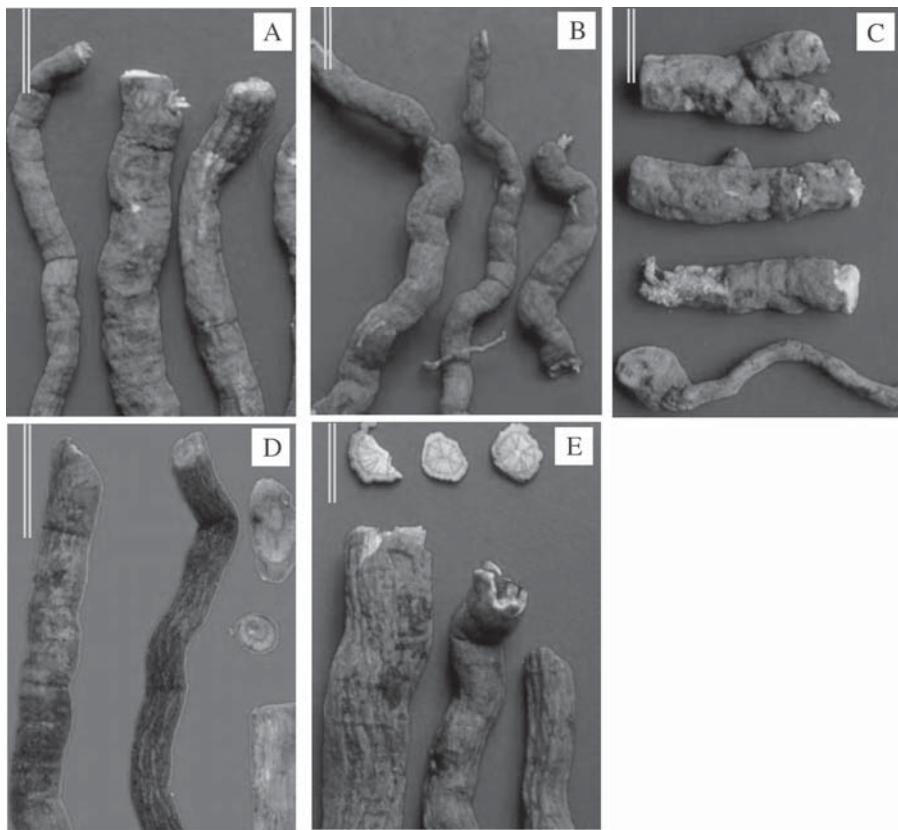


Figure 2 Macroscopic characteristics of Krai-krue samples; 1 scale unit in each illustration equal 2 cm.

- A. samples from Jao- grom-per herbal store
- B. samples from Tai-hua-jan drugstore
- C. samples from Vechapong Co., Ltd.
- D. sample collected from Sakhon Nakhon province
(*A. pierrei* Lec.)
- E. sample collected from Loei province
(*A. tagala* Cham.)

Microscopic studies

The transverse section

The cross sections of all crude drug samples (the roots of *Aristolochia pierrei* Lec., *A. tagala* Cham. collected from the wild, and the purchased samples) exhibited similar microscopic features. The outer layers were composed of several rectangular sclereid cork cells. The cortex appears as a zone of thin-walled parenchymatous cells containing starch granules and sclerified cells.

Stone cells were evenly scattered as stripes in the inner layer of cork cells and the outer part of cortex layer near the phellogen, which were only found in the roots of *A. tagala* Cham. In addition, stone cells of the root sample of *A. pierrei* Lec., Krai-krue sample from Tai-hua-jan drugstore and the sample purchased from Vechapong Co.,Ltd. were found densely scattered as single, pair, and/or crowned in the cortex and the stele, while those of the roots of *A. tagala* Cham. were not abundant.

Table 1 Comparative macroscopic features of Krai-krue, (A) = sample from Jao-grom-per herbal store; (B) = sample from Tai-hua-jan drugstore; and (C) = sample from Vechapong Co., Ltd., (D) = *A. pierrei* Lec. and (E) = *A. tagala* Cham. roots.

Features	Krai-krue (A)	Krai-krue (B)	Krai-krue (C)	Root of <i>A. pierrei</i> Lec.(D)	Root of <i>A. tagala</i> Cham.(E)
Diameter (cm)	0.3-1.3	0.4-1.5	0.5-2.2	0.3-1.2	0.4-3.1
Number of rays	>10	>10	>10	4-15	4-10
Colour	yellowish brown	brown	dark brown	dark brown	dark brown
Longitudinal wrinkles	present	present	present	present	present
Texture	hard and brittle	hard and brittle	hard and brittle	hard and brittle	hard and brittle
Fracture	uneven; bark, light brown; wood, bright yellow with light brown	uneven; bark, light brown; wood, light yellow with dark yellowish	uneven; bark, dark brown; wood, bright yellow with light brown	uneven; bark, dark brown; wood, light yellow with dark yellowish	uneven; bark, dark brown; wood, yellowish white with light- brown branched ray
Rootlet scars	present	present	present	present	present

The size of cork cells in the cork layers (ranging from 5-30 layers) was similar in all samples. The ratio of cortex region and stele region could be classified into three groups: group one ; cortex region<stele region root found in *A. tagala* Cham. [2:5], the sample from Tai-hua-jan drugstore [4:7], and the sample from Vechapong Co., Ltd [2:3]) : group two; cortex layer=stele region root found in *A. pierrei* Lec. [1:1] and group three; cortex region>stele region found in the sample from Jao-grom-per herbal store [4:3]). However, these ratios may be varied significantly due to aging and the cutting position of the roots.

Branched or band of trachea elements and fibrous tissues of xylem tissues in the root samples of *A. tagala* Cham., *A. pierrei* Lec., and in the Krai-krue sample from Jao-grom-per herbal store were distinctly furrowed. The sample from Tai-hua-jan drugstore and the sample from Vechapong Co., Ltd. were, however, seen only as a long band of xylem tissues. The furrowed or

banded of xylem tissues may be also due to the age and the cutting position of the samples.

The stele consisted of non-distinguishable phloem tissues and a broad zone of secondary xylem tissues. The secondary xylem was composed of large pore vessels, xylem fibers, and xylem parenchyma cells. In all samples, 5 or more 20 tapes were found in radial-arranged of medullary rays. These rays were rather large, and composed of non-lignified parenchyma cells containing starch granules. The piths of all samples were mostly observed as non-lignified parenchyma cells containing compressed starch grains and derived from the extension of primary xylem.

Furthermore, pores were also observed at macroscopic level, as xylem vessels. Pores in the roots of *A. tagala* Cham. were, however, the largest. In contrast, smaller pores appeared as scalariform lines were observed in Krai-krue samples purchased from Tai-hua-jan drugstore and those purchased from Vechapong Co., Ltd. In

addition, vascular cambiums were distinct in the root samples of *A. tagala* Cham. The cross section views of all crude drugs are shown in Figure 3.

The anomalous type (large xylem tissues with tiny phloems) of medullary rays is an important character justifying their similarity. Although the rays may be found separated by a vascular cambium, this may not always be the case. This anomalous structure was axed with lobed or furrowed xylem, which obviously affected the appearance of medullary and/or xylem rays. In addition, cell division of xylem tissues may occur more than phloem tissues in plants growing in arid environment especially among climbing herbs in

the tropical forest (Metcalfe and Chalk, 1985 and 1987; Mauseth, 1991; Huber, 1993; Esau, 2002; Junsongduang, 2003).

The powdered drugs

The powdered drugs of the five crude drug samples were also resembled. Both simple and compound starch granules, spherical to ovoid shape, usually with a well-marked hilum, were found in abundance. Sclereids with irregularly thickening wall and simple pit were also common. These sclereids were isodiametric or elongated in shape, but occasionally occurred with stone cells. The fragments of cork tissue with polygonal (surface view), rectangular (section view), dark

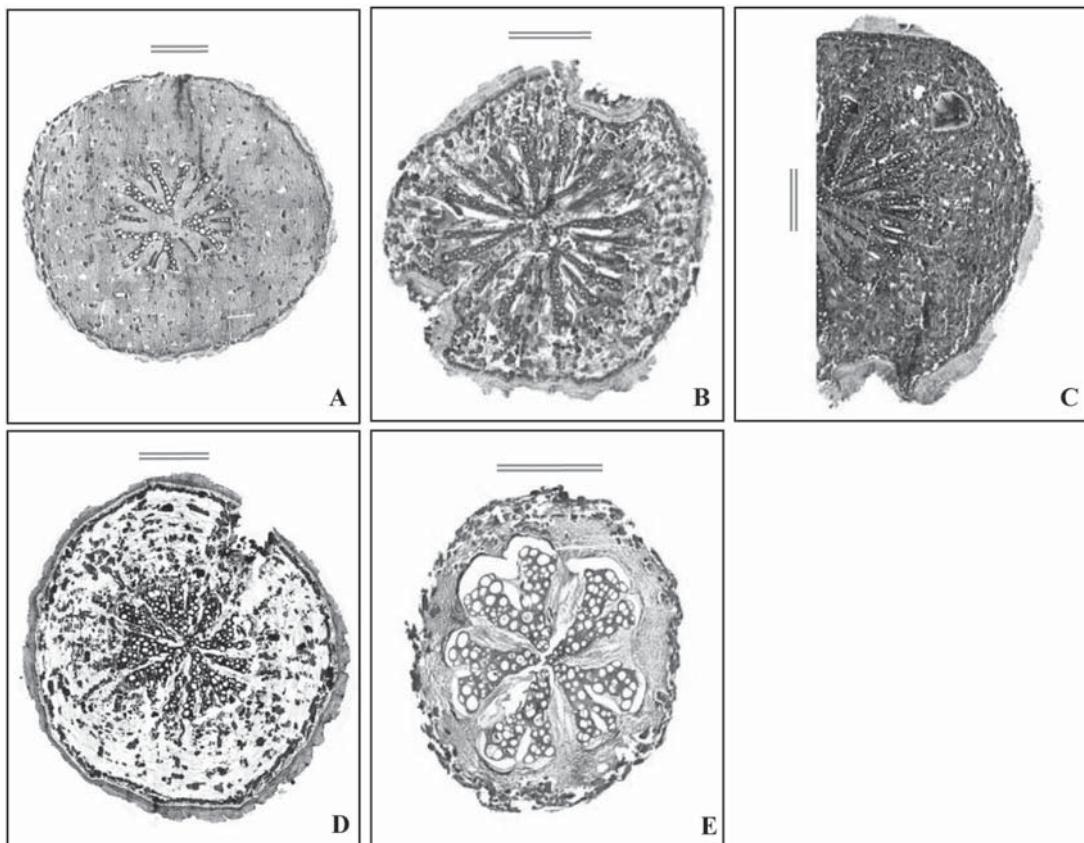


Figure 3 Photographic images of transverse sections of the Krai-krue samples from different sources. A. from Jao-grom-per herbal store, B. from Tai-hua-jan drugstore, C. from Vechapong Co., Ltd., D. root of *Aristolochia pierrei* Lec. and E. root of *A. tagala* Cham. (bar equivalent to 2 mm, x50).

brownish cells were observed. The large vessels with elongated pits occurred either singly or in small groups associated with other xylem elements. Moreover, vessels, both with and without tail, were also observed. Single and groups of unseptate fibres, with large and small simple pits, were mostly with thickening and lignified wall. The powdered samples of all crude drugs are shown in Figure 4.

Statistical analysis

Table 2 demonstrated the mean \pm standard error (SE), statistic significance among

means of more than two groups as determined by one-way analysis of variance (ANOVA).

Diameter of parenchyma and starch grain were not significantly different among groups of all samples ($p=0.057$ and $p=0.137$). Statistical significant difference ($p<0.05$) in mean width and mean length of cork and xylem vessel was, however, observed in all samples. The widths of xylem fibre and sclereids were also significantly different in all samples ($p<0.05$), but the lengths were not significantly different ($p=0.528$ and $p=0.227$).

The mean sizes of parenchyma, starch

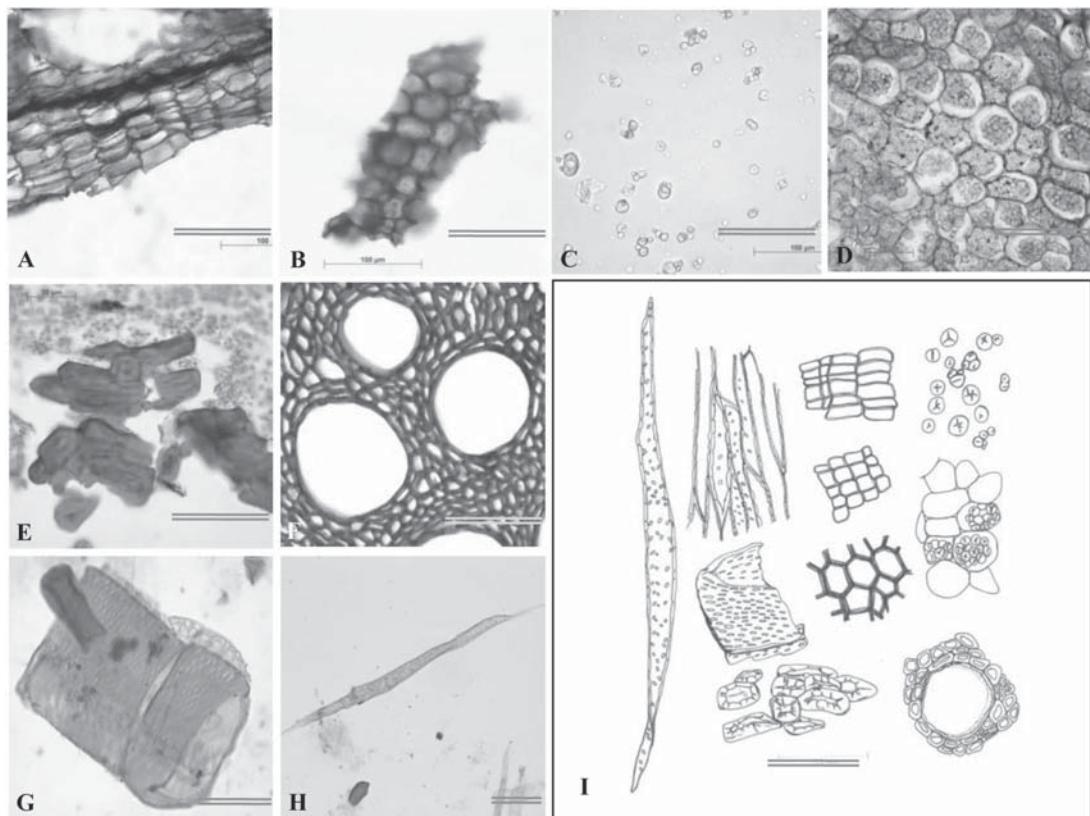


Figure 4 Microscopic images identification of Krai-krue samples (each scale unit equivalent to 100 μm). A. a fragment of corks in transverse view (x400), B. a fragment of corks in surface view (x400), C. simple and compound starch granules (x400), D. parenchyma cells containing starch granules (x400), E. stone cells (x400), F. a group of xylem tissues in transverse section (x400), G. a large pitted vessel (x400), H. a fibre (x200) and I. illustrated line-drawings of powdered drugs (x400).

grain, sclereid, cork, xylem fibre, and xylem vessel size from all samples were not affected by the external factors such as ages of the roots collected, sites and season of harvesting, and/or post-harvesting processes. Also, the data was scattered in an abnormal curve. Therefore, using ANOVA one-way parameter as an indicator for statistical difference among groups may be inappropriate.

In significantly different cases, the data was further analyzed by the *post hoc* Scheffe multiple comparison procedure. A *p*-value of <0.05 was considered significantly different between each pair. The width and length of corks were not significantly different within groups of the four samples obtained from Jao-grom-per herbal store, Tai-hua-jan drugstore, Vechapong Co., Ltd. and the roots of *A. pierrei* Lec. ($p>0.05$). The width of fibres and xylem vessels were also not significantly

different among these four samples ($p>0.05$). However, no significant difference was observed in the lengths of fibres and xylem vessels, including those of the root of *A. tagala* Cham. ($p>0.05$). In addition, the lengths of sclereid cells were not significantly different within groups of all samples ($p>0.05$). The widths of sclereid cells were not significantly different within all samples ($p>0.05$), except the roots of *A. tagala* Cham. purchased from Tai-hua-jan drugstore ($p<0.05$).

From these statistical evidences, the five samples appeared to be significantly different, ($p<0.05$) as seen from the width of corks, length of corks, width of xylem vessels, length of xylem vessels, width of xylem fibres, width of sclereids, but not significantly different between groups ($p>0.05$), in the size of parenchyma and starch grains, length of xylem fibres and sclereids.

Table 2 Comparison of microscopic characters (SE \pm) of the samples in sectional views and in the powdered form.

Measurement (mm, n=20)	The commercial Krai-krue			Roots of <i>A. pierrei</i> Lec.	Roots of <i>A. tagala</i> Cham.	Sig.
	Jao-grom- per herbal store	Tai-hua-jan drugstore	Vechapong Co., Ltd.			
Diameter of parenchyma cell in cortex layer	65.09 \pm 2.21	60.34 \pm 2.25	57.69 \pm 2.80	65.58 \pm 2.95	57.55 \pm 2.34	0.057
Diameter of starch grain	11.43 \pm 0.83	10.37 \pm 0.80	11.27 \pm 1.03	8.77 \pm 0.41	10.93 \pm 0.82	0.137
Sclereid	w	39.35 \pm 3.21	47.21 \pm 3.04	41.41 \pm 2.50	39.22 \pm 3.11	30.06 \pm 1.94
	l	70.95 \pm 6.28	81.01 \pm 6.49	75.59 \pm 7.52	79.45 \pm 6.06	61.79 \pm 5.76
Cork	w	26.54 \pm 2.11	25.43 \pm 1.62	29.23 \pm 1.51	27.76 \pm 1.15	17.87 \pm 0.92
	l	49.59 \pm 2.32	45.03 \pm 2.44	49.79 \pm 2.14	43.28 \pm 1.99	34.48 \pm 2.08
Xylem fibre	w	18.43 \pm 1.54	20.57 \pm 1.91	18.17 \pm 1.27	17.89 \pm 0.66	27.43 \pm 2.31
	l	596.27 \pm 31.61	576.37 \pm 20.54	560.67 \pm 24.25	567.11 \pm 24.08	620.35 \pm 33.06
Xylem vessel	w	87.98 \pm 6.40	70.43 \pm 5.46	105.07 \pm 8.61	88.24 \pm 7.49	180.08 \pm 11.23
	l	164.28 \pm 10.48	146.80 \pm 9.36	152.87 \pm 10.03	189.69 \pm 9.83	151.85 \pm 12.67
Amount of cork cell layers		8-16	18-25	25-30	5-27	5-15

w=width, l=length.

Moreover, there was no significant difference in total cells analysis between the four Krai-krue samples obtained from Jao-grom-per herbal store, Tai-hua-jan drugstore, Vechapong Co., Ltd., and the roots of *A. pierrei* Lec. at 95% confident interval level ($p>0.05$).

Chemical analysis

TLC chromatograms

The resulting thin-layered chromatograms of all samples are shown in Figure 5. They were resembled in five solvent systems. The presence of aristolochic acids was also clearly

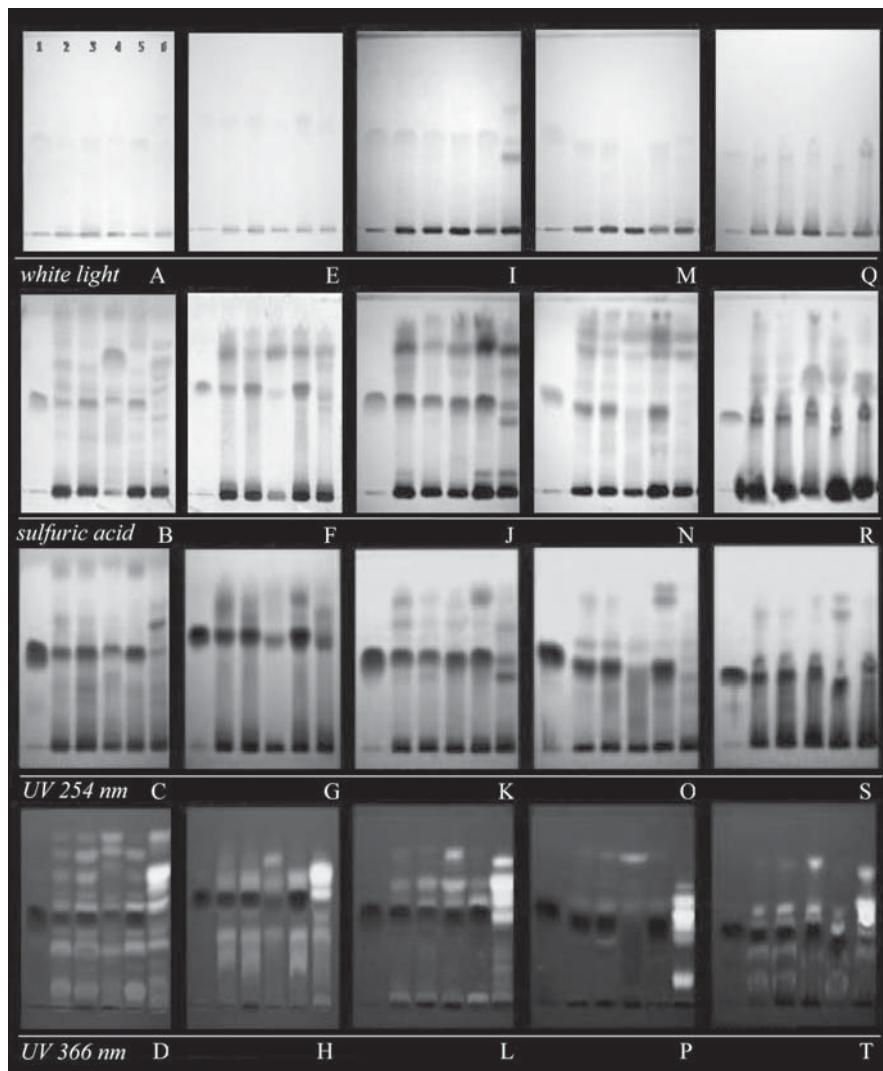


Figure 5 Thin layer chromatograms of the crude drug samples in mobile phase. Lane 1: standard mixture of aristolochic acid I and aristolochic acid II. Lanes 2-4: extracts of the crude drug Krai-krue from Jao-grom-per herbal store, Tai-hua-jan drugstore, and Vechapong Co., Ltd., respectively. Lanes 5-6: dried root extracts of *A. pierrei* Lec. and *A. tagala* Cham., respectively. A-D, dichloromethane: methanol (90:10); E-H, ethylacetate: methanol (92:8); I-L, diethylether: methanol (99:1); M-P benzene: acetone (55:45); and Q-T, benzene: methanol (99:1).

demonstrated. The TLC results indicated that the three Krai-krue samples from Jao-grom-per herbal store, Tai-hua-jan drugstore and Vechapong Co., Ltd. are the same as two dried roots of *A. pierrei* Lec. and *A. tagala* Cham. from the natural habitats. However, the results also revealed the common presence of aristolochic acid derivatives in two dried roots of *A. pierrei* Lec. and *A. tagala* Cham. which could be used as a phytochemical identification.

In addition, it is worth noting that there are two morphologically alike plants, namely, *Aristolochia* crude drugs commonly used in Chinese herbal medicine: *Aristolochia fangchi* Y.C.Wu ex L.D.Chou et S.M.Hwang (Radix Aristolochiae Fangchi) and *A. debilis* Sied. et Zucc. (Radix Aristolochiae), both of which are the official names assigned in the Pharmacopoeia of the People's Republic of China (1997). These two plant drugs are known to cause nephrotoxicity (Nortier *et al.*, 2000; U.S. FDA., 2000) due to aristolochic acids and derivatives, commonly found in this genus. The possible toxicity of these herbal plants and, hence, Krai – krue itself, should be warned to the consumer and drug manufacturers.

Phylogenetic analysis

Figure 6 showed the phylogenetic tree based on the similarity index, macroscopic and microscopic features, TLC fingerprints, the presence and absence characters. From the dendrogram, similarity coefficients among the five samples were 0.64-0.98 and the samples could be clustered into two groups: 1. the roots sample of *A. pierrei* Lec., Krai-krue from Jao-gromper herbal store, Krai-krue from Tai-hua-jan drugstore, and Krai-krue purchased from Vechapong Co., Ltd (0.94£r0.98); and 2. the roots sample of *A. tagala* Cham. ($r=0.64$). This is the first report on the identity of Krai-krue based on the collective data as related to *A. pierrei* Lec.

CONCLUSIONS

Krai-krue is one of the crude drug commonly employed in Thai traditional herbal medicine. It is available in herbal dispensaries nationwide in the form of hard cut roots. The botanical origin of this plant drug is so far unknown.

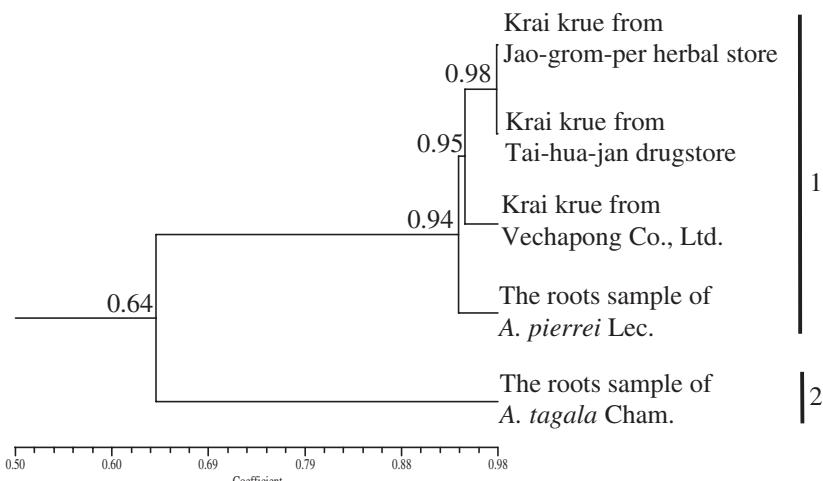


Figure 6 The dendrogram established from the thin-layered chromatograms, macroscopic and microscopic features of all samples.

To unmask the plant origin of Krai-krue, the crude drug samples were purchased from three herbal dispensaries in Bangkok. The herbs were traced back to their original collecting sites in the deep forests of Loei, and Sakon Nakhon provinces of Thailand. The identity of Krai-krue was reconfirmed with the locals and traditional healers in the implemented areas. The herbarium specimens collected from these sites are of the same plant, and identified as *Aristolochia pierrei* Lec. and *A. tagala* Cham. (Aristolochiaceae). These plants are scarcely found in the natural environment and could not meet the high demand in the future. Artificial cultivation is, therefore, needed to increase the yield and active constituents for sustainable uses.

The morphologies of Krai-krue samples from three herbal drugstores in Bangkok; roots of *Aristolochia pierrei* Lec. and *A. tagala* Cham. from the forest were comparatively similar. The microscopic investigations of these samples indicated that they may be originated from the same plant genus. Furthermore, studies on thin-layered chromatographic of these crude drugs referred the botanical origin of Krai-krue to *Aristolochia pierrei* Lec. The similarity coefficient, macroscopic and the microscopic features, and the TLC fingerprint patterns indicated that Krai-krue from Jao-gromper herbal store, Tai-hua-jan drugstore, and Vechapong Co., Ltd; and the roots sample of *A. pierrei* Lec. are closely related and could be grouped in the same cluster, while the root sample of *A. tagala* Cham. belongs to a different cluster.

ACKNOWLEDGEMENTS

This research project was partly supported by the grants from the Graduate School, the Faculty of Pharmaceutical Sciences, Khon Kaen University. We are grateful to Mrs. Leena Phuphathanaphong of the Bangkok Forest Herbarium for her valuable discussion and

suggestions on the genus *Aristolochia*; Mr. Piya Mokkamul (Khon Kaen University) for photographs of the crude drugs and Professor Shoyama from the Faculty of Pharmaceutical Science, Kiushu University, Japan, to support standard mixture of aristolochic acid I and aristolochic acid II.

LITERATURE CITED

Berlyn, G.P. and J.P. Miksche. 1976. **Botanical and Microtechnique Cytochemistry**. The Iowa University Press, Ames, Iowa. 326 p.

Bolton, S. 1997. **Pharmaceutical Statistics: Practical and Clinical Applications**. 3rd ed. Marcel Dekker, New York. 737 p.

Department of Medical Science, Ministry of Public Health. 1995. **Thai Herbal Pharmacopoeia**. Vol I. Prachachon Co., Ltd., Bangkok. 140 p.

Department of Medical Sciences, Ministry of Public Health. 2000. **Thai Herbal Pharmacopoeia** Vol II. Prachachon Co., Ltd., Bangkok. 152 p.

Esau, K. 2002. **Plant Anatomy**. 2nd ed. Rajendra Ravindra, New Delhi, India. 767 p.

Huber, H. 1993. Aristolochiaceae. pp. 129-137. In K. Kubitzki, J.G. Rohwer and V. Bittrich (eds.). **The Families and Genera of Vascular Plants**. Springer -Verlag, Berlin, Germany.

Jacob, C. 1988. **Statistical Power Analysis for the Behavioral Sciences**. 2nd ed. Department of Psychology, New York University, New York. 567 p.

Junsongduang, A. 2003. **Comparative Anatomy of Aristolochiaceae in Thailand**. M.S. Thesis. Khon Kaen University, Khon Kaen.

Mauseth, J.D. 1991. **Botany an Introduction to Plant Biology**. 2nd ed. Saunders College Publishing, Philadelphia. 75 p.

Metcalfe, C.R. and L. Chalk. 1979. **Anatomy of the Dicotyledons: Leaves, Stem, and Wood in Relation to Taxonomy with Notes on Economic Uses**. 1st ed. The Clarendon,

Oxford, Great Britain. 276 p.

Metcalfe, C.R. and L. Chalk. 1985. **Anatomy of the Dicotyledons: Wood Structure and Conclusion of the General Introduction.** 2nd ed. Vol 2. The Clarendon, Oxford, Great Britain. 297 p.

Nortier, J.L., C.A. Bieler and D. Abramowicz. 2000. Urothelial carcinoma associated with use of a Chinese herb (*Aristolochia fangchi*). **N. Engl. J. Med.** 342(23): 1686-1692.

Picheansoonthon, C., M. Chawalit and V. Jiravongse. 2001. **An Explanation of King Narai Remedies: The Special Edition Commemorated the King 72 Birthday Anniversary (December 5, 1999).** Ammarin Printing and Publishing Co., Ltd. (Public Company), Bangkok. 777 p.

Picheansoonthon, C., S. Tiyaworantan and V. Jirawongse. 2002. Botanical origin of Kushta So. **Pharmacop. News** 9(1): 1-10.

Picheansoonthon, C. and V. Jirawongse. 2003. Origin of the word "Kushta" and Kushta used in Thai herbal remedies. **J. Roy. Inst. Thai.** 28(1): 118-124.

Picheansoonthon, C. and V. Jirawongse. 2003. The botanical origin of Tian yaowapanee. **J. Roy. Inst. Thai.** 28(3): 746-756.

Picheansoonthon, C. and N. Vichai. 2004. The botanical origin of Chan-daeng. **J. Roy. Inst. Thai.** 29(1): 25-34.

Pongboonrod, S. 1979. **Thai and Exotic Plants- Indications for Thai and Exotic Herbs .** Kasem Bannakich Book Store, Bangkok. 600 p.

Sinthusarn, U. 1999. **Herbs in Jao-grom-per Herbal Store.** 1st ed. Thammasarn Printing Company, Bangkok. 344 p.

The Pharmacopoeia Commission of the People's Republic of China. 1997. **Pharmacopoeia of the People's Republic of China.** Vol 1. Chemical Industry Press, Beijing. 545p.

U.S. FDA. 2000. **Letter to health care professionals FDA concerned about botanical products, including dietary supplements, containing aristolochic acid.** Retrieved 2002, Dec 23, from <http://www.cfsan.fda.gov/~dms/ds-bot12.html>

Vuthithammavech, V. 1997. **Encyclopedia of Herbs .** O.S. Printing House, Bangkok. 618 p.

Wanitbancha, K. 2002. **Data Analysis with SPSS for Window .** 5th ed. Chulalongkorn University Publishing, Bangkok. 631 p.