# CHAPTER II
# LITERATURE REVIEWS

## 2.1 Plant samples

### 2.1.1 *Aristolochia* spp

#### 2.1.1.1 Morphology of *Aristolochia* plants

*Aristolochia* plants belong to Aristolochiaceae family or birthwort family or Krachauseeda family in Thai. The taxonomic description of *Aristolochia* was reported by Leena Phuphathanaphong (1987) as follows:

"Prostrate, scrambling, climbing herb or woody climber. Leaves entire or 3-lobed, palmately or pinnately nerved. Flowers solitary, racemose or paniculate, irregular; perianth basally expanded into the utricle, then contracted into a slender tube; limb 1-2 lipped. Ovary slightly 6-angular and 6-locular; ovules many in each cell. Stamens 6, adnate to the style to form a gynostemium. Stigmatic lobes 3 or 6. Capsule septicidal, dehiscing from base upwards, 6-valved. Seeds numerous, flat, winged or not, verrucose."

According to Tem Smitinand (2014), there are 22 species of *Aristolochia* found in Thailand as shown in Table 1.

Table 1 *Aristolochia* plants found in Thailand.

| No. | Scientific name | Local name |
|---|---|---|
| 1 | *Aristolochia arenicola* Hance | กอมก้อยลอดขอน (ตะวันออกเฉียงเหนือ) |
| 2 | *A. baenzigeri* B.Hansen & Phuph. | กระทุงบวบเหลี่ยม |
| 3 | *A. curtisii* King ex Gamble | กระเช้าภูเก็ต |
| 4 | *A. dinghoui* Favio González & O. Poncy | นกกระจิบ (ประจวบคีรีขันธ์) |
| 5 | *A. grandis* Craib | นกขมิ้น (เหนือ) |
| 6 | *A. hansenii* Phuph. | กระเช้าเชียงราย. |
| 7 | *A. helix* Phuph. | กระเช้าหนู (ใต้) |
| 8 | *A. gigantea* Mart. | ไก่ฟ้าใหญ่ (กรุงเทพ), Brazillian Dutchmant's Pipe, Giant Pelican Flower |
| 9 | *A. kerrii* Craib | กระเช้าปากเป็ด (เหนือ), เครือไก่น้อย (เชียงใหม่) |
| 10 | *A. kongkandae* Phuph. | กระเช้าคลองพนม |
| 11 | *A. labiata* Willd.<br>syn. *A. brasiliensis* Mart. & Zucc. | นกกระทุง (กลาง), Mottled Dutchmant's Pipe, Rooster Flower |
| 12 | *A. littoralis* Paradi.<br>syn. *A. elegans* Mast. | นกกระทุง (เชียงใหม่), นกกระทุงปากบาน, เหนียงนกกระทุง (กรุงเทพมหานคร), Calico flower |
| 13 | *A. longeracemosa* B.Hansen & Phuph. | ไก่แจ้ (เหนือ) |
| 14 | *A. macrophylla* Lam.<br>syn. *A. durior* Hill | นกกระทุงใหญ่ (กลาง), Broadleafed Birthwort |
| 15 | *A. perangustifolia* Phuph. | กระเช้าใบแคบ (general) |
| 16 | *A. pierrei* Lecomte | กระเช้าผีมด (เชียงใหม่), หนอนตาย (กลาง) |
| 17 | *A. poomae* Phuph. | กระเช้านกกระสา (general) |
| 18 | *A. pothieri* Pierre ex Lecomte<br>syn. *A. siamensis* Craib | กระเช้าถุงทอง (กลาง) |
| 19 | *A. ringens* Vahl | ไก่ฟ้า (กรุงเทพมหานคร), Gaping Dutchman's Pipe, Pelican flower |
| 20 | *A. tagala* Cham. | กระเช้าผีมด, กระเช้ามด, กระเช้าสีดา (กลาง), ปุลิง (เชียงใหม่), หาบกะเชอ (ขอนแก่น), Indian Birthwort |
| 21 | *A. versicolor* S.M. Hwang | กระทุงขน (เหนือ) |
| 22 | *A. yalaensis* Phuph. | กระเช้ายะลา (general) |

Among these *Aristolochia* plants, seven of them including *A. gigantea* Mart., *A. kerrii* Craib, *A. littoralis* Paradi., *A. pierrei* Lecomte, *A. pothieri* Pierre ex Lecomte, *A. ringens* Vahl and *A. tagala* Cham. have been chosen as plant materials in this study because they are widely used as medicinal plants in many countries (Heinrich, Chan et al. 2009). In addition, another three *Aristolachia* species, *A. tentaculota* Schmidt in Fedde, *A. grandiflora* Sw. and *A. anguicida* Jacq., were also included in this investigation. The other species are excluded because they are very rare or completely absent. The botanical descriptions of *A. kerrii* Craib, *A. littoralis* Paradi., *A. pierrei* Lecomte, *A. pothieri* Pierre ex Lecomte, *A. ringens* Vahl and *A. tagala* Cham. were reported by Leena Phuphathanaphong (1987) whereas those of *A. anguicida* Jacq., *A. gigantea* Mart., *A. grandiflora* Sw. and *A. tentaculata* Schmidt in Fedde were reported as follows (Pfeifer 1966):



1) ***A. kerrii* Craib** (Aristolochiaceae) is called as "กระเช้าปากเป็ด".

"Climber, stem glabrous. Leaves: petioles *2.3-3.2* cm, glabrous; lamina ovate-lanceolate, triangular, triangular-ovate to broadly ovate, 5.2-7.4 by *4.5-6.6* cm, base truncate or ± cordate, margin entire, apex acute to acuminate, glabrous above, puberulous below, gland-dotted on both sides, palmately 5-nerved, venation finely reticulate, obscure above, prominent below. Inflorescences axillary, fascicled racemose 1.5-3 cm long, puberulous; bracts lanceolate, 0.5-1.2 by 0.2-0.5 cm longitudinally veined, densely gland-dotted. Flowers with 0.*8-1.1*cm pedicel and ovary 2-3 by 0.5-1 mm. Perianth 1.3-2 cm, purple, inside cream; utricle spherical or ovoid, 2.9-4.7 by 2.7-4.1 mm, tube 3.1-7.5 by 1.3-2 mm, bent on transition from utricle, limb sagittate, 0.7-1.4 by 0.4-1.2 cm, Anthers ellipsoid, 0.5-0.6 by 0.8-1 mm. Gynostemium 0.8-1.8 by 1.2-3.5 mm, stigmatic lobes 6, short, obtuse. Fruit ovoid, 1.8-2.2 by 1.6-1.8 cm;

pedicel 1.8-2.2 cm. Seeds obovate, flat, not winged, 4-4.5 by 2.3-2.6 mm, verrucose on both sides."

2) ***A. littoralis*** **Paradi.** (Aristolochiaceae) is called as "เหนียงนกกระทุง".

"Woody climber, stem glabrous. Leaves with pseudo-stipules, leaf-like, amplexicaul, ± 1.2 by 2 cm; petiole 3.5-4 cm, slender, glabrous; lamina triangular 4-5.5 by 6-7 cm, base cordate, margin entire, apex rounded or obtuse, glabrous on both sides, but young leaves pubescent, palmaterly finely 3-nerved, venation reticulate, obscured. Flowers solitary, large; pedicel and ovary 7-8.5 cm. Perianth white or greenish with purple-black mottlings; utricle obliquely ellipsoid, 2.5-4 by 1.2-1.5 cm, tube bent upwards, 1.2-2 by ± 0.5 cm, limb orbicular, 5-7 cm diam. Anthers linear, ± 4 by 1 mm. Gynostemium ± 5.8 by 4.5 mm, stigmatic lobes 6, flattened, outside apically pubescent, with margins recurved outwards. Fruit not seen."



3) ***A. pierrei*** **Lecomte** (Aristolochiaceae) is called as "หนอนตาย".

"Climber, stem glabrous. Leaves: petioles 0.9-1.5 (-3.5) cm, grooved above, hairy along edges, glabrous beneath; lamina lanceolate to broadly lanceolate, 8.5-13.8 by 2.8-4.6 cm, base cordate, margin entire, slightly recurved, hairy above, apex acute or tapering acute, uper surface finely pubescent, lower surface puberulous, with scattered gland-dots on both sides, palmately 5-nerved, lateral nerves 3-4, venation reticulate, conspicuous above, prominent below. Inflorescences racemose, 3.5-7 cm, tomentose; bracts lanceolate or narrowly lanceolate, 0.8-1.2 by 0.35-0.45 cm both sides hairy. Flowers with 0.4-1 cm pedicels, slender, ovary 3.7-4.5 by 0.5-1 mm. Perianth 2-3.5 cm, utricle spherical or ovoid, 3.5-4.5 by 3-4 mm, purple inside, tube 5-8.5 by ± 1 mm, greenish light brown, limb oblong, 1-1.5 by 0.45-0.55 cm, purplish-brown. Anthers

oblong ± 0.4 by 0.2 mm. Gynostemium 1.8-2.1 mm, stigmatic lobes 6, long conical, obtuse. Fruit ovoid, 2-2.5 by 1.8-2 cm; pedicel 3.2-4.5 cm. Seeds broadly obcordate or triangular, winged, 4.7-5.1 by 5.5-6 mm, verrucose on both sides."

4) ***A. pothieri*** **Pierre** ex Lecomte (Aristolochiaceae) is called as "กระเช้าถุงทอง".

"Climber, stem laxly adpressed puberulous. Leaves: petiole 3.5-5.2 cm, puberlous; lamina entire or 3-lobed, broadly ovate, lobes less than half the length of the lamina, 11-11.5 by 12-13.6 cm, base deeply cordate, apex acute, acuminate or obtuse, lateral lobes obtuse, laxly pubescent and gland-dotted on both sides, palmately 3-nerved with transverse nerves conspicuous above and prominent beneath. Inflorescences axillary panicles, 6 cm, densely puberulous; bracts ovate or lanceolate, ± 1 by 0.5 mm. Flowers with pedicels 6-7 mm, ovary 6-8 by 1-1.2 mm, densely pubescent. Perianth 2.4-4.4 cm, brown, purplish, reddish, straight; utricle spherical or ovoid, 3.5-7.5 by 3.3-4 mm; tube 0.8-1.6 by 0.1-0.2 cm; limb obovate, oblong or spathulate, 1.3-1.8 by 0.5-0.7 cm. Anthers ellipsoid, 0.7-0.9 by 0.7-0.9 mm. Gynostemium 2-2.2 by 2.7-3 mm, stigmatic lobes 6, conical, obtuse. Fruit ovoid, 4-4.5 by 2.5-3 cm; pedicel 5-6 cm. Seeds triangular-obcordate, thin, winged, 7.5-8 by 6.7-7 mm, verrucose on one side the other side smooth, brown."

5) ***A. ringens*** **Vahl** (Aristolochiaceae) is called as "ไก่ฟ้า".

"Climber, stem glabrous; the whole plant gland-dotted. Leaves with pseudo-stipules, leaf like, reniform, unequal, the bigger one 1.5-2.2 by 2-3 cm, the smaller on 9.6-1.5 by 1-2 cm; petiole 3-8 cm, slender, glabrous; lamina reniform 5-10 by 7.5-14.5 cm, base deeply cordate, margin entire, apex rounded, ± cuspidate, glabrous,

but young leaves slightly pubescent, palmately nerved, nerves branched near the margin, venation reticulate. Flowers solitary, large, dark red or dark purple; pedicels ± 6.5 cm, ovary 2.5-4.5 by 0.1-0.2 cm. Perianth 17.5-27 cm, utricle obliquely ellipsoid, 4-7.5 by 3-4 cm; tube bent upwards, 2-2.5 by 0.6-0.8 cm; limb 2 lipped, upper lip spathulate, 4.5-6 cm, reticulate, lower lip oblong-lanceolate, 9-15 cm, apex obtuse. Anthers linear, 5.5 by 0.8 mm. Gynostemium ± 8 by 5 mm, stigmatic lobes 6, long conical, obtuse. Fruit oblong, 7-7.5 by 3-3.5 cm; pedicel 10-12 cm, strongly 6-ribbed. Seeds including wing obovate 9-12 by 5-7.5 mm, seed proper obcordate, 3-3.5 by 2.5-3.5 mm, brown with light brown verrucae, the other side dark brown, wing light brown."



6) ***A. tagala*** **Cham.** (Aristolochiaceae) is called as "กระเช้าผีมด".

"Climber, young stem pubescent, glabrescent. Leaves: petioles 3-6 cm, grooved above, pubescent along grooved surface, glabrous below; lamina ovate to ovate-lanceolate, 9.5-16.5 by 5.8-7.6 cm, base deeply cordate, margin entire ± recurved, apex acuminate, mature leaves glabrous on both sides, nerves and veins ± pubescent, laxly gland-dotted; palmately 3-nerved, pinnately 3-4 nerved along the midrib, veins transverse, venation reticulate, obscurely above, prominent below. Inflorescences paniculate or racemose, 6-13.5 cm, pubescent to nearly glabrous; bracts ovate, 1.8-3 by 1-1.5 mm, base cuneate, margin ciliate, apex acute, puberulous on both surfaces. Flowers with pedicel 5-8 mm, ovary 5-9.7 by 1-1.5 mm. Perianth 3.6-5 cm long, greenish, dark purple hairy inside, laxly hairy outside; utricle globular, 5-6.5 by 5-7 mm, tube 0.6-1.2 by 1-1-.5 mm, limb linear-lanceolate, 1.6-2.5 by ± 0.8 cm. Anthers ellipsoid ± 1 by 0.8-1.7 mm. Gynostemium 3-4 by 3.5-4 mm, stigmatic lobes 6, long conical, obtuse. Fruit ovoid, 3.3-5.5 by 2.7-4.3 mm; pedical 2.2-5.8 cm. Seeds broadly oboavate ±

obcordate, winged, 5-7.5 by 7-7.5 mm, laxly verrucose on one side, the other side smooth, light brown."

7) *A. anguicida* Jacq. (Aristolochiaceae)

"Glabrous lianas. Leaves membranous, broadly triangular, acute to obtuse at the apex, basally deeply cordate, 5-7 cm broad, 7-9 cm long, smooth above, beneath with emersed veins. Pseudostipules usually present on strong stems, amplexicaul. Flowers solitary in the leaf axils, ebracteolate, rectilinear, purple, green and yellow, the utricle ovoid, gibbous, 1 cm long, syrinx strongly inequilateral, the tube straight, 1.5 cm long, the limb 1-lobed, narrowly triangular, smooth, tightly revolute after anthesis, 1 cm wide, 1.5-2.0 cm long, uunappendaged. Gynostemium deeply 6-lobed, 3 mm high, 2 mm broad, the anthers 6, equidistant. Fruits short, thick-cylindric, 3 cm long, 2 cm wide, dehiscence acropetal, septifragal, the hypanthium 1.5 mm long. Seeds numerous, flat, 3 mm wide, 4 mm long, 1 mm thick."



8) *A. gigantea* Mart. (Aristolochiaceae)

"Large strong-growing lianas. Leaves broadly ovate-triangular, acuminate, basally subtruncate, 10-15 cm broad, 12-16 cm long, deep green, glabrous above, beneath white-tomentose. Pseudostipules absent. Flowers cauliflorous, ebracteolate, geniculate, purple and yellow-orange, the utricle sublacrimiform, gibbous, 10 cm long, syrinx absent, the tube not sharply differentiated from the utricle and limb, U-shaperd, ca 4 cm long, annulus absent, the limb 1-lobed, abruptly spreading from the tube, broadly cordate, ca 14 cm wide, 16 cm long, unappendaged. Gynostemium 6-lobed, 1 cm high, 4 cm broad, the anthers 6, equidistant. Fruits large, glaucous, 8 cm long,

2.5-3.0 cm wide, dehiscence acropetal, septifragal, the hypanthium curved, 5 mm long. Seeds numerous, flat, 5 mm wide, 7 mm long, very thin, papery."

**9) *A. grandiflora* Sw.** (Aristolochiaceae)

"Strong-growing, glabrescent lianas. Leaves triangular-cordate, apex acute to acuminate, basally deeply cordate, 8-15 cm broard, 10.2 cm long, deep green, smooth above, beneath strigose in juvenile leaves, becoming smooth with age, paler. Pseudostipules absent. Flowers solitary in leaf axils, bracteolate, more or less twice-geniculate (once at the tube flexure and again at the annulus), variously blotched with purple, white, yellow, red and green, very variable in size over a vast range, but commonly very large, the utricle lacrimiform, gibbous, 6-18 cm long, syrinx cylindric, as long as 4 cm, directed obliquely into the utricle, the tube bent at its middle, 7-15 cm long, annulus thin, sharp-edged, the limb abruptly spreading from the annulus and tube, 1-lobed, 20-50 cm or more wide, 0.5-3.0 m of the limb. Gynostemium 6-lobed, coroniform, 1.5 cm high, 1.0 cm broad, the anthers 6, equidistant. Fruits cylindric, 10 cm long, 4 cm wide, dehiscence acropetal, septifragal, hypanthium absent. Seeds numerous, triangular, flat, 1 cm wide, 1.2 cm long, 2 mm thick."

**10) *A. tentaculata* Schmidt in Fedde** (Aristolochiaceae)

"Glabrous small lianas. Leaves cordate-orbiculate, slightly emarginated, cordate-auriculate, 2-6 cm broad, 3-8 cm long, smooth above, beneath paler with emersed veins. Pseudostipules absent. Flowers solitary in the leaf axils, often on small-leafed, short, lateral shoots, bracteolate, subgeniculate, purple, green and yellow, the utricle gibbous-obconic, 1 cm long, syrinx inequilaterl, annular, the tube straight, narrow at first, thence flaring into the limb, 2-3 cm long, the limb broadly lanceolate,

sparsely long-fimbriate along the lateral margins, 2.5 cm wide, 6-8 cm long, unappendaged. Gynostemium 6-lobed, 4 mm high, 4-5 mm broad, the anthers 6. Fruits cylindric, 5 cm long, 2.5 cm wide, dehiscence acropetal, septifragal, the hypanthium straight, 2 mm long. Seeds numerous, 6.5 mm wide, 8 mm long, thin, papery."

2.1.1.2 Bioactivity of *Aristolochia* plants

*Aristolochia* plants have been used in many countries around the world as important medicinal plants. Seven *Aristolochia* species including *A. indica* L. (Asia), *A. serpentaria* L. (North America), *A. debilis* Sieb & Zucch. (China), *A. acuminata* Lam (including *A. tagala* Cham.) (India), *A. trilobata* L. (Central/South America, Caribbean), *A. clematitis* L. (Europe) and *A. bracteolata* Lam. (Africa) are widely reported for various therapeutic purposes. The most commonly medicinal use is the treatment of gastrointestinal problems, especially diarrhea. The other traditional uses of birthworth species are anti-snake venom, treatment of female reproductive system conditions and sexually transmitted diseases, treatment of central nervous system conditions, dermatological problems and cardiovascular ailments (Heinrich, Chan et al. 2009).



2.1.1.3 Chemical constituents of *Aristolochia* plants

The plants belonging to the family Aristolochiaceae normally produce aristolochic acids (AAs) such as AAI, AAII, aristolactams (ALs). Aristolochic acids are a family of nitrophenanthrene carboxylic acids. The amount of AAs varies depending on the plant species, habitat, harvesting time and other factors. Concentrations ranged from 3 to 12,980 ppm for AA I and from not detected to 6,325 ppm for AA II (NTP 2008). However, the two major substances found in whole parts of *Aristolochia* plants are aristolochic acid I (aristolochic acid A) and its demethoxylated derivative, aristolochic acid II (aristolochic acid B). The structures of AAI and AAII were shown in Figure 1 (NTP 2011).

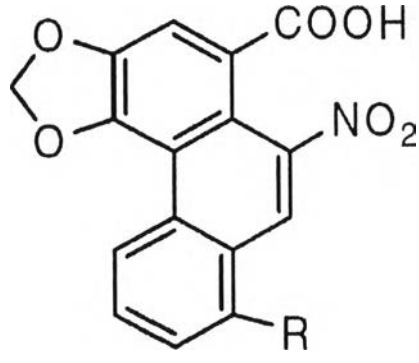


Figure 1 Structure of aristolochic acid I (AAI) (R = $OCH_3$) and AAII (R = H)

There are other common compounds found in this genus such as alkaloids (AA III, AA IIIa, AA IV, aristolactams) (Holzbach and Lopes 2010, Lu, Wei et al. 2012), mono-, sesqui-, di-, triterpens (ß-caryophyllene, isocaryophyllene, E-caryophyllene, bicyclogermacrene, ß-sitosterol) (Kumar, Prasad et al. 2003, Silva-Brandão, Solferini et al. 2006).



2.1.1.4 Toxicity of *Aristolochia* plants

Exposure to aristolochic acid has been reported throughout the world. (NTP 2008). The first report is a cluster of patients with progressive renal interstitial fibrosis associated with urothelial malignancies after taking pills from slimming clinic in Belgium in 1992. The incident was caused by the substitution of Chinese herb labelled as Han Fang Ji (roots of *Stephanio tetrondra*) by Guang Fang Ji (roots of *Aristolochia fangchi*). The disease was called as "Chinese herb nephropathy (CHN)" (Vanherweghem, Tielemans et al. 1993). Since then, many cases from various countries were continuously reported, for instance, four cases in France from intake of slimming pills containing *Aristolochia fangchi* instead of *Stephania tetrandra*, two cases in England after treatment of eczema with Guan Mu tong (stem of *Aristolochia manshuriensis*) instead of Chuan Mu Tong

(stem of *Clematis armandii* and *C. montana*) or Bai Mu Tong (stem of *Akebia quinata* and *A. trifoliata*) and one case in Spain after chronic intake of a tea containing *Aristolochia pistolochia* (IARC 2002).

From many studies and case reports, consumption of *Aristolochia* plants causes severe toxicities including renal fibrosis, irreversible nephropathy, end-stage renal failure and kidney cancer which are due to aristolochic acids and its derivatives through DNA adduct mechanism (Nortier, Martinez et al. 2000, IARC 2002, Chan, Luo et al. 2007, Shibutani, Dong et al. 2007). According to the International Agency for Research on Cancer (IARC) Monographs (2015) (IARC 2015), aristolochic acid and plants containing them are classified as group 1 agent. The group 1 agent is carcinogenic to humans by sufficient evidence of carcinogenicity in humans and/or experiential animals and strong evidence in exposed humans that the agent acts through a relevant mechanism of carcinogenicity (IARC 2015).



2.1.2 Krai-Krue herbs

2.1.2.1 Morphology of Krai-Krue herbs

Krai-Krue herbs is slightly brown hard root derived from many climbers with characteristic odor and bitter taste. It is yellow-brown in powder form. Sources of Krai-Krue have been reported as *Raphistemma pulchellum* (Roxb) Wall (ข้าวสารดอกใหญ่) (Apocynaceae), *Aristolochia indica* L. (กระเช้าสีดา) (Aristolochiaceae), *A. pothieri* Pierre ex Lecomte (กระเช้าถุงทอง) (Aristolochiaceae) (Vuthithammavech 1997, Picheansoonthon, Chawalit et al. 2001) and *Jasminum* spp. (Picheansoonthon, Chawalit et al. 2001) and also can be substituted by dried root of *Gymnopetalum integrifolium* Kurz. (ขี้กาขาว/ ขี้กาแดง) (Cucurbitaceae) (Vuthithammavech 2003).

According to microscopic, morphological and chemical profiling approaches, Krai-Krue is derived from dried roots of the three *Aristolochia* species, *A. pothieri*

Pierre ex Lecomte (Athikomkulchai and Ruangrungri 2001), *A. pierrei* Lecomte and *A. tagala* Cham. (Sathornviriyapong, Picheansoonthon et al. 2007).

Therefore, the plant samples were additionally collected in this study including *Raphistemma pulchellum*, *Jasminum sambac*, *J. adenophyllum* and *Gymnopetalum integrifolium*. The botanical description of *Raphistemma pulchellum* was reported in Flora of China (1995). That of *Jasminum sambac* was reported by Chang Mei-chen, Qiu Lian-qing and Peter S. Green (1996), *J. adenophyllum* by Peter Shaw Green (2000) and *Gymnopetalum integrifolium* by Willem J.J.O. De Wilde and Brigitta E.E. Duyfjes (2008) as follows:



1) ***Raphistemma pulchellum* (Roxb) Wall** (Apocynaceae) is called as "ข้าวสารดอกใหญ่".

"Stems to 8 m, terete, glabrous. Petiole 4-12 cm, with apical adaxial gland cluster; leaf blade ovate, 6-20 × 4-15 cm, sparsely adpressed pubescent to glabrous, base deeply cordate, apex acute-acuminate; lateral veins 6 or 7 pairs. Peduncle 3.7-13 cm. Pedicel 1.2-4 cm. Sepals ovate-oblong, 3-4 mm, ciliate. Corolla yellowish white; tube 1.2-1.8 cm, limb 3-4 cm in diam.; lobes shorter than tube, oblong, glabrous. Corona lobes white, 1-1.2 cm. Ovaries glabrous. Follicles ca. 16 × 4 cm. Seeds ovate; coma to 4 cm. Fl. Jun-Aug, fr. Sep-Dec."

2) ***Gymnopetalum integrifolium* Kurz.** (Cucurbitaceae) is called as "ขี้กาขาว/ขี้กาแดง".

"Climbing or creeping herb, rooting at the nodes, to 5 m long, stem (densely) grey or brownish hairy. Probact lanceolate, acute, unlobed or (deeply) 2- or 3-lobed, (1-)1.5-2.5 cm long, sometimes absent, green-yellow, late-caducous. Tendrils

unbranched or unequally 2- branched near the base. Leaves: blade circular, or reniform, or broadly ovate in outline, or 5- angular, 2-11 cm diam., subglabrous above, densely coarse-pubescent below, at least on the veins, when fresh bullate above, cystoliths in older leaves present, base deeply cordate, margin entire, finedly dennate-mucronate or ± coarsely lobulate or wavy-dentate, apex rounded or subacute, 5 palmately veined, reticulation distinct below; petiole 1-5 cm long. Male inflorescences: flowers solitary or in bracteates racemes; bracts 1-2 cm long, lobed, base cuneate, sessile. Male flowers: densely grey (or brown) pubescent; pedicel 2-12 cm long for solitary flowers, 1(-2) cm long in the racemes, persistent, at apex faintly articulate; receptacle-tube (strongly) narrowed below insertion of stamens, 15-20 (-30) by 6-7 (at throat) mm, outside and inside pubescent, throat inside yellow; sepals narrowly triangular, lanceolate, entire or ± lobed, recurved, (4-)5-8 mm long, green; petals obovate, ± clawed, ca. 2 by 1.5 cm, distinctly veined; stamens inserted ca. 10 mm below throat; filaments 2-2.5 mm long, ± glabrous, synandrium 8-12 by 2-2.5 mm. connectives not enlarged, apex of synandruim flat, narrow,, hairy, bright yellow when fresh; disc consisting of 3 short linear bodies. Female flowers; solitary, resembling male flowers; pedicel 1-3 cm long; ovary ellipsoid, 8-10 by 6-7 mm, long-pubescent; receptacle-tube cylindrical, ca. 10 by 5 mm; style 7-10 mm long, stigmas erect, ca. 2 mm long, ± included; disc at base of the tube, very low and minute (nectariferous?) or absent. Fruits short ellipsoid or globose, (2-)3-4 cm long, (orange-) red, at first sparsely hairy, later on glabrous; fruiting pedicel 1-3(-5) cm long. Seeds (elliptic) oblong, 6-9 by 2.5-3 by 1.5-2 mm, faces small, almost smooth, demarcated by groove from broad, rounded margin."

3) ***Jasminum adenophyllum* Wall. Ex C.B. Clarke** (Oleaceae) is called as "มะลิวัลย์".

"Woody climber, young shoots glabrous or scattered puberulent. Leaves elliptic to oblong-elliptic, 6-15 cm long, 2.5-7 cm broad, base attenuate onto the grooved petiole; apex slightly aceminate; glabrous; 4 or 5 primary veins on each side of the midrib, raised below, but not reticulate, slightly sunk above, joining to form a distinct submarginal vein; 3-4 tufted domatia in the axils of the primary nerves with the midrib below; petioles 5-20 mm long, glabrous or pilose above. Inflorescence axillary or terminal on short shoots, 1- to 3(-5)-flowered, glabrous; bracts linear, 2 mm long; glabrous; pedicels 1-4 cm long. Calyx tube 2 mm long; lobes 5-14 mm long, somewhat filiform, glabrous. Corolla white, fragrant; tube (9-)15-20 mm long; lobes 8 or 9, 15-20 mm long, 2-3.5 mm broad. Fruit spheroidal, 7x15 mm."



4) ***Jasminum sambac* (L.) Aiton** (Oleaceae) is called as "มะลิลา".

"Shrubs erect or scandent, to 3 m. Branchlets terete or slightly compressed, sometimes hollow, sparsely pubescent. Leaves opposite, simple; petiole 2-6 mm, articulate, pubescent; leaf blade orbicular to elliptic or obovate, 4-12.5 × 2-7.5 cm, papery, glabrous except for tufted hairs at vein axils abaxially, both ends blunt, sometimes base subcordate; primary veins 4-6 on each side of midrib. Cymes terminal, (1 or)3(or 5)-flowered; bracts subulate, 4-8 mm. Flowers very fragrant. Pedicel 0.3-2 cm. Calyx glabrous or sparsely pubescent; lobes 8-9, linear, 5-7 mm. Corolla white; tube 0.7-1.5 cm; lobes oblong to suborbicular, 5-9 mm broad. Berry purple-black, globose, ca. 1 cm in diam. Fl. May-Aug, fr. Jul-Sep. $2n = 26$*."

2.1.2.2 Traditional use of Krai-Krue herbs

Krai-Krue is used as a common ingredient in several Thai folk medicinal formulas for various purposes such as tonic, muscle relaxant, diuretic, antipyretic, analgesic, anti-rheumatism, immunostimulant, emmenagogue, abortion and liver enhancer (Vuthithammavech 1997, มูลนิธิฟื้นฟูส่งเสริมการแพทย์ไทยเดิมอายุรเวทวิทยาลัย(ชีวกโกมารภัจจ์) 2535, พระยาวิชยาธิบดี(กล่อม) 2546). It has been used as an ingredient in 10 herbal recipes on the list of Herbal Medicinal Products A.D. 2006 of Thailand, for example, Ya hom Nawakod (ยาหอมนวโกฐ), Ya hom Inthajuk (ยาหอมอินทจักร์), Ya Ummaruekawatee (ยาอำมฤควาที), Ya Tatbunjob (ยาธาตุบรรจบ), Ya Wisumpayayai (ยาวิสัมพยาใหญ่), Ya Munthatat (ยามัณฑธาตุ), Ya Kheawhom (ยาเขียวหอม), Ya Treehom (ยาตรีหอม), Ya Prasaganplu (ยาประสะกานพลู), Ya Prasajettapungkee (ยาประสะเจตพังคี) (Health 2006).



2.2 Assessment for identification of the medicinal plants

2.2.1 Genetic assessment

Genetic assessment of medicinal plants can be classified into three categories, namely hybridization-based method, polymerase chain reaction-based and sequencing-based. First, the hybridization-based method is a technique that measures the degree of nucleotide similarity when two complementary single-stranded nucleic acids anneal into a double-stranded nucleic acid by hydrogen bonds formation. Second, the PCR-based method is carried out by amplification of the region(s) of interest in the genome. Then gel electrophoresis is performed to size and/or score the amplification products. Examples are sequence characterized amplified regions (SCAR), simple sequence repeat (SSR) analysis, PCR-restriction fragment length polymorphism (PCR-RFLP), amplified fragment length polymorphism (AFLP) and random amplified polymorphic DNA (RAPD). Nucleotide sequences are needed for some techniques of PCR-based method. Third, the sequencing-based method is a technique that obtains

nucleotide sequences of organism and establishes centered database such as GenBank. Searching and comparison to the database are performed for identification of unknown organism (Yip, Chau et al. 2007). The advantages of genetic assessment are that: it can be performed with a small amount of sample, the sample can be analyzed without prior nucleotide sequence, and it can be succeeded within a short time. Besides, the molecular techniques is also performed very well at sensitivity, reliability, reproducibility, and running costs (Heubl 2010).

Application of genetic assessment for accurate identifications for plant samples have been reported, such as randomly amplified polymorphic DNA (RAPD) (Khan, Mirza et al. 2010, Mir, Koul et al. 2013), amplified fragment length polymorphism (AFLP) (Mir, Koul et al. 2013), sequence characterized amplified region (SCAR) (Marieschi, Torelli et al. 2012), multiplex amplification refractory mutation system (MARMS) (Wang, Kwon et al. 2011), polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) (Suwanchaikasem, Phadungcharoen et al. 2013) and DNA barcode (Ogata, Uchiyama et al. 2013, Chen, Zhu et al. 2014).



Currently, genetic assessment by sequencing-based techniques has been widely developed for the identification and authentication of medicinal plant species. The latest focused approach is DNA barcoding which is DNA sequencing of standardized DNA region(s) for each organism (Hollingsworth, Graham et al. 2011).

DNA sequencing is a powerful process involving the order of four bases, adenine (A), thymine (T), cytosine (C), and guanine (G), in DNA samples that can be used to identify species, analyze phylogenetic relationships, population genetics, and evolutionary processes. It is also widely used in many other fields such as archaeology, anthropology, genetics, biotechnology, molecular biology, and forensic sciences (França, Carrilho et al. 2002). In addition, this technique can be used to determine the sequence of individual genes, clusters of genes or entire genomes (Shendure and Ji 2008).

DNA barcoding is one of sequencing-based techniques that involve using the analysis of short standardized regions of genome and establishment of centralized sequence database of organism. It has been proposed for identifying and authenticating unknown biological material to species level (Group 2009). Up to the present, there is no universal DNA region for species discrimination among all organisms (Stoeckle 2003). A Consortium for the Barcode of Life (CBOL) Plant Working Group proposed four DNA regions including large subunit of ribulose-bisphosphate carboxylase *(rbc*L) and maturase K (*mat*K) as the core barcode, and nuclear internal transcribed spacer (ITS or ITS2) and *trn*H-*psb*A spacer as the additional barcode for standard land plant barcode (Hollingsworth 2011). This technique is included in the one of raw material identification steps in The United States Pharmacopoeia and Chinese Pharmacopoeia (Song, Yao et al. 2009, Li, Cao et al. 2011).



Multiplex PCR is a modified PCR-based technique that is based on the amplification of two or more DNA targets. The process uses multiple primers in a single reaction mixture (Da-cheng, Shi-lin et al. 2010). It has been widely applied for species identification of several organisms, including microorganisms (Oliveira and de Lencastre 2002, Lucignano, Ranno et al. 2011), genetically modified crops (Forte, Di Pinto et al. 2005), and medicinal plants (Lee, Doh et al. 2008, Jigden, Wang et al. 2010). However, studies of multiplex PCR for the authentication of *Aristolochia* Krai-Krue have not been reported. Recently, nucleotide polymorphisms based on ITS sequences have been widely used for the development of molecular markers for medicinal plant identification (Lee, Kim et al. 2012).

2.2.2 Chemical assessment

Many chromatographic and spectroscopic techniques have been used for both authentication and quality control purposes of herbal materials, for instance, thin layer chromatography (TLC), near infrared (NIR), gas chromatography (GC), high-performance

liquid chromatography (HPLC), gas chromatography–mass spectroscopy (GC–MS), high-performance liquid chromatography- mass spectroscopy (HPLC–MS) (Liang, Xie et al. 2004, Xie, Chen et al. 2006).

HPTLC is a method of choice because it is a simple, reliable, and rapid analytical technique of chromatography. It has been widely used as a screening tool and routinely used for qualitative assessment of chemical constituents of botanical materials by detection of specific peaks or zones due to known or unknown components of the extract. (Reich and Schibli 2007). HPTLC is recognized as one of chromatographic fingerprint in herbal standardization (Kulkarni, Patil et al. 2014). The technique has been successfully applied in many fields of research such as pharmaceutical analysis, environmental analysis, food and clinical laboratories (Shewiyo, Kaale et al. 2012). With retard to *Aristolochia* plants or suspected *Aristolochia*-containing products, this method is recommended as a preliminary screening test for aristolochic acids (Blatter and Reich 2004, Li, Au et al. 2012, Phadungrakwittaya, Akarasereenont et al. 2012, Li, Au et al. 2014).