

## CHAPTER II

### LITERATURE REVIEW

#### 2.1 Species classification

*Limnophila aromatica* (Lam.) Merr. and *L. geoffrayi* Bonati belong to the genus *Limnophila* R.Br. Previously they belonged to family Scrophulariaceae. Currently they have been transferred to Plantaginaceae based on molecular phylogenetic evident (Stevens, P. F., 2024). This genus has been classified based on taxonomic ranges according to POWO (2025) as follow:

Kingdom:	Plantae
Phylum:	Streptophyta
Class:	Equisetopsida
Subclass:	Magnoliidae
Order:	Lamiales
Family	Plantaginaceae
Genus	<i>Limnophila</i> R.Br.
Species	<i>Limnophila aromatica</i> (Lam.) Merr. <i>Limnophila geoffrayi</i> Bonati

Smitinand, T. and Larsen, K. (1990) reported 19 species of *Limnophila* found in Thailand. *Limnophila aromatica* is called Phak Ka Yaeng, including Ka om, Kha yaeng, Phak Pha, Phak Luem Phua, Ma-Om and Yan Nang Yai as other vernacular names (Forest and Plant Conservation Research Office, 2024). It has never found in nature in Thailand although it is distributed from India to Australia and Japan (POWO, 2025). Currently it has become an economic plant which is cultivated as a substitute in rice fields. The morphology of Ka Yaeng is shown in Figure 2.1. *Limnophila aromatica* is an annual or perennial herb. Its stems are erect, 30-70 cm high, simple to much branched, glabrous or glandular, base decumbent and rooting from nodes. Leaves are mainly 3 in whorls and sessile. Leaf shape is ovate-lanceolate to lanceolate-elliptic, 1-5 cm long

and 0.3-1.5 cm broad and margin serrate and with pinnately venation. Flowers arise from the leaf axils or on terminal branches, usually solitary. It has a pedicel 0.5-2 cm long and glabrous or glandular. Calyx is green, 4-6 mm long, divided into 5 narrowly lobes and glabrous or glandular pubescent. Corolla is violet, 1-1.3 cm long, sub-bilaterally symmetry, with sparsely and finely glandular hairs and inside white villous. Style apex is dilated. Stigma is short and 2-lamellate. Capsule is ovoid, ca. 6 mm long (eFloras, 2008).

*Limnophila geoffrayi* has vernacular name as Ka Om, including Phak kha yaeng and Ang-om as other vernacular names (Forest and Plant Conservation Research Office, 2024). It is distributed in Cambodia, Laos, Thailand, and Vietnam. The first specimens for scientific naming were collected in Cambodia. In Thailand, it is found in the north, in Phitsanulok Province, and the northeastern region in Nong Khai, Maha Sarakham, Khon Kaen, and Nakhon Ratchasima provinces.

*Limnophila geoffrayi* is an aquatic annual herb, that is fragrant and prefers to grow in open, moist places. Stems are slender and erect, 10–35 cm high. Branches are simple or laxly branched near the base with densely hirsute. Leaves are mainly opposite, sessile, semi-amplexicaul, oblong-lanceolate, oblong, or ovate-elliptic, 1–3 x 0.3–1 cm width, acute to obtuse at apex, attenuate to base, crenate-serrate, scabrid or glabrous above, glabrous and punctate beneath and pinnately nerved. Flowers born in axillary or terminal racemes with 2–10 flowers. Peduncles are slender, 1.5–6 cm long and sparsely hirsute to glabrous. Pedicels are 1–4 mm long and sparsely hirsute to glabrous. Bracts are small and narrowly lanceolate, ca. 0.5 mm long. Bracteoles are filiform, acuminate, and 1–2 mm long. Calyx is about 3.5 mm long, sparsely short-hirsute, or glabrescent and striate at maturity. Calyx lobes are lanceolate, 2.5 mm long. Corolla is white, violet, or pink, 10–13 mm long, glabrous outside and villous posteriorly inside. The upper lip of corolla is broadly orbicular, emarginate and the lower lip is 3-lobed, orbicular. Capsule is ellipsoidal, 3 x 1.5 mm long (Smitinand, T. and Larsen, K., 1990). The morphology of Ka Om is shown in Figure 2.2.



Figure 2.1 *Limnophila aromatica* A. Habit, B. Floral branches, C. Flowers.

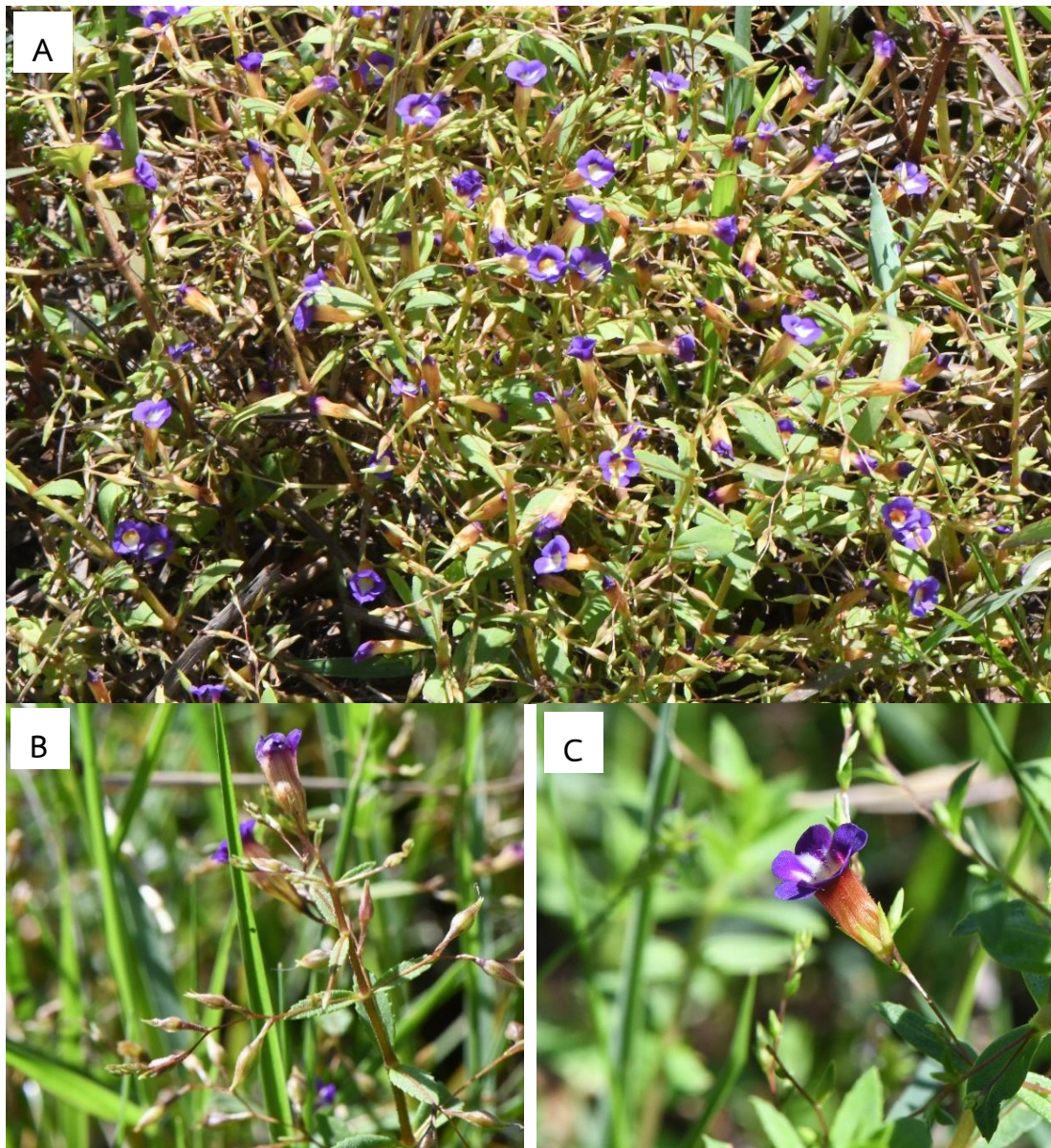


Figure 2.2 *Limnophila geoffrayi* A. Habit, B. Floral branches, C. Flowers.

## 2.2 Floral ecology

### 2.2.1 Flower part and shape

Flower is a major diagnostic feature of angiosperm, modified reproductive shoot (Simpson, M. G., 2019). Typically, a flower is comprised sepals, petals, stamens and pistil, arranged from external to internal. All part attached to the receptacle, part of the stem. It composes of sterile parts, sepals and petals and reproductive part, stamens and pistil. It composes of an ovary at its basal part and a long neck called the style with stigma at the top. Inside the ovary, there is one or more ovule, which become to seed after fertilization (Urry, L. A., Cain, M. L., Wasserman, S. A., Minorsky, P. V., and Reece, B. O., 2011)

The flower shape in angiosperm exhibits so diverse, depended on species. The feature of flowers is related to attracting the visitors, especially non environment vector of pollination. Every floral organ is generally thought to play a more or less specific role in pollination. Functional flower features are present according to specific plants (Willmer, P., 2011 quoted in Faegri, K., and Van Der Pijl, L. (1979). Willmer distinguished types of the flower in relation to actual visitors into 11 types as follow:

1. Open disk
2. Tubular flowers with radial symmetry
3. Tubular flowers with bilateral symmetry
4. Bell-shaped flowers
5. Spherical flowers
6. Keel flowers
7. Iris-type flowers
8. Orchid flower
9. Brush blossoms
10. Composite flowers
11. Trap flowers

The flower shape of *Limnophila* is a small tubular flower with bilateral sub-symmetry. The lower one consists of 3 lobes and the upper one consists of 2 lobes. The upper most corolla lobes are not expanded apically as a hood, part of stamens

protection, like Lamiaceae or some of Scrophulariaceae and Plantaginaceae. However, their tubular flower with bilateral sub-symmetry could be classed in this type.

### 2.2.2 Arrangement of stigma and stamen

Plants have evolved to guarantee successful reproduction through selfing, which is typically linked to a deficit of pollinators. In Botany, Herkogamy refers to the position of the anthers in a flower that is on a different plane from the position of the stigma of the same flower. As a result, self-pollination does not occur on the same flower. Herkogamy is divided into two forms: the anther is lower than the pistil (approach herkogamy), and the anthers are higher than the top of the pistil (reverse herkogamy) (Cardoso, J. C. F., Viana, M. L., Matias, R., Furtado, M. T., Caetano, A. P. D. S., Consolaro, H., and Brito, V. L. G. D., 2018). The arrangement of the stigma and stamen is related to self-pollination or cross-pollination, depending on species. In *Limnophila*, it has 4 stamens arranged in 2 pairs. The longer one is above the stigma and the shorter one is under the stigma, considered as non-herkogamy, due to the position of stamens and stigma promote self-pollination.

### 2.2.3 Anther dehiscence

The term "anther dehiscence" describes as the opening of the anther that allows pollen grains to break out (Beauzamy, L., Nakayama, N., and Boudaoud, A., 2014). Anther dehiscence has 4 types, longitudinal, poricidal, transverse, and valvular. The most prevalent and historical form of dehiscence is longitudinal dehiscing along the long axis of the thecae and along a suture. Other varieties are uncommon and unique to certain groups, such as having a pore at one end of the thecae, poricidal organisms dehisce via transverse, dehiscing at an angle to the Ericaceae; theca's long axis and valvular, dehiscing through a pore-like in the Lauraceae, tissue flap-covered (Simpson, M. G., 2019).

## 2.3 Floral phenology

Plant phenology is the time of different phases in the plant life cycle, such as leaf budding, blooming period, fruiting period, etc. (Stucky, B. J., Guralnick R., Deck, J.,

Denny, E. G., Bolmgren, K., and Walls, R., 2018). Floral phenology is the study of a flower's life cycle, starting from the date of opening, anthesis, to ending with wilting (Gentry, A. H., 1974). The phenology of the flowers will change depending on the species and other factors such as the habitat, length of the flowering season, seed predatory behavior, etc. (Willmer, P., 2011). It is possible to study phenology at several scales, including phylogenetic characteristics, populations, communities, single flowers, individual plants and dioecious plants (Dafni, A., 1992). An example of a phenology study of plant species which having floral characteristics similar to *Limnophila* is *Penstemon penlandii* W.A.Weber, Plantaginaceae. It is an endangered endemic plant in the state of Colorado United States. The flowers are tubular about 1.5 cm long and 0.5 cm wide. It has 2 short stamens and 2 long stamens. Flower phenology study showed that the anthers of the longer stamens break during 9:00-9:30 hrs. while the anthers of the shorter stamens break during 10:30-13:00 hrs. Bloom on the 3<sup>rd</sup> or 4<sup>th</sup> day before the flowers drop. The phenology of this type of flower affects pollination by insects, especially when pollinators visit the flowers (Tepedino, V. J., Sipes, S. D., and Griswold, T. L., 1999).

Opening and closure of the flower are an important trait of a reproductive syndrome because it allows pollen removal by vectors. Different traits such as diurnal and nocturnal and single or repeated opening depend on species. Cell expansion happens when osmotic solution levels increase causing the opening. Temperature, light intensity and humidity results in opening and depends on species (Van Doorn, W. G. V., and Van Meeteren, U. V., 2003).

## 2.4 Pollination system

Pollination is the process by which pollen grains fall on the surface of the stigma. This results in the germination of pollen tubes. There is the growth of the pollen tube to meet with the egg and fertilization occurs. There are three types of pollination.

1. Self-pollination means pollination in the same flower and is a transfer of pollen that can be found in flowers of two sexes in the same plant (bisexual flower).

2. Geitonogamous-pollination means pollen transfer from one flower to another in the same plant which relies on vectors to facilitate pollination (Richards, A. J., 1997). They can be found in both bisexual flowers and unisexual flowers.

3. Cross-pollination means the transfer of pollen from one flower to the top of the pistil of another flower. It is found in dioecious species. The general flowering plants have also this form of pollination. It can be found in monoecious plants, especially when anthers and stigma do not function at the same time or there are other barriers between anther and stigma. This type of pollination promotes high genetic diversity (Cardoso, J. C. F., Viana, M. L., Matias, R., Furtado, M. T., Caetano, A. P. D. S., Consolaro, H., and Brito, V. L. G. D., 2018). Most flowering plants that require vectors facilitate pollinators. Those vectors are usually insects. Hence, flowers are the structures of flowering plants that play an important role in helping each plant to reproduce successfully. The flowers can attract insects to help pollinate them. Mainly because flowers provide food for insects such as nectar or pollen, etc. In addition, insects help to pollinate may because that flowers can provide refuge for insects to escape from bad weather or predators. It is a spawning ground for females and in some cases, that can be a source of warmth for insects in cold climates (Willmer, P., 2011). There are various vector pollination patterns which are 1) pollination by flies, 2) pollination by butterflies and moths, 3) pollination by birds, 4) pollination by bats, 5) pollination by nonflying vertebrates and other oddities, and 6) pollination by bees, defined by Willmer.

## 2.5 Breeding system

The breeding system or reproductive system is a form of seed acquisition process through the fertilization of male gametes and female gametes by pollinators. There are various forms of pollination (Dafni, A., 1992), including:

1. Autogamy means the fertilization of male gametes and female gametes from pollinators in the same flower (self-pollination) that occurs in nature without the help of vectors. However, in the case where vectors such as insects are needed to help pollinate, it is called facilitated autogamy (Lloyd, D. G., and Webb, C. J., 1992).

2. Geitonomy means the fertilization of male gametes and female gametes, from the transmission of pollen from one flower to another in the same tree. This breeding system type resulting plants having more genetically diverse than autogamy plants due to the possibility of mutations in the proliferative tissues of the non-reproductive organs (vegetative organ) and organs that perform reproductive functions (Barrett S. C. H., and Harder L. D., 2017). However, it is less successful than autogamy because it requires vectors to facilitate pollination (Eckert, C. G., 2000).

3. Xenogamy means the fertilization of male gametes and female gametes from cross-pollinating by using vectors such as wind, water, and animals such as birds, bats, ants, or insects (Richards, A. J., 1997), which will give plants high genetic diversity. This breeding system results in increasing the survival rate in nature even more because they can adapt to a variety of environments (Simpson, M. G., 2006).

4. Cleisogamy means the fertilization of male gametes and female gametes from pollinators in the same flower (self-pollination) that occurs in immature flowers (Lord, E. M., 1981). It is an adaptive form of reproduction for the lowest cost but highest yield of plants with low insect pollination or in conditions that are restricted to growth (Culley T. M., and Klooster, M. R., 2007).

## **2.6 Seed germination**

An ovule undergoes fertilization to become a reproductive unit called a seed. A seed's endosperm, embryo, and seed coat (testa) are its three primary structural components (Smith, P., 2018). Seed germination is the physiological process achieving of the embryo emerging from the seed coat. The imbibition, water absorption by seed, starts to activate the metabolism led to expansion of the embryo and produce the radicle throughout the seed (Bewley, J. D., Bradford, K., Hilhorst, H., and Nonogaki, H., 2013). Deterioration of seed quality may mean a loss of seed quality, strength, and viability of seeds due to inappropriate environmental factors such as temperature, humidity, or a too-high ratio of oxygen to carbon dioxide. Deterioration in seed quality can occur both before, during, and after harvest (Khan, F. A., Bhat, S. A., Narayan, S., Maqbool, R., Murtuza, I., and Khan, F. U., 2017).

Preparing seeds before planting by soaking seeds in water for a period of time appropriate for that type of seed can help stimulate seed germination. This is because water is an important contributor to various processes within the seed, such as protein synthesis or various substances that help the germination process. Soaking the seeds in water will help make the seeds with thick, hard shells softer. This makes water and air permeate into the seeds more easily than usual (Khammapana, L., Mulalin, S., and Tangteerawatana, S., 2019). Furthermore, researchers discovered that soaking seeds in water can enhance the germination of seeds exposed to unsuitable environmental conditions like salinity and drought. Different plants combined with different environmental conditions will result in different germination efficiencies (Farahani, H. A., and Maroufi, K., 2011), such as in the report of Casenave, E. C. and Toselli, M. E. (2007), who studied the method of preparing seeds before planting by soaking cotton seeds in water. Under conditions where rainfall and temperature fluctuate unevenly, the amount of rainfall and the temperature are important factors that hinder the germination of cotton seeds. Studies have shown that soaking cotton seeds in water can accelerate cotton seed germination. A study by Tiwari, R. K. S., Chandra, K. K., and Dubey, S. (2018) studied techniques for breaking seed dormancy and the germination efficiency of six important medicinal plants. It was found that soaking seeds in water for 24 hours increases seed germination of those six species is effective in three species, *Asparagus racemosus*, *A. moschatus*, and *Psoralea corylifolia*.

Gibberellins are hormones that stimulate plant growth and development. It plays an important role in stimulating seed germination, stimulates the development of the embryo from the division of tissue cells until it develops into various components such as roots, stems, and leaves. It also acts to stimulate the production of hydrolytic enzymes such as  $\alpha$ -amylase in the aleurone layer in various grains to help stimulate germination (Gupta, R. and Chakrabarty, S. K., 2013). From these properties, studies on the use of gibberellins have been reported to stimulate the germination of various plant species. For example, Hilooğlu, M., Sozen, E., Yucel, E., and Kandemir, A. (2018) studied seed germination of *Verbascum calycosum*, an endemic plant of Erzincan, Turkey, in the family Scrophulariaceae. The seeds are very small, approximately 1-1.3 mm. The seed shells are brown-black. It is very hard and seems like water and air

cannot penetrate the seeds. The results showed that the germination rate mostly increased in the method of soaking seeds in gibberellic acid (GA<sub>3</sub>) 100  $\mu$ M (39%) and 200  $\mu$ M (54.5%). This is the most effective method for breaking dormancy in this type of seed.

## 2.7 Seed dormancy

There are many definitions but it most often refers to the failure of a seed that cannot germinate. Although environmental conditions such as water, temperature, light, and air are conducive to seed germination (Baskin, C. C. and Baskin, J. M., 2014). Seed dormancy can be divided into 3 main mechanisms as follows: 1) physical dormancy is a seed with a shell, usually in a thick, hard, impermeable form, 2) physiological dormancy is a seed that cannot germinate until some chemical change occurs, and 3) morphological dormancy is the seed that scatters when the fruit with seeds have fallen off to help with propagation but are not yet fully developed. As a result, when the seeds fall into areas with suitable conditions, they will not be able to germinate immediately. It requires further development until it can eventually germinate (Smith, P., 2018). Each species has a different method for overcoming seed dormancy. There is some research conducted a study to break the dormancy of seeds from plants in the same family as *Limnophila*. Kirmizi, S., Gülerüz, G., Arslan, H., Sakar, F. S., and Kocabıyık, G. A. (2010) used moist chilling, gibberellic acid, and scarification to break the dormancy of *Pedicularis olympica* seeds, a plant that grows in wet places but has very small seeds. The results showed that the highest germination rate and mean germination times were significantly lower in the seeds treated with 250 ppm GA<sub>3</sub>. In addition, Jang, G. H., Chung, J. M., Rhie, Y. H., and Lee, S. Y. (2022) studied on breaking seed dormancy of *Veronicastrum sibiricum*, the result showed about 90% of the seeds germinated after the GA<sub>3</sub> treatment successfully broke physiological dormancy.

There is information suggesting that storing seeds at room temperature for a certain period can break seed dormancy such as the study of the effect of newly obtained and one-year-old seeds of four heathland species on seed germination by Valbuena, L., and Vera, M. L. (2002). The results of the experiment indicated that seeds

collected one year ago germinated better than freshly collected seeds. In addition, the method of using time to help break seed dormancy is also simple and beneficial to villagers because it is easy to follow.

## 2.8 Effect of BAP on shoot generation of *L. aromatica*

Plant tissue culture is an *in vitro* technique whereby plant tissue fragments, or explants, are grown in an artificial medium under aseptic conditions. Promote cell proliferation and plant regeneration, entails cultivating explants—such as shoot tips, root tips, calluses, seeds, embryos, pollen grains, ovules, or even a single cell—isolated from the original plant on a sterile nutrient medium (Hasnain, A., Naqvi, S. A. H., Ayesha, S. I., Khalid, F., Ellahi, M., Iqbal, ... Abdelhamid, M. M. A., 2022). At present, there are studies on the induction of shoot regeneration *in vitro* of *L. aromatica*. In addition, Karatas, M. and Aasim, M. (2015) conducted an *in vitro* study on the induction of shoots in *L. aromatica*, using both shoot and leaf fragments. They incorporated these fragments into the Murashige and Skoog (MS) medium, varying the benzyl adenine (BAP) concentration from 0.25-2 mg/l for 8 weeks. The results showed that BAP helped to induce better shoot formation. Shoots per explant ranged from 19.61 to 44.22, and high concentrations of BAP decreased the number of shoots per explant. Dogan, M. (2019) did a study on *L. aromatica* shoot induction in a lab setting using a shoot tip in MS medium with varying amounts of BAP, GA, and KIN. They also looked at the effects of white, red, and blue LED lights over 8 weeks. The results showed the maximum number of shoots/explants and the longest shoot lengths of LED lights in a 1:2:1 ratio in MS medium supplemented with 0.10 and 0.20 mg/l BAP. Tissue culture of *L. geoffrayi* has not yet been studied.