CHAPTER 2

LITERATURE REVIEW

2.1 Bioinsecticide

Botanical pesticides is a subcategory of " Biopesticide" that are defined as "Any naturally occurring organisms or products originating from living organisms and which have high level of activity against one or more pests, inducing mortality, regulating pest growth and development and biological changes leading to the death or reduction of pest populations" (Rodcharoen and Wongsiri, 1997).

Bioinsecticides come from living organisms such as plants or microorganisms. Bioinsecticides are useful against several insect pests in yards and gardens, such as ants, beetles, caterpillars, fleas, flies, leafhoppers and mosquitoes. Nicotine, from the tobacco plant, is an example of a bioinsecticide. Pyrethrum, derived from the chrysanthemum, is another. The advantage of using bioinsecticides is that they often completely break down into nontoxic compounds within hours or days when exposed to sunlight. The potential of these chemicals to contaminate ground water is less than that of some synthetic insecticides. (The Spokane Country Office of Washington State University, 1997)

The application of botanical pesticides, primarily of plant origin, preceded by a long time that of synthetic pesticides. In the period between 1900 and the 1940s only nicotine, anabasine, pyretrins, rotenone and quassia were used in addition to inorganic pesticides, and their application virtually ceased on the discovery and large-

scale economic production of synthetic pesticides. However, in recent decades, the importance of certain representatives of this group has increased. This was partly due to the enhanced ability to separate these phytochemicals and determine their structures and biological activities and partly because of their advantage from the point of view of toxicology and environmental protection (Matolcsy *et al.*, 1988).

2.2 *Stemona* plants

The genus *Stemona*, previously named as *Roxburghia* (Prain, 1905) belongs to the Stemonaceae family (monocotyledon), which is classified under the order of Dioscoreales. This family also includes the genus *Croomia* and *Stichoneuron* (Fig. 2.1) (Duyfjes, 1993; Pilli and Ferreira de Oliveira, 2000).

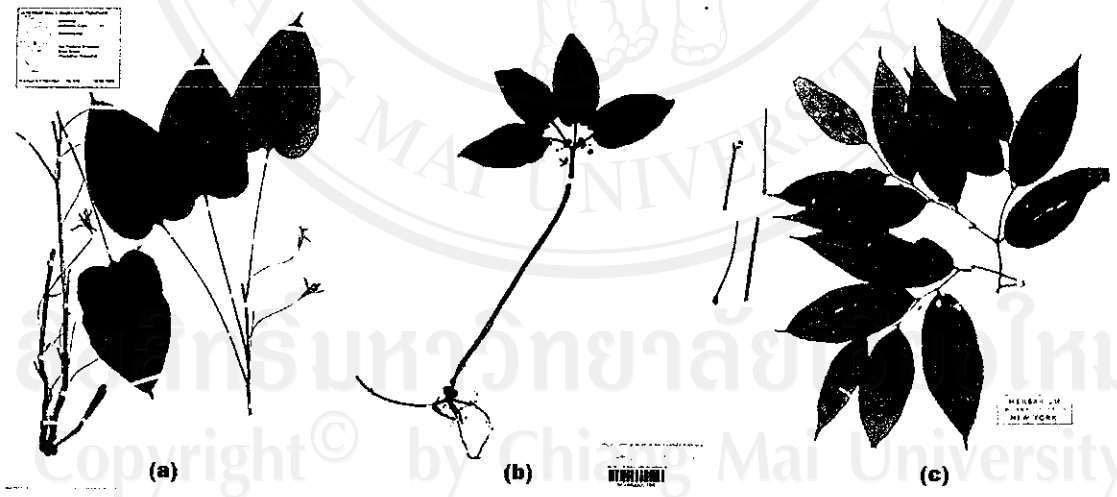


Figure 2.1 Stemonaceae (a) *Stemona* (b) *Croomia* (c) *Stichoneuron*

The *Stemona* genus is distributed through Southeastern Asia, Malaysia and Northern Australia (Duyfjes, 1993; Pilli and Ferreira de Oliveira 2000; Brem *et al.* 2002). *Stemona* plants are called by different names depending upon the regions that

they are found for example, 'Bai bu' in China, 'Bach bo' in Vietnam and 'Non-Tai-Yak' in Thailand. There are at least 25 species in this genus and 10 species have been found in Thailand (Maxwell, 1991; Rungrojsakul, 2001) but only a few of them have been studied phytochemically (Brem *et al.*, 2002; Seger *et al.*, 2004).

2.2.1 *Stemona* alkaloids structural classification

The *Stemona* alkaloid structures contain a characteristic pyrrolo[1,2-*a*]azepine nucleus. The *Stemona* alkaloids have been classified according to their structural features into 8 groups i.e. stenine (**I**), stemoamide (**II**), tuberostemospironine (**III**), stemonamine (**IV**), parvistemoline (**V**), stemofoline (**VI**), containing the pyrrolo[1,2-*a*]azepine nucleus characteristic of the majority of the *Stemona* alkaloids, stemocurtisine (**VII**) which displays the pyrido[1,2-*a*]azepine nucleus, and a miscellaneous group (**VIII**) either lacking or featuring a hidden pyrrolo[1,2-*a*]azepine moiety (Fig. 2.2) (Pilli *et al.*, 2005).

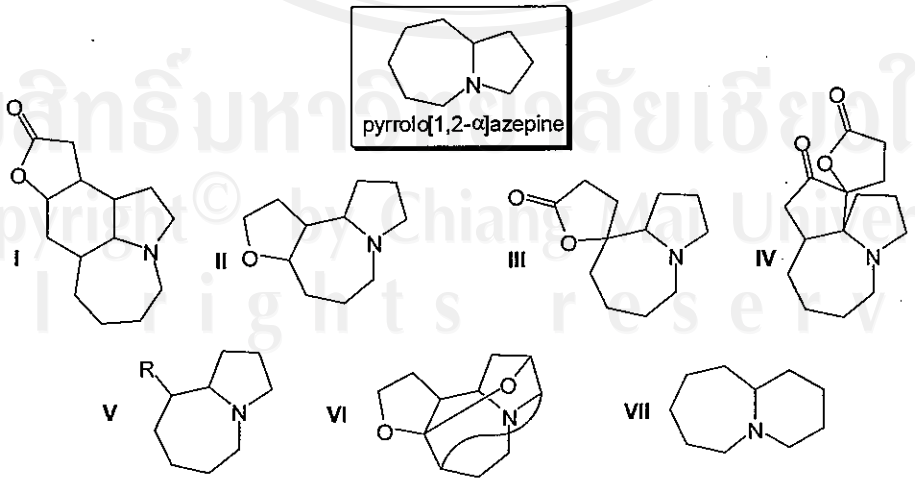


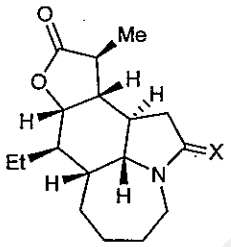
Figure 2.2 *Stemona* alkaloid groups

Stenine group

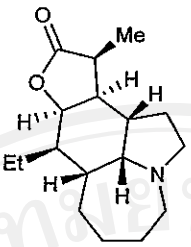
The structural characteristic of this group is the tetracyclic furo[2,3-*h*]pyrrolo[3,2,1-*jk*]benzazepin-10(2H)-one nucleus (**I**, Fig. 2.2). Currently, fourteen *Stemona* alkaloids: stenine (**1**), 2-oxostenine (**2**), isostenine (**3**), also named as neostenine, tuberostemonine (**4**), tuberostemonine A (**5**), tuberostemonine J (**6**), tuberostemonine H (**7**), tuberostemonol (**8**), neotuberostemonol (**9**), didehydrotuberostemonine (**10**), also named as bisdehydrotuberostemonine, bisdehydroneotuberostemonine (**11**), *epi*-bisdehydroneotuberostemonine J (**12**) also designated as *epi*-bisdehydrotuberostemonie J, neotuberostemonine (**13**), also named as tuberostemonine LG, and oxotuberostemonine (**14**) (Fig. 2.3) had been reported.

Stemoamide group

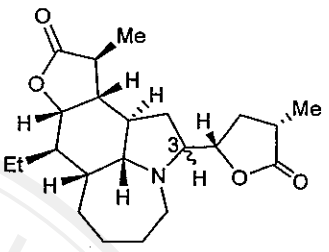
The *Stemona* alkaloids which have the tricyclic 2H-furo[3,2-*c*]pyrrolo[1,2-*a*]azepine nucleus (**II**, Fig. 2.2) are classified as belonging to the stemoaminde group. Thirteen alkaloids (Fig. 2.4): stemoamide (**15**), stemonine (**16**), neostemonine (**17**), bisdehydroneostemonine (**18**), protostemonine (**19**), dehydroprotostemonine (**20**), oxyprotostemonine (**21**), didehydroprotostemonine (**22**), isoprotostemonine (**23**), stemocochinin (**24**), tuberostemoamide (**25**), sessilifoliamide A (**26**), and stemoninine (**27**) are members of this group.



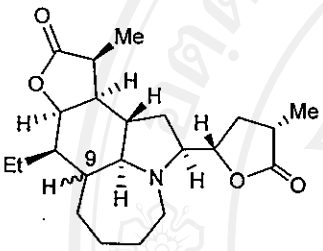
Stenine (**1**) X=H, H
2- Oxostenine (**2**) X=O



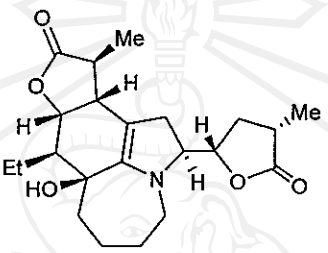
Isostenine (**3**)



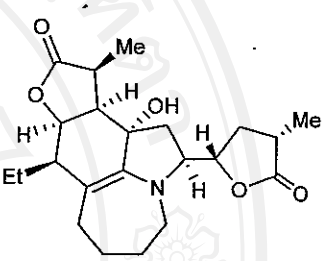
Tuberostemonine (**4**) H3α
Tuberostemonine A (**5**) H3β



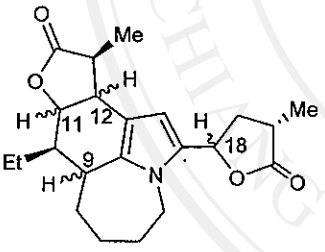
Tuberostemonine J (**6**) H9α
Tuberostemonine H (**7**) H9β



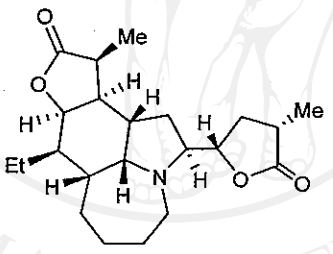
Tuberostemonol (**8**)



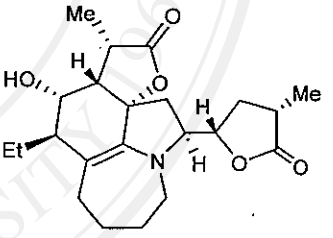
Neotuberostemonol (**9**)



Didehydrotuberostemonine (**10**)
H9β, H11β, H12β, H18β
Bisdehydroneotuberostemonine (**11**)
H9β, H11α, H12α, H18β
epi-Bisdehydroneotuberostemonine J (**12**)
H9α, H11α, H12α, H18α

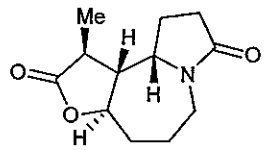


Neotuberostemonine (**13**)

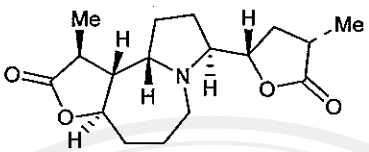


Oxotuberostemonine (**14**)

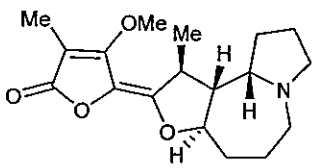
Figure 2.3 *Stemona* alkaloids of the stenine group



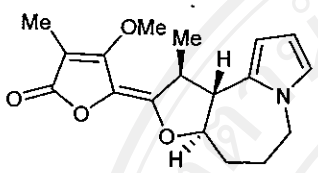
Stemoamide (**15**)



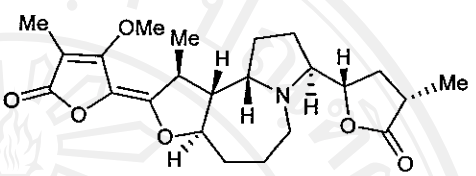
Stemonine (**16**)



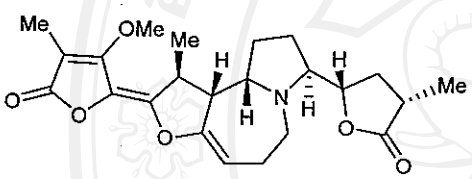
Neostemonine (**17**)



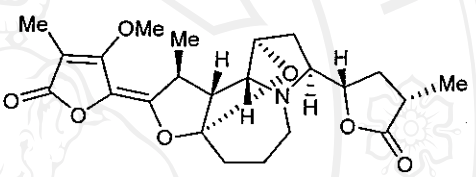
Bisdehydroneostemonine (**18**)



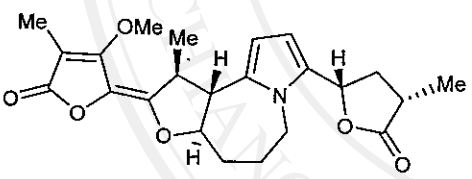
Protostemonine (**19**)



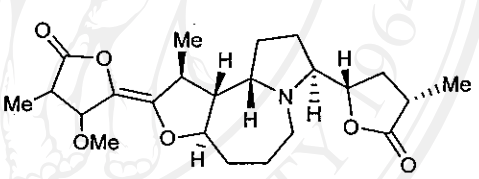
Dehydroprotostemonine (**20**)



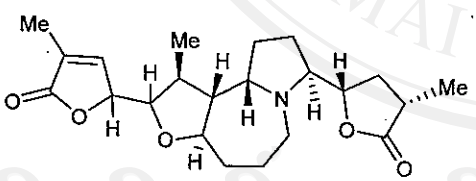
Oxyprotostemonine (**21**)



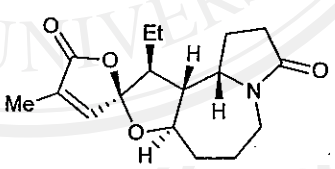
Didehydroprotostemonine (**22**)



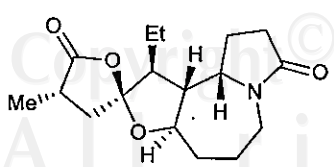
Isoprotostemonine (**23**)



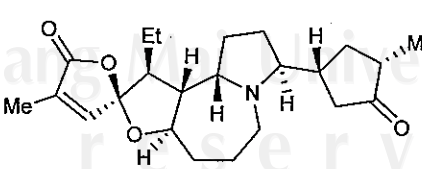
Stemocochinin (**24**)



Tuberostemoamide (**25**)



Sessilifoliamide A (**26**)



Stemoninine (**27**)

Figure 2.4 *Stemona* alkaloids of the stemoamide group

Tuberostemospironine group

The tuberostemospironine group of *Stemona* alkaloids is characterized by a spiro[furan-2-(5*H*),9′[9*H*]pyrrolo[1,2-*a*]azepin]-5-one nucleus which displays a spiro γ-lactone at C9 of the basic ring system (**III**, Fig. 2.2) and comprises seven members: tuberostemospironine (**28**), croomine (**29**), stemospironine (**30**), stemotinine (**31**), isostemotinine (**32**), stemonidine (**33**), and didehydrocroomine (**34**) (Fig. 2.5).

Stemonamine group

The structural characteristic of this group is the tetracyclic spiro[1*H*-cyclopenta[*b*]pyrrolo[1,2-*a*]azepine-11(10*H*),2′(5′*H*)-furan]-5′,10-dione skeleton with a spirolactone ring at C12 (**IV**, Fig. 2.2), which may be found in both absolute configurations. The stemonamine group includes the following *Stemona* alkaloids: stemonamine (**35**), isostemonamine (**36**), stemonamide (**37**), isostemonamide (**38**), maistemonine (**39**), and oxymaistemonine (**40**) (Fig. 2.6).

Parvistemoline group

The parvistemoline alkaloids lack the B-C ring fusion and have a hexahydro-2,6-dimethyl-5-oxofuro[3,2-*b*]furan-3-yl moiety attached to C-9 in the pyrrolo[1,2-*a*]azepine nucleus (**V**, Fig. 2.2). This group comprises the alkaloids parvistemoline (**41**), parvistemonine (**42**), didehydroparvistemonine (**43**), sessilifoliamide B (**44**), sessilifoliamide C (**45**), sessilifoliamide D (**46**), and neostemodiol (**47**) (Fig. 2.7).

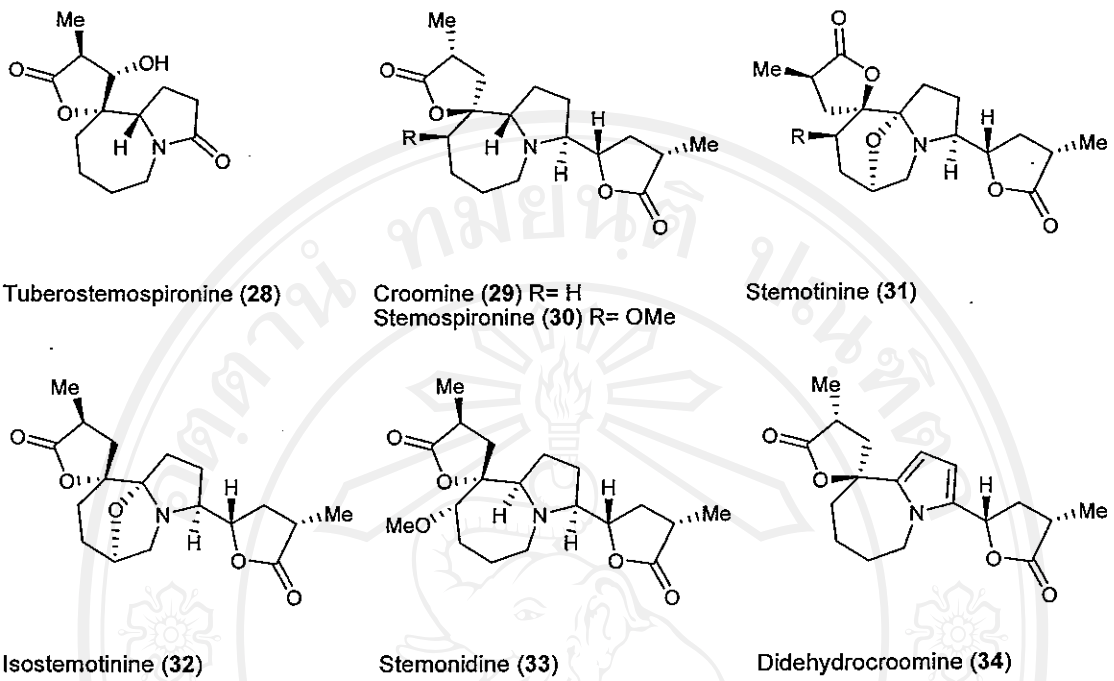


Figure 2.5 *Stemona* alkaloids of the tuberostemospironine group

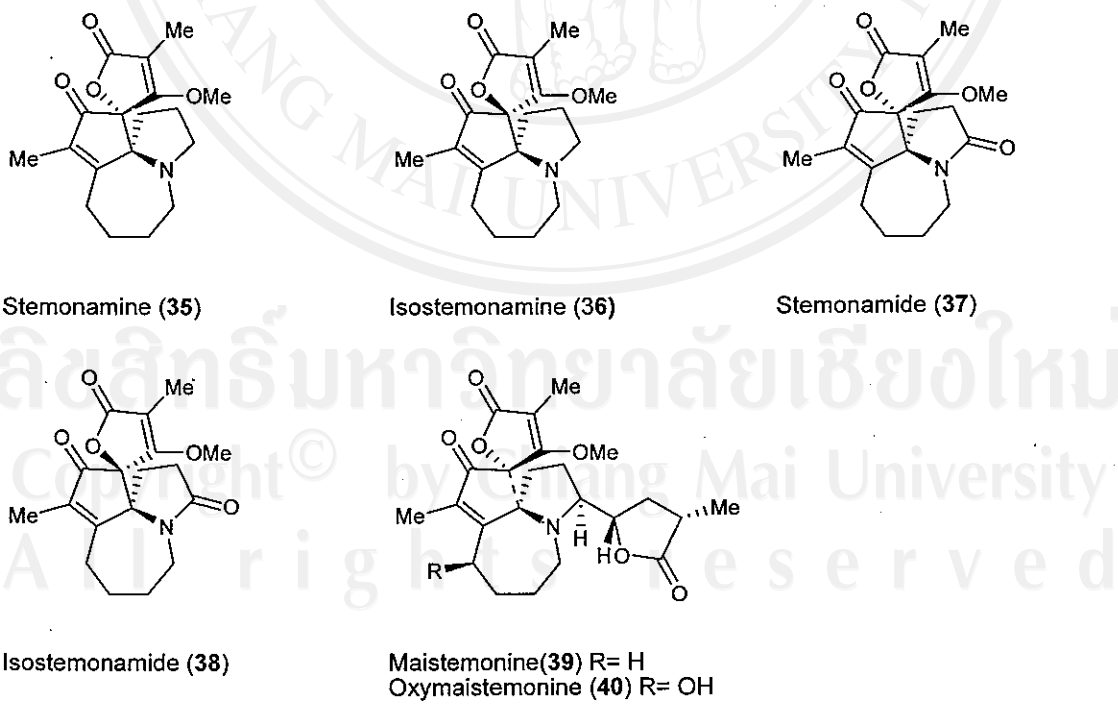
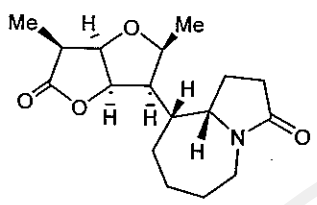
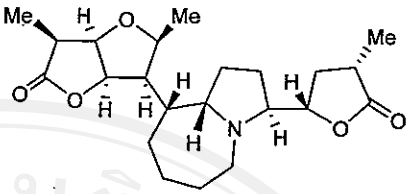


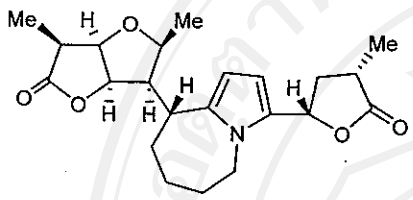
Figure 2.6 *Stemona* alkaloids of the stemonamine group



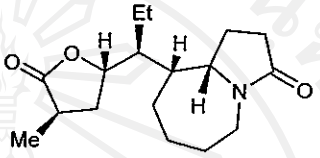
Parvistemoline (**41**)



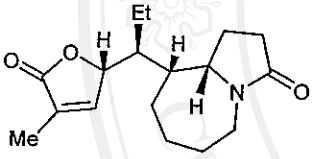
Parvistemonine (**42**)



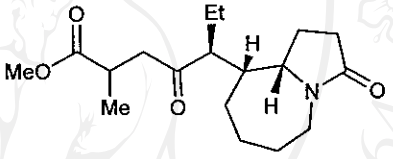
Didehydroparvistemonine (**43**)



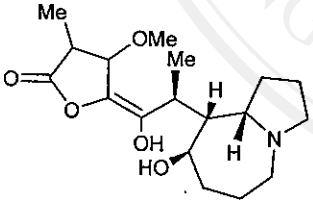
Sessilifoliamide B (**44**)



Sessilifoliamide C (**45**)



Sessilifoliamide D (**46**)

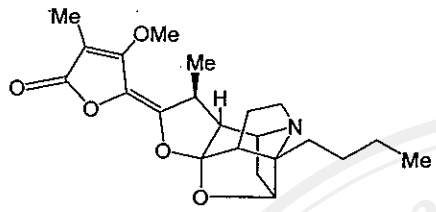


Neostemodiol (**47**)

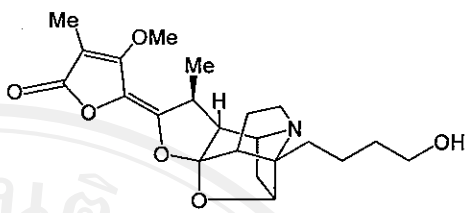
Figure 2.7 *Stemona* alkaloids of the parvistemoline group

Stemofoline group

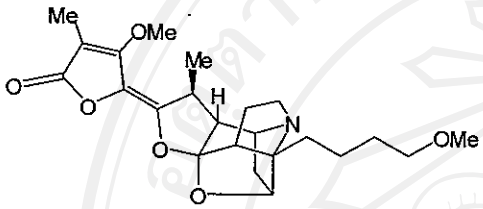
They feature a pentacyclic skeleton with an oxygen bridge between C2 and C8 and a carbon-carbon bond between C3 and C7 of the parent pyrrolo[1,2-*a*]azepine ring system with a β-methoxy-α-methyl-α,β-unsaturated γ-butyrolactone appended at C11 (**VI**, Fig. 2.2), except for stemoburkilline (**57**) which displays a tetracyclic core resulting from the formal cleavage of the C11-oxygen bond. In fact, the removal of the oxygen atom bridging C2 and C8, the bond between C3 and C7, and the side chain at C3 of alkaloids **48-50** formally leads to the stemoamide alkaloid neostemonine (**17**). Ten alkaloids have been reprorted: stemofoline (**48**), oxystemofoline (**49**), methoxystemofline (**50**), 2′*S*-hydroxystemofoline (**51**), 16,17-didehydro-16(*E*)-stemofoline (**52**), also named as didehydrostemofoline, didehydrostemofoline, 1′,2′-didehydrostemofoline or asparagamine A, 16,17-didehydro-4(*E*),16(*E*)-stemofoline (**53**), parvistemoninine (**54**), parvistemoninol (**55**), (11*S*,12*R*)-dihydrostemofoline (**56**), and stemoburkilline (**57**) (Fig. 2.8).



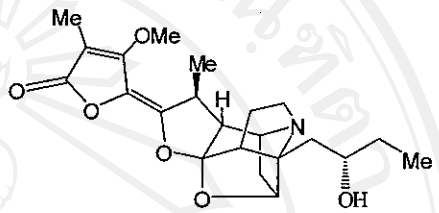
Stemofoline (**48**)



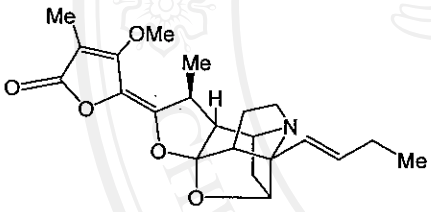
Oxystemofoline (**49**)



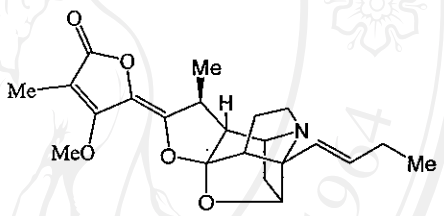
Methoxystemofoline (**50**)



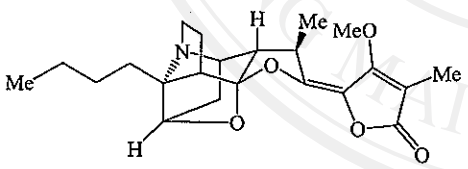
2′*S*-Hydroxystemofoline (**51**)



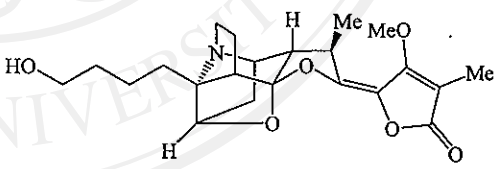
16,17-Didehydro-16(*E*)-stemofoline (**52**)



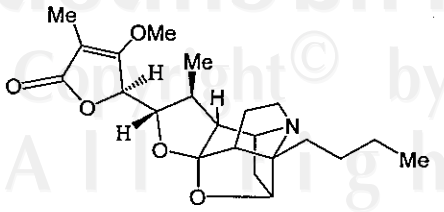
16,17-Didehydro-4(*E*),16(*E*)-stemofoline (**53**)



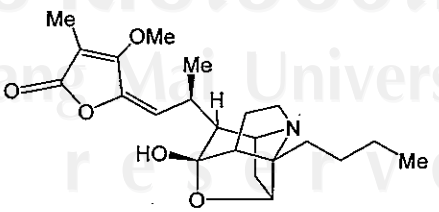
Parvistemoninine (**54**)



Parvistemoninol (**55**)



11(*S*),12(*R*)-Dihydrostemofoline (**56**)



Stemoburkilline (**57**)

Figure 2.8 *Stemona* alkaloids of the stemofoline group

Stemocurtisine group

The stemocurtisine group is characterized by the presence of the pyrido[1,2-*a*]azepine nucleus (**VII**, Fig. 2.2). This group comprises six alkaloids: stemocurtisine (**58**), also named as pyridostemin, stemokerrin (**59**), methoxystemokerrin-*N*-oxide (**60**), oxystemokerrin (**61**), oxystemokerrin-*N*-oxide (**62**), and stemocurtisinol (**63**) (Fig. 2.9).

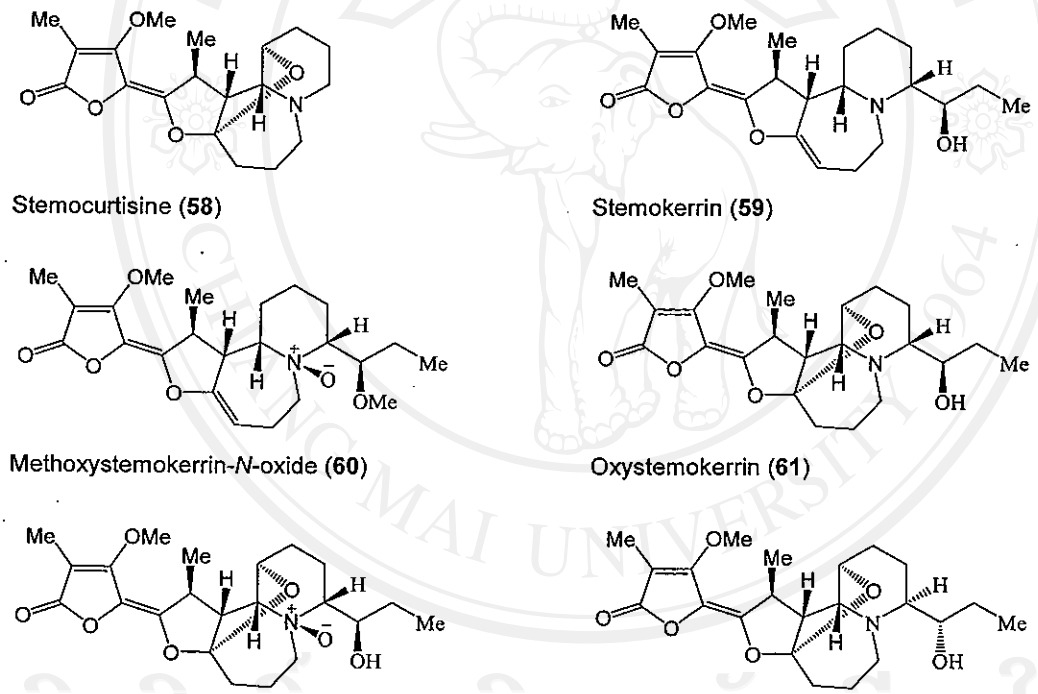
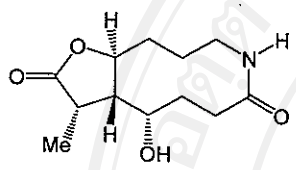


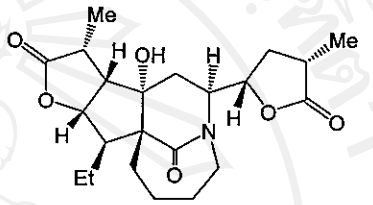
Figure 2.9 *Stemona* alkaloids of the stemocurtisine group

Miscellaneous group

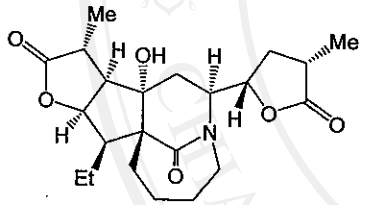
The miscellaneous group includes five *Stemona* alkaloids: parvistemoamide (**64**), tuberostemoninol (**65**), neotuberostemoninol (**66**), tuberostemonone (**67**), and parvineostemonine (**68**) (Fig. 2.10).



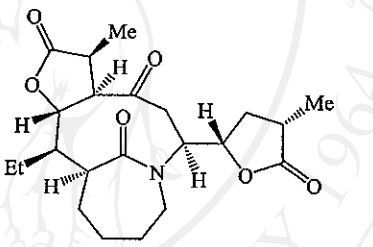
Parvistemoamide (**64**)



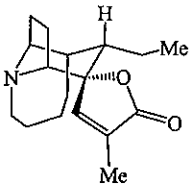
Tuberostemoninol (**65**)



Neotuberostemoninol (**66**)



Tuberostemonone (**67**)



Parvineostemonine (**68**)

Figure 2.10 *Stemona* alkaloids of the miscellaneous group

2.2.2 *Stemona* alkaloids review

Most phytochemical studies have focused on the isolation and structural elucidation of *Stemona* alkaloids which are very rich in the roots and also found in leaves, stem and rhizomes (Duyfjes, 1993). The *Stemona* alkaloids that have been reported are provided in Table 2.1.

Table 2.1 Stemona alkaloids from *Stemona* spp. (Pilli *et al.*, 2005)

Stemonaceae species	Group	*Stemona* alkaloids
S. tuberosa Lour.	Stenine (**I**)	Stenine (**1**)
		Isostenine/Neostenine (**3**)
		Tuberostemonine (**4**)
		Tuberostemonine J (**6**)
		Tuberostemonine H (**7**)
		Tuberostemonol (**8**)
		Neotuberostemonol (**9**)
		Didehydrotuberostemonine (**10**)
		Bisdehydroneotuberostemonine (**11**)
		epi-Bisdehydroneotuberostemonine J (**12**)
		Neotuberostemonine/Tuberostemonine LG (**13**)
		Oxotuberostemonine (**14**)
	Stemoamide (**II**)	Stemoamide (**15**)
		Tuberostemoamide/Stemoninoamide (**25**)
	Tuberostemospironine (**III**)	Tuberostemospironine (**28**)
		Stemotinine (**31**)
		Isostemotinine (**32**)
		Stemonidine (**33**)
	Miscellaneous (**VIII**)	Tuberostemoninol (**65**)
		Neotuberostemoninol (**66**)

Table 2.1 (continued)

Stemonaceae species	Group	*Stemona* alkaloids
S. tuberosa Lour.	Miscellaneous (**VIII**)	Tuberostemonone (**67**)
S. sessilifolia	Stenine (**I**)	Stenine (**1**)
Franch. & Sav.		2-Oxostenine (**2**)
		Tuberostemonine (**4**)
		Tuberostemonine A (**5**)
		Neotuberostemonol (**9**)
	Stemoamide (**II**)	Tuberostemoamide/Stemoninoamide (**25**)
		Sessilifoliamide A (**26**)
		Stemoninine (**27**)
	Stemonamine (**IV**)	Maistemonine/Protostemotinine (**39**)
	Parvistemoline (**V**)	Sessilifoliamide B (**44**)
		Sessilifoliamide C (**45**)
		Sessilifoliamide D (**46**)
	Miscellaneous (**VIII**)	Tuberostemonone (**67**)
		Oxotuberostemonine (**14**)
S. collinsae Craib.	Stenine (**I**)	Isostenine/Neostenine (**3**)
		Bisdehydroneotuberostemonine (**11**)
		Neotuberostemonine/Tuberostemonine LG (**13**)
	Stemofoline (**VI**)	Stemofoline (**48**)
		2′*S*-Hydroxystemofoline (**51**)
		16,17-didehydro-16(*E*)-stemofoline/
		Didehydrostemofoline (**52**)
		16,17-didehydro-4(*E*),16(*E*)-stemofoline (**53**)
S. japonica Miq.	Stemoamide (**II**)	Stemonine (**16**)
		Neostemonine (**17**)
		Bisdehydroneostemonine (**18**)

Table 2.1 (continued)

Stemonaceae species	Group	*Stemona* alkaloids
S. japonica Miq.	Stemoamide (**II**)	Protostemonine (**19**)
		Didehydroprotostemonine/
		Bisdehydroprotostemonine (**22**)
		Isoprotostemonine (**23**)
	Tuberostemospironine (**III**)	Stemospironine (**30**)
		Stemonidine (**33**)
	Stemonamine (**IV**)	Stemonamine (**35**)
		Isostemonamine (**36**)
		Stemonamide (**37**)
		Isostemonamide (**38**)
		Maistemonine/Protostemotinine (**39**)
	Parvistemoline (**V**)	Neostemodiol/Stemodiol (**47**)
	Stemofoline (**VI**)	Stemofoline (**48**)
S. cf. pierrei	Stemoamide (**II**)	Stemonine (**16**)
Gagnep.		Protostemonine (**19**)
S. cochinchinensis	Stemoamide (**II**)	Protostemonine (**19**)
Gagnep.		Stemocochinin (**24**)
	Stemofoline (**VI**)	Stemofoline (**48**)
		2′-Hydroxystemofoline (**51**)
S. mairei K. Krause	Stemoamide (**II**)	Protostemonine (**19**)
	Stemonamine (**IV**)	Maistemonine/Protostemotinine (**39**)
		Oxymaistemonine (**40**)
S. kerrii Craib.	Stemoamide (**II**)	Protostemonine (**19**)
		Dehydroprotostemonine (**20**)
		Oxyprotostemonine (**21**)
		Stemocochinin (**24**)

Table 2.1 (continued)

Stemonaceae species	Group	*Stemona* alkaloids
S. kerrii Craib.	Stemocurtisine (**VII**)	Stemokerrin (**59**)
		Methoxystemokerrin-*N*-oxide (**60**)
		Oxystemokerrin (**61**)
		Oxystemokerrin-*N*-oxide (**62**)
S. curtisii Hook.f.	Stemoamide (**II**)	Dehydroprotostemonine (**20**)
		Oxyprotostemonine (**21**)
		Stemocochinin (**24**)
	Stemofoline (**VI**)	Stemofoline (**48**)
		2′*S*-Hydroxystemofoline (**51**)
	Stemocurtisine (**VII**)	Stemocurtisine/Pyridostemin (**58**)
		Oxystemokerrin (**61**)
		Stemocurtisinol (**63**)
S. parviflora Wright C. H.	Parvistemoline (**V**)	Parvistemoline (**41**)
		Parvistemonine (**42**)
		Didehydroparvistemonine (**43**)
	Stemofoline (**VI**)	Stemofoline (**48**)
		Oxystemofoline (**49**)
		Methoxystemofoline (**50**)
		Parvistemoninine (**54**)
		Parvistemoninol (**55**)
	Miscellaneous (**VIII**)	Parvistemoamide (**64**)
		Parvineostemonine (**68**)
Stemona sp.	Stemoamide (**II**)	Stemoninine (**27**)
	Parvistemoline (**V**)	Parvistemonine (**42**)
	Stemocurtisine (**VII**)	Stemocurtisine/Pyridostemin (**58**)
		Oxystemokerrin (**61**)

Table 2.1 (continued)

Stemonaceae species	Group	*Stemona* alkaloids
Stemona burkillii Pain	Stemocurtisine (VII)	2′*S*-Hydroxystemofoline (**51**)
		Stemofoline (**48**)
		11(*S*),12(*R*)-Dihydrostemofoline (**58**)
		Stemoburkilline (**59**)
C. heterosepala Okuyama	Tuberostemospironine (III)	Croomine (**29**)
C. japonica Miq.	Tuberostemospironine (III)	Croomine (**29**)
		Didehydrocroomine (**34**)

2.2.3 Biological activities

The root extracts of *Stemona* species have been widely used as insecticides on agriculture pests and as anthelmintic agents for domestic animals. Moreover, these extracts have also used in the treatment of various respiratory diseases and used as anticough agents in China and Japan (Pilli *et al.*, 2000; Brem *et al.*, 2002;Ye *et al.*, 1994a; 1994b; 1998).

Many investigations have described the insecticidal properties of the *Stemona* species. Pilli and Ferreira de Oliveira (2000) have reported that stemonine, stemospironine and stemofoline have insecticidal activity against the fourth instar *Bombyx mori* (silkworm larvae). Additionally, other *Stemona* alkaloids such as neostemonine and isoprotostemonine were also reported to have antifeeding activity against last-instar larvae of *Spodoptera litura*. Jiwajinda *et al.* (2001) found two new alkaloids, 16,17-didehydro-16(*E*)-stemofoline and its isomer at C-4, 16-17-didehydro-4(*E*)-16(*E*)-stemofoline which displayed higher insecticidal and antifeedant activities

against the diamondback moth larvae than stemofoline. Brem *et al.* (2002) indicated that the methanolic leaf and root extracts from *S. collinsae* showed very high insect toxicity compared to *Aglaia* species and pyrethrum extract and azadirachtin. Whereas, *S. tuberosa* extracts from the root and leaves showed no activity. Moreover, *S. collinsae* extracts also showed strong antifeedant activity against fifth instar larvae in leaf disk choice, whereas *S. tuberosa* showed repellency activity. Additionally, it was found that the antiinsect properties of both species were based on pyrrolo[1,2-*a*]azepine alkaloids, namely 1′,2′-didehydrostemofoline which was the major compound of the root of *S. collinsae* and stemofoline that displayed contact toxicity and antifeedant activity. While, tuberostemonine was the major alkaloid in the root of *S. tuberosa* which demonstrated repellency but had no toxic effects. On the other hand, four new stenine-type *Stemona* alkaloids that are tuberostemonine JI, tuberostemonine HI, epi-bisdehydrotuberostemonine J and neostenine and also neotuberostemonine, which is a known compound from *S. tuberosa,* displayed antitussive activity in guinea pig (Chung *et al.*, 2003). Oxyprotostemonine, an alkaloid isolated from *S. curtisii*, showed strong larvicidal activity on mosquito larvae (*Anopheles minimus* HO). While, pure compounds, stemocurtisine and stemocurtisinol, and ethanolic crude extract from *S. curtisii* also showed the larvicidal activity on mosquito larvae (Mungkornasawakul *et al.*, 2004).

For fungicidal activity from extracts of *Stemona* species, the literature indicated that fifteen new stilbenoids and four dihydrostilbenes were extracted from a methanolic extract of *Stemona collinsae* roots and showed antifungal activity against *Cladosporium herbarum*. While, other antifungal stilbenoids were found from *S. cf. pierrei* (Kostecki *et al.*, 2004).

2.3 Insecticidal properties of alkaloids

Many alkaloids are well-known botanical pesticides such as nicotine, pyrroles and stemofoline (Godfrey, 1995; Sakata, *et al.* 1978; Dalpiaz *et al.*, 2001; Brem *et al.*, 2002). Many compounds in this group have shown antifeedant and repellent activities. Diterpenoid and norditerpenoid alkaloids were tested against *Tribolium casteneum* (Herbst.) in order to assess their repellent activity. Of these 29 tested alkaloids, 21 compounds showed promising insect repellent activity, while eight of them were not found to be active. Alkaloids were also obtained from *Delphinium*, *Consolida* and *Aconitum* species. The highest activity was found in hetisine, a diterpene alkaloid (59.37% mortality) and the lowest activity in another diterpene alkaloid, venulol (31.25% mortality) (Ulubelen *et al.,* 2001). Moreover, a new alkaloid, *N*-methyl-6-β-(deca-1′,3′,5′-trienyl)-3-β-methoxy-2-β-methylpiperidine, which was isolated from stem bark of *Microcos paniculata*, showed good insecticidal activity against *Aedes aegypti* second instar larvae (Bandara *et al.,* 2000). In the same year, the sesquiterpene pyridine alkaloids of *H. excelsa* showed at 100 ppm, 100% insecticidal activity against *Nilaparvata lugens* (Fujimoto *et al.,* 2000). Moreover, three insecticidal sesquiterpene pyridine alkaloids were isolated from the root bark of *Euonymus verrucosoides*, *E. fortunei* and *E. phellomana* by bioassay-guided fractionation (Zhu *et al.*, 2002). As well as the known sesquiterpene pyridine alkaloids from the leaves of *Maytenus chiapensis*, the alkaloids, wilfordine, alatusinine and euonine exhibited strong antifeedant activity against *Spodoptera littoralis*. (Nunez *et al.,* 2004). This activity was also found in the alkaloid extracts obtained from the bark of the African medicinal plant *Fagara macrophylla* which gave three alkaloids, oblongine, tembetarine and magnoflorine and the flavonoid,

hesperidin. Previously this plant was tested for antifeedant activity in a binary-choice bioassay and the acridone alkaloid xanthoxoline was found to have a potent antifeedant activity against larvae of both *Spodoptera frugiperda* and *S. littoralis*. While, the polar fractions of an ethanolic extract obtained from the bark of the African medicinal plant *Fagara macrophylla* led to the isolation and identification of the alkaloids and were tested for antifeedant activity in a binary-choice bioassay. (Tringali *et al.*, 2001). Besides, 1-hydroxy-3-methoxy-*N*-methyl-acridone, arborinine, tembetarine and magnoflorine were antifeedant against *S. frugiperda*. Additionally, root extracts from *Stemona collinsae* and *S. tuberosa* demonstrated insecticidal activity. In leaf disk choice tests against fifth instar larvae, *S. collinsae* showed strong antifeedant activity, whereas *S. tuberosa* was characterized by remarkable repellency. The antiinsect properties of both species were based on pyrrolo[1,2-*a*]azepine alkaloids, from which 1′,2′-didehydrostemofoline (asparagamine A) was the major compound of the roots of *S. collinsae*, exhibiting the highest toxicity in feeding assays (Brem *et al.*, 2002; Jiwajinda *et al.*, 2001). Furthermore, the screening for novel naturally occurring insecticides from Chinese traditional medicine, the methanolic extract of the fruits of *Evodia rutaecarpa* Benth, and the bark of *Phellodendron* were found to give insecticidal activity against larvae of *Drosophila melanogaster* Meigen. Three alkaloids, evodiamine, rutaecarpine, and rhetsinine, from the fruits of *Evodia rutaecarpa* Benth, and two alkaloids, berberine and palmatine, from the bark of *Phellodendron amurense* Rupr. also had insecticidal activity (Miyazawa *et al.*, 2002). Additionally, a bioactivity-directed investigation of an extract of the New Zealand clubmoss, *Lycopodium varium*, collected on subantarctic Campbell Island, has led to the isolation of the alkaloid huperzine A as the major antifeedant and insecticidal

component. Huperzine A showed weak insecticidal activity against the Australian carpet beetle, *Anthrenocerus australis* (LD_{50} 110 ppm), the Australian sheep blowfly, *Lucilia cuprina* (LD_{50} 2380 ppm), and the webbing clothes moth, *Tineola bisselliella* (LD_{50} 630 ppm). Feeding by *A. australis* was reduced by 97% at 63 ppm (Ainge *et al.*, 2002).

2.4 Plant selection criteria (Rates, 2001; Souza Brito, 1996)

The different criteria to approach the selection of a plant for insecticidal activity studies can be based on the following:

Literature reviews: The selected species are chosen based on their interesting properties that have been reported previously in the literature.

Chemotaxonomy: Plant species are selected in relation to their chemical category of compounds in a genus or family.

Ethnophamacology: The selection is based on their antipest properties used by farmers and or local people.

Random collection: This selection method often leads to the discovery of new chemical structures, including those that are biologically active or are not.

Thus, the most successful strategies for the discovery of new plant-derived compounds include plant selection along the guidelines of the above mentioned criteria, extract preparation using different solvent systems, initial primary biological screening of crude extracts and separation and screening of photochemicals, especially bioassay-guided separations.

There are about 10 species of *Stemona* growing in Thailand, there habitat is deciduous and green forests. The characteristic of each species is different and these

have been described in the literature (Maxwell, 1991; Duyfjes, 1993). For chemical investigation, the major components were *Stemona* alkaloids (Pilli and Ferreira de Oliveira, 2000).

In this study, according to the insecticidal properties reviews, three different *Stemona* spp. i.e. *S. curtisii* and 2 unknown species were studied in order to select the most appropriated plant specie to develop a new insecticidal formulation and to search for new active compounds.

***Stemona curtisii* Hook F.**

S. curtisii (Fig. 2.11) is distributed in Sri Lanka (rare), Thailand and Malaysia. It is a glabrous twiner and has 10 cm long tuberous roots, which form a bundle. It has alternate, seldom opposite leaves. Flowers are tepars pink, brown pink and brownish red. This species can be found near waterfalls, the shore and on the riverbanks (Duyfjes, 1993). A few biological activities have been reported. The effect of the crude root extract on the action potential of the frog sciatic nerve and its toxicity on house fly larvare (*Culex p. fatigans* and *Aedes aegypti*) have been reported (Prucksunand *et al.*, 1977). In addition, the larvicidal acivity of the crude root extract and that of compounds, stemocurtisine, stemocurtisinol and oxyprotostemonine on mosquito larvae (*Anopheles minimus* HO) have been reported (Mungkornasawakul *et al.*, 2004).

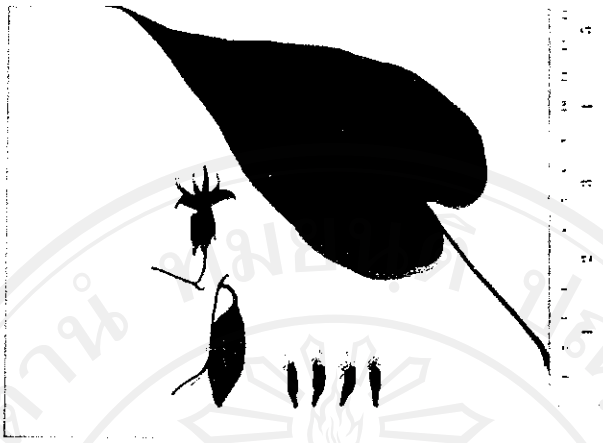


Figure 2.11 *S. curtisii*

2.5 Herbal plant extraction techniques

There are 4 steps of extraction i.e. raw material preparation, the extraction process, the evaporation process and packaging (Department of Environmental Quality Promotion, Ministry of Natural Resources and Environment, 2006).

2.5.1 Raw material preparation

Drying: In general, 3-4 days drying in the sun is preferred. However, a large area is required. If it rains, the material will be damaged. Application of an oven can speed up the drying and allows working with a large quantity of plant material within a shorter period.

Grinding or cutting into small pieces: Grinding or cutting the material into small pieces will facilitate the extraction. They should not be kept in large amount since the active substances will decompose due to the interaction with moisture and the outside environment. Materials should be prepared not so long before extraction.

2.5.2 Extraction process

The plant material was macerated in 95% ethanol (plant material: ethanol = 1g: 4ml) for 3-4 days until the extract color became dark brown. The extract was

filtered to separate the extract and plant material. The same batch of plant material underwent this extraction process three times.

2.5.3 Evaporation process

Evaporation was performed to eliminate the alcohol from the extract. The evaporation tank consisted of 2 chambers. The outer chamber contained hot circulating water transferring heat to the inner chamber containing the dilute extract. Evaporation was done under a vacuum and the temperature was regulated as not to exceed 55 °C. The crude extract obtained was dark brown and slightly viscous.

2.5.4 Packaging

The plant extract was kept in an opaque container and under nitrogen to avoid degradation of the active compounds caused by light and oxygen. The cap or lid was sealed tightly to prevent microbial growth and contamination. Details of the product, including the production date were included on the labels of samples for quality assurance purposes.

An additive or preservative was sometimes added to improve and maintain the efficiency of the extract and to prolong the shelf life.

2.6 Bioassays

The approach for the detection of biological activity of natural product mixtures can be divided into two groups for screening purposes: general screening bioassays and specialized screening bioassays. The aims of the screening program depended on either a general screening or a specific assay which the effect on the target organism can be found.

2.6.1 General screening bioassays

The two most popular general screening bioassays are the brine shrimp lethality test and the crown-gall tumor inhibition test (Ghisalberti, 1993; McLaughlin, 1991). The first technique is an in *vivo* lethality test on a tiny crustacean, the brine shrimp (*Artemia salina*). Since its introduction in 1982 (Meyer *et al.*, 1982), this test has been used for the detection in *vivo* of active antitumour agents and pesticides produced by plants. However, it can also be used to evaluate plants for different pharmacological activities (McLaughin, 1991). The details of the crown-gall tumor inhibition test have been described in the literature (McLaughlin, 1991). Herbicidal, insect-antifeedant, larvicidal and molluscidal activities can been determined by simple bioassays, which can function as surrogate assays to isolate bioactive compounds from plant extracts. However, it should be reminded that primary bioassays provide only preliminary information, which always should be checked in more appropriate specialized bioassays.

2.6.2 Specialized screening bioassays

Specialized screening bioassays can be subdivided according to the target organisms which are used in the model (Hostettmann *et al.*, 1995). These can be lower organisms (e.g. microorganisms), insects, mollusks, isolated intact cells of human or animal origin, isolated organs or vertebrates, or whole animals.

Specialized bioassays must be sensitive in a dose dependent fashion to standard compounds that are known to possess the desired therapeutic property. Additionally, the relative potency of known active agents in the bioassay should be comparable to their relative potency in clinical use and the bioassay should be selective (Vogel and Vogel, 1997).

2.6.3 Assay for detecting insecticidal activity

Topical application

Many methods have been used for detecting insecticidal activity. A common method is topical application. The insecticide is dissolved in a relatively nontoxic solvent, such as acetone, and is applied at a chosen location on the body surface as small, measured droplets. Topical application as is practiced today was made possible by the invention of the micrometer-driven syringe.

Injection method

This method is used for applying the exact amount of insecticide inside the body of an insect. For this, very fine stainless steel needles of 27 or 30 gauge (0.41 or 0.30 mm in diameter) are used. Small glass needles of 0.1-0.16 mm in diameter may be used for injection into smaller insects. The insecticide is commonly dissolved in propylene glycol or peanut oil and an injection is made into the body cavity.

Dipping method

This method is used with small plant-feeding insects, store-product insects, housefly larvae, insect eggs, red spiders etc. The insects are dipped in aqueous solutions, emulsions, or suspensions of the chemical or target substances for a short period of time. In this case, the LC_{50} is used to express the results.

Contact or residual method

The insecticide in a volatile solvent is applied to a glass container such as a vial or a jar. The solvent is allowed to evaporate by rotating the container so that the insecticide is spread evenly over the entire surface leaving a residual film. Alternatively, the insecticide is applied evenly on a glass, filter paper, wood panel or other types of building materials and allowed to dry before exposing the insects to the

residual deposits. The deposits are express as milligrams or grams of active ingredient per square meter.

Feeding and drinking method

This method was used to evaluate the toxicity of ingested chemicals. The target substances were added drink and then fed to insects.

2.7 Formulation of insecticides

Formulation is the processing of a compound by such methods that will improve its properties of storage, handing, application, effectiveness and safety to the applicator and the environment, and profitability. Formulation is the final physical condition in which the insecticide is sold commercially. In most cases, it must be diluted according to the formulator's instructions before used. The price for a given weight of chemical depends largely on the type of formulation, the most expensive being the pressurized aerosol. The common formulations (Perry *et al.*, 1998) were defined as following:

2.7.1 Emulsifiable concentrates (EC)

More than 75% of all insecticide formulations are applied as sprays. The majority of these are water emulsions prepared from emulsifiable concentrations, which are solutions of the technical-grade material in an appropriate organic solvent with enough emulsifier added to allow the concentration to mix readily with water for spraying. When an emulsifiable concentrate is added to water, the emulsifier causes the oil to disperse uniformly throughout the water phase, giving a milky appearance when agitated. This oil in water suspension is a normal emulsion.

Emulsifiable concentrates, if properly formulated, should remain stable without further agitation for several days after dilution with water. If a precipitate forms after 24 h, a small amount of detergent or emulsifier can be added and mixed thoroughly to improve its quality.

Most domestic insecticides are formulated as emulsifiable concentrates and have a shelf life of approximately 3 years.

2.7.2 Flowables (F or L)

A flowable or liquid can be mixed with water to form a suspension in a spray tank.

2.7.3 Wettable powders (WP or W)

Wettable powder formulations are made by combining the active ingredient with a fine powder. They look like dusts, but they are made to mix with water. These formulations need continuous agitation to maintain a suspension and are thus difficult for home gardeners to use. When mixing a WP, one first mixes the measured quantity with a small amount of water with stirring and then adds it and additional water to a spray tank. The spray tank must be frequently shaken to maintain the suspension.

2.7.4 Soluble powder (SP)

A soluble powder formulation is made from an active ingredient in powder form that dissolves in water.