CHAPTER 1
INTRODUCTION

1.1 General introduction

One of major problems in agricultural countries is the pest management. For several decades, chemical pesticides have been considered the predominant method of controlling pests in many countries (Khambay and Jewess, 2000). The synthetic pesticides are known to be very stable in almost conditions that may cause harm to human and environment (Bessette, 2006; Marrs and Ballantyne, 2004; Plimmer, 2003; Wilson, 2003). To reduce the hazard to human, environment and purchase cost, using natural pesticides from plant is an alternative strategy for pest control. Searching for new pest controlling agents from natural products such as plant secondary metabolites has gained popularity among researchers in countries with a strong herbal tradition. Large number of plants have been shown to possess insecticidal activity (Curtis *et al.*, 1989; Sukumar *et al.*, 1991; Yang and Tang, 1988), such as neem, tobacco, syringa, hellebore, quassia, camphor, turpentine, bearberry, calamus, nepal ivy, garlic, pepper fruit, pyrethrum, derris, and stemona (Benezet *et al.*, 1986; Bessette, 2006; Charleston *et al.*, 2006; Egwunyenga *et al.*, 1998; Kaltenegger *et al.*, 2003; Long, 1998; Sae-yun *et al.*, 2006; Tewary *et al.*, 2005). Insecticidal compounds from plants such as pyrethrin, rotenone, nicotine, azadirachtin, salannin, veratrine, and ryanodine have been extensively used and developed in the form of pesticide products for handling qualities enhancement (Balandrin, 1985; Martin *et al.*, 2004; Pedigo, 1996).

Stemona spp. have long been recognized for its bioactivities (Kaltenegger *et al.*, 2003). It has been used in traditional Asian medical practices for the treatment of inflammatory-related diseases such as cough relief, and anti-asthmatics (Do, 2001; Vo, 2001). The root tubers of *Stemona* have been examined phytochemically and were shown to contain various pyrido[1,2-a]azepine alkaloids and related derivatives with high insect toxicity or insect-repelling activity (Brem *et al.*, 2002; Kaltenegger *et al.*, 2003; Mungkornasawakul *et al.*, 2004b; Pilli and Oliveira, 2000). *Stemona* might also provide selective agricultural pesticides without halmful side effects toward non-target mechanisms (Brem *et al.*, 2002).

Figure 1. *Stemona curtisii* Hook f.

Stemona curtisii is a herbaceous plant found in the Southern region of Thailand. Stem are slender, twining, and climbing high. Leaves are alternate, oval or broadly oval in outline, tip with an abrupt, narrow point, base broadly heart-shape, usually about 4 inch × 3 inch, but and sometime reaching twice this size, ribs 9-13, and leaf-stalk to about 3 inch long. Flowers are nodding, in a cluster at the end of a slender stalk up to about 3.5 inch long, the individual flower-stalks to about 0.75 inch long, and jointed in the middle. The perianths are about 1 inch, narrow, deep purple pink, the lobes narrowly elliptical in outline, and their tips spreading. There are stamens with short stalks, and a long, narrow appendage projecting above the anthers. The capsules are about 1 inch long with a short beak (Henderson, 1974).

This plant is known in the Thai vernicular as "Non Tai Yak" and has previously been used by rural people of Thailand as a useful herbal medicine for treatment

of respiratory disorders and cancer (Limtrakul *et al.*, 2007). Kaltenegger *et al.* (2003) investigated the insecticidal capacities of the crude extract from *Stemona* species. *S. curtisii* extract was clearly proved to be high active with LC_{50} values of 9 ppm against neonate larvae of *Spodoptera litoralis*. This high activity can be attributed to the major compound stemofoline similar to that found in *Stemona collinsae* (Brem *et al.*, 2002).

Nowadays, the *Stemona* products have not been developed. Thai agriculturists usually soak the roots of *Stemona* spp. in water and use the resulting milky solution for spraying in their field. However, this method is inconvenient and the effectiveness in pest control cannot be predicted, since the amount of active substance may be varied and unstable.

To make the *Stemona* spp. extract convenient and effective to use, a suitable type of formulation must be developed. Therefore, several aspects such as extraction, conservation of the extracts, stability and cost should be taken into consideration for the formulation development. All these aspects are understood when the main substances contained in the extract are identified and monitored. However, the present knowledge is lacked in a high-performance liquid chromatographic (HPLC) method for determination of the major component among its related derivatives of the *S. curtisii* extract. Specially, the quantification of the marker is well known to be the one of the most important process in the formulation development. Attempts were made to develop HPLC method for quantitative determination of the major compound in the *S. curtisii* extract and examine the stability of this compound. Furthermore, water dispersible granules and emulsifiable concentrate containing *S. curtisii* extract were prepared and evaluated for their physico-chemical properties and stabilities.

Hence, the objectives of this present study were:

1. To prepare *S. curtisii* extract, separate, and determine the major compound in the extract
2. To develop the natural pesticide water dispersible granules and emulsifiable concentrate containing *S. curtisii* extract
3. To study the physicochemical properties and stability of the developed formulations

1.2 Review of literatures

1.2.1 Pesticides

The word pesticide is an all-inclusive term that includes a number of individual chemicals designed specifically for the control of certain pests. Table 1 shows the pesticides as classified by their target species, with 21 generic terms for each specific purpose. However, six of these generic words end with -cide, which means "to kill" or "killer" (Bohmont, 2000).

Table 1. Pesticides as classified by their target species (Bohmont, 2000)

Types of pesticides	Target species
Acaricide	Mites, ticks
Algicide	Algae
Attractant	Insects, birds, other vertebrates
Avicide	Birds
Bactericide	Bacteria
Defoliant	Unwanted plant leaves
Desiccant	Unwanted plant tops
Fungicide	Fungi
Growth regulator	Insect and plant growth
Herbicide	Weeds
Insecticide	Insects
Miticide	Mites
Molluscicide	Snails, slugs
Nematicide	Nematodes
Piscicide	Fish
Predacide	Vertebrates
Repellents	Insects, birds, other vertebrates
Rodenticide	Rodents
Silvicide	Trees and woody vegetation
Slimicide	Slime molds
Sterilants	Insects, vertebrates

Most of the specific pesticides (insecticides, herbicides, or fungicides) are specific to the pest for which they are intended to control. The term pesticide, as defined by federal and state laws in the United States, also includes chemical compounds known as growth regulators that stimulate or retard the growth of plants; those known as desiccants that speed the drying of plants and are used as an aid in mechanical harvesting of cotton, soybeans, and other crops; and those chemicals known as defoliants that remove leaves and aid in the harvesting of potatoes and certain other crops. The term pesticide also applies to compounds used for repelling, attracting and sterilizing insects. The last two groups do not fit with the original definition, hence, they are not end with "-cide". Some pesticides can be used for several purposes. For example, some insecticides can also be nematicides and bird repellent. Since, pesticides will kill all life forms of specific target therefore, they need to be used with extreme care and caution (Bohmont, 2000).

1.2.2 Pesticide formulation

Nowadays the natural pesticides as original are not applicable, due to they cannot be added directly to water or mixed in the field with solids. Since any naturally occurring pesticide is a mixture of ingredients including not only the active component but also the additives. The manufacturer must modify the product by combining it with other materials such as solvents, adjuvants and fillers. This mixture of active and additive is called a pesticide formulation (Bohmont, 2000; Morgan, 1992).

The natural pesticides are usually formulated in a manner that (1) increase pesticide effectiveness in the field, (2) improves safety features, and (3) enhances handling qualities (Martin *et al.*, 2004).

Pesticide formulations are classified as solids or liquids on the basis of their physical state in the container at the time of purchase (Table 2). A formulation can contain more than one active ingredient and many have to be further diluted with an appropriate carrier (e.g., water) prior to use.

Table 2. Classification of pesticide formulations (Bohmont, 2000; Martin *et al.*, 2004; Morgan, 1992)

Physical state	Formulation	Description
Solid	Dusts	- manufactured by the sorption of an active ingredient onto a finely-ground, solid inert material such as talc, clay, nut, hull or chalk - size of individual dust particle is variable - relatively easy to use because no mixing is required and the application equipment (e.g., hand bellows and bulb dusters) is lightweight and simple
	Wettable powders	- Active ingredient is sorbed on finely divided solids, typically mineral clays. - contained wetting and dispersing agents - diluted with water and applied as a liquid spray - upon dilution, a suspension is formed in the spray tank
	Pellets	- Active ingredient is combined with inert materials to form slurry (a thick liquid mixture). This slurry is then extruded under pressure through a die and cut at desired lengths to produce a particle that is relatively uniform in size and shape. - typically used in spot applications
	Granules	- manufactured by the sorption of an active ingredient onto a large particle, solid inert material such as clay, sand or ground plant materials - applied dry - usually are intended for soil applications where they have the advantage of weight to carry them through foliage to the ground below

Table 2. Classification of pesticide formulations (continued)

Physical state	Formulation	Description
Solid (continued)	Dry flowables or water dispersible granules	- manufactured in the same way as wettable powders except that the powder is aggregated into granular particles - diluted with water and applied in a spray - upon dilution, a suspension is formed in the spray tank
	Soluble powders	- mixed with water prior to spraying - dissolve in the spray tank, and form a true solution
Liquid	Liquid flowables	- manufactured in the same way as wettable powders with the additional step of mixing the powder, dispersing agents, wetting agents, etc., with water before packaging - diluted with water before use
	Solutions or water-soluble concentrates	- water-soluble active ingredients dissolved in water for sale to the applicator for further dilution prior to field application - form a true solution in the spray tank and require no agitation after they are thoroughly dissolved
	Emulsifiable concentrates	- A homogeneous liquid formulation which forms an emulsion on mixing with water - consist of an oil-soluble active ingredient dissolved in an appropriate oil-based solvent to which is added an emulsifying agent - emulsifiable concentrates are mixed with water and applied as a spray - form an emulsion in the spray tank

Table 2. Classification of pesticide formulations (continued)

Physical state	Formulation	Description
Aerosols and Fumigants	Aerosols	- A delivery system that moves the active ingredient to the target site in the form of a mist of very small particles: solids or liquid drops. - Particles can be released under pressure or produced by fog or smoke generators. - useful for indoor insect control, as coverage is thorough - difficult to confine the aerosol to the target area and danger of inhalation
	Fumigants	- Active ingredient is delivered to the target site in the form of a poison gas. - Some active ingredients are liquids and become gases under high pressure. - Some active ingredients are volatile liquids when enclosed in an ordinary container and become gases during application. - Others are solid that release gases when applied under conditions of high humidity or in the presence of water vapor. - completely fill a space and many have tremendous penetrating power - The most hazardous pesticide products to use due to their extreme inhalation danger.

Water dispersible granules are also called dry flowable, which is a formulation of granular dimensions contain granules made up of finely divided solids (Brecke and Unruh, 2003). Water dispersible granule formulations consist primarily of wetting agent, dispersant, diluent and a binder other than the active ingredient. Disintegrant and antifoam are added as supplements, when necessary (Ho *et al.*, 2007). Granules

present low inhalation hazard to applicator, good flowability, and can be measured easily, in contrast to dust (Martin *et al.*, 2004). Azatin® 0.265E (Fermone) is an example of botanical pesticide in water dispersible granule formulation consisted of azadiractin, an extract from *Azadirachta indica*.

Emulsifiable concentrated pesticides are a general formulation used in the field (Chan *et al.*, 2007). Emulsifiable concentrates are concentrated oil solutions of technical grade material with enough emulsifier added to make the concentrate mix readily with water for application and are applied as water emulsions (Kim *et al.*, 2003; Ware, 1983). There are natural pesticides which were developed as emulsifiable concentrates for effectiveness pesticides such as pyrethrum from *Chrysanthemum cinerariaefolium*, neem oil from *Azadirachta indica*, essential oil extract from *Chenopodium ambrosioides* (Edgecomb *et al.*, 2006; Murphy, 2005; Waghmare *et al.*, 2007). Some of them present commercially such as Neemarin® (Biotech International Ltd.), Neem soil conditioner® (IndiaMART InterMESH Ltd), Neem 47® (Nim Distribution Center AB), Pyrellin® EC Insecticide (Webb Wright cooperation), Evergreen® Crop Protection EC 60-6 (McLaughlin Gormley King Co. Inc.), PyGanic® Crop Protection EC 5.0 II (McLaughlin Gormley King Co. Inc.) and Natural Pyrethrin EC pesticide® (Beijing Austin Trading).

1.2.3 *Stemona* plant

Stemona represents the largest genus of the small monocotyledonous family, Stemonaceae. Many species prefer a seasonal climate and occur as perennial climbers or subshrubs with tufted tuberous roots in rather dry vegetation ranging from continental Asia and Japan through Southeast Asia to tropical Australia (Duyfjes, 1993; Tsi and Duyfjes, 2000). Various species from the genus *Stemona* (Stemonaceae) have long been used in traditional Asian medical practices (such as Chinese, Japanese, Vietnamese, and Thai) to treat respiratory diseases, antifungal, insecticides, and anticancer (Pilli and Oliveira, 2000). "Baibu", the dried tuber root of *Stemona sessilifolia* (Miq.) Miq., *Stemona japonica* (Bl.) Miq., or *Stemona tuberosa* Lour., are listed in the Chinese Pharmacopoeia and used to relieve cough and kill insects and worms (Chinese Pharmacopoeia Commission, 2000). In Vietnamese folk medicine, *S. tuberosa* Lour., *S.*

collinsae Craib, *Stemona saxorum* Gagnep., *Stemona pierrei* Gagnep., *and Stemona cochinchinensis* Gagnep. have been used for cough relief and as antiasthmatics (Do, 2001; Vo, 2001). The roots of *S. japonica* and *S. sessilifolia* have been used as insecticides and anthelmintics in the East, and are known to possess antitubercular and antitussive activities (Cong *et al.*, 1995; Ye *et al.*, 1994).

More recently, extracts and pure alkaloids derived from extracts of the leaves and roots of *S. collinsae* and *S. tuberosa* were shown to have antifungal, insect toxicity, antifeedant, and repellent activities (Brem *et al.*, 2002; Kaltenegger *et al.*, 2003; Pacher *et al.*, 2002). There are recent reports of free radical scavengers of four new dehydrotocopherols (chromenols) isolated from the roots of various Stemonaceae species including *S. curtisii*, *S. tuberosa* and *S. collinsae* (Brem *et al.*, 2004). In addition, the root extracts of *S. tuberosa* showed an enhancement of apoptosis for its anti-tumor effect on medullary and thyroid carcinoma cells which is known to be relative resistant to chemotherapy (Rinner *et al.*, 2004). Moreover, there was a report of *S. curtisii* extract modulated P-glycoprotien activity that may play an importance role as a P-glycoprotien modulator and may be effective in the treatment of multidrug-resistance cancers (Limtrakul *et al.*, 2007). *Stemona* spp. have been phytochemical studies and a number of pure compounds were evaluated for biological activities. The chemical constituents found in *Stemona* spp. are demonstated in Table 3.

Table 3. Chemical constituents of *Stemona* spp.

Chemical substance	Bioactivity	*Stemona* sp.	References
Alkaloids			
bisdehydro-neotuberostemonine	-	*S. collinsae*	Pham *et al.*, 2002
bisdehydroneostemoninine	-	*S. tuberosa*	Lin *et al.*, 2006
bisdehydroneotuberostemonine	-	*S. mairei*	Cai and Luo, 2007
bisdehydrostemoninine	antitussive activity	*S. tuberosa*	Lin *et al.*, 2006
bisdehydrostemoninine A	-	*S. tuberosa*	Lin *et al.*, 2006
bisdehydrostemoninine B	-	*S. tuberosa*	Lin *et al.*, 2006
bisdehydrotuberostemonine	-	*S. mairei*	Cai and Luo, 2007

Table 3. Chemical constituents of *Stemona* spp. (continued)

Chemical substance	Bioactivity	*Stemona* sp.	References
Alkaloids (continued)			
epi-bisdehydro-tuberostemonine J	-	*S. tuberosa*	Chung *et al.*, 2003
cochinchistemoninone	-	*S. saxorum*	Wang *et al.*, 2007c
croomine	antitussive activity	*S. tuberosa*	Jiang *et al.*, 2006; Xu *et al.*, 2006
dehydroprotostemonine	insecticidal activity (*Spodotera littoralis*, LC_{50}=6.1ppm)	*S. kerrii* *S. curtisii* *S. saxorum*	Kaltenegger *et al.*, 2003; Wang *et al.*, 2007c
11(*S*),12(*R*)-dihydrostemofoline	-	*S. burkillii*	Mungkornasakul *et al.*, 2004a
2-oxostenine	-	*S. mairei*	Cai and Luo, 2007
saxorumamide	-	*S. saxorum*	Wang *et al.*, 2007c
16,17-didehydro-16(*E*)-stemofoline	insecticidal activity (3rd instar larvae of *Plutella xylostella*, LD_{50}=0.63μg/cm^2; *S. littoralis*, LC_{50}=0.8ppm)	*S. collinsae*	Jiwajinda *et al.*, 2001; Kaltenegger *et al.*, 2003
16,17-didehydro-4(*E*)-16(*E*)-stemofoline	-	*S. collinsae*	Jiwajinda *et al.*, 2001
epoxytuberostemonone	-	*S. mairei*	Cai and Luo, 2007
6-hydroxycroomine	-	*S. tuberosa*	Schinnerl *et al.*, 2005
2′-hydroxystemofoline	insecticidal activity (*S. littoralis*, LC_{50}=30ppm)	*S. burkillii* *S. burkillii* *S. collinsae* *S. cochinchinensis* *S. curtisii*	Kaltenegger *et al.*, 2003; Mungkornasakul *et al.*, 2004a; Schinnerl *et al.*, 2007
isobisdehydrostemoninine	-	*S. tuberosa*	Lin *et al.*, 2006
isomaistemonine	-	*S. saxorum*	Wang *et al.*, 2007c
isoprotostemonine	-	*S. saxorum*	Wang *et al.*, 2007c
isosaxorumamide	-	*S. saxorum*	Wang *et al.*, 2007c
isostemotinine	-	*S. tuberosa*	Xu *et al.*, 1982

Table 3. Chemical constituents of *Stemona* spp. (continued)

Chemical substance	Bioactivity	*Stemona* sp.	References
Alkaloids (continued)			
isostemonamine	-	*S. saxorum*	Wang *et al.*, 2007c
isostenine	-	*S. collinsae*	Pham *et al.*, 2002
methoxystemokerrin-*N*-oxide	insecticidal activity (*S. littoralis*, LC_{50}= > 100ppm)	*S. kerrii*	Kaltenegger *et al.*, 2003
maireistemoninol	-	*S. mairei*	Cai and Luo, 2007
maistemonine	-	*S. saxorum*	Wang *et al.*, 2007c
neotuberostemonine	antitussive activity	*Stemona sp.*	Adams *et al.*, 2005;
		S. collinsae	Pham *et al.*, 2002;
		S. tuberosa	Chung *et al.*, 2003;
		S. mairei	Cai and Luo, 2007
neotuberostemonone	-	*S. mairei*	Cai and Luo, 2007
neostenine	antitussive activity	*S. tuberosa*	Chung *et al.*, 2003
neotuberostemoninol	-	*S. tuberose*	Jiang *et al.*, 2002;
		S. mairei	Cai and Luo, 2007
neotuberostemonol	-	*S. tuberosa*	Jiang *et al.*, 2002
oxyprotostemonine	insecticidal activity (*S. littoralis*, LC_{50}=159ppm)	*S. curtisii*	Kaltenegger *et al.*, 2003; Mungkornasakul *et al.*, 2004b; Wang *et al.*, 2007c
		S. kerrii	
		S. saxorum	
oxystemokerrin	insecticidal activity (*S. littoralis*, LC_{50}=5.9ppm)	*S. curtisii*	Kaltenegger *et al.*, 2003; Schinnerl *et al.*, 2007; Wang *et al.*, 2007c
		S. kerrii	
		S. saxorum	
oxystemokerrilactone	-	*S. saxorum*	Wang *et al.*, 2007c
oxystemokerrin-*N*-oxide	insecticidal activity (*S. littoralis*, LC_{50}=12.5ppm)	*S. curtisii*	Kaltenegger *et al.*, 2003; Schinnerl *et al.*, 2007; Wang *et al.*, 2007c
		S. kerrii	
		S. saxorum	

Table 3. Chemical constituents of *Stemona* spp. (continued)

Chemical substance	Bioactivity	*Stemona* sp.	References
Alkaloids (continued)			
protostemonine	insecticidal activity (*S. littoralis*, LC_{50}=17.7ppm)	*S. pierrei* *S. sessilifolia* *S. japonica* *S. parviflora* *S. curtisii* *S. kerrii* *S. cochinchinensis* *S. saxorum*	Cong *et al.*, 1995; Kaltenegger *et al.*, 2003; Kostecki *et al.*, 2004; Schinnerl *et al.*, 2007; Wang *et al.*, 2007c
sessilifoliamide I	-	*S. sessilifolia*	Hitotsuyanagi *et al.*, 2007
sessilifolines A	-	*S. sessilifolia*	Qian and Zhan, 2007
sessilifolines B	-	*S. sessilifolia*	Qian and Zhan, 2007
sessilistemonamines A	acetylcholinesterase (AchE) inhibition IC_{50}= 68.8 μM	*S. sessilifolia*	Wang *et al.*, 2007a
sessilistemonamines B	acetylcholinesterase (AchE) inhibition IC_{50}= 17.1 μM	*S. sessilifolia*	Wang *et al.*, 2007a
sessilistemonamines C	-	*S. sessilifolia*	Wang *et al.*, 2007a
sessilistemonamines D	-	*S. sessilifolia*	Wang *et al.*, 2007b
stemoburkilline	-	*S. burkillii*	Mungkornasakul *et al.*, 2004a
stemocochinin	insecticidal activity (*S. littoralis*, LC_{50}=170ppm)	*S. curtisii* *S. kerrii* *S. cochinchinensis* *S. saxorum*	Kaltenegger *et al.*, 2003; Wang *et al.*, 2007c
stemocurtisine (pyridostemin)	insecticidal activity (*S. littoralis*, LC_{50}=149ppm)	*S. curtisii*	Schinnerl *et al.*, 2007; Mungkornasakul *et al.*, 2003
stemocurtisinol	-	*S. curtisii*	Mungkornasakul *et al.*, 2004b; Schinnerl *et al.*, 2007

Table 3. Chemical constituents of *Stemona* spp. (continued)

Chemical substance	Bioactivity	*Stemona* sp.	References
Alkaloids (continued)			
stemofoline	insecticidal activity (3^{rd} instar larvae of *P. xylostella*, LD_{50}=5.50μg/cm^2); *S. littoralis*, LC_{50}=2.0ppm)	*S. burkillii* *S. collinsae* *S. parviflora* *S. cochinchinensis* *S. curtisii*	Kaltenegger *et al.*, 2003; Mungkornasakul *et al.*, 2004a; Jiwajinda *et al.*, 2001; Schinnerl *et al.*, 2007
stemokerrine	insecticidal activity (*S. littoralis*, LC_{50}=58ppm)	*S. kerrii* *S. saxorum*	Kaltenegger *et al.*, 2003; Schinnerl *et al.*, 2007; Wang *et al.*, 2007c
stemokerrin-*N*-oxide	-	*S. saxorum*	Wang *et al.*, 2007c
stemonamine	-	*S. saxorum*	Wang *et al.*, 2007c
stemonidine	-	*S. tuberosa*	Xu *et al.*, 1982
stemonine	-	*S. pierrei*	Kostecki *et al.*, 2004
stemoninine	antitussive activity	*S. tuberosa*	Jiang *et al.*, 2006; Xu *et al.*, 2006
stemotinine	-	*S. tuberose* *S. mairei*	Xu *et al.*, 1982; Cai and Luo, 2007
stichoneurine A	-	*S. tuberosa*	Schinnerl *et al.*, 2005
stichoneurine B	-	*S. tuberosa*	Schinnerl *et al.*, 2005
tuberospironine	-	*S. tuberosa*	Jiang *et al.*, 2006
tuberostemonine	-	*S. tuberosa*	Adams *et al.*, 2005
tuberostemonine A	-	*S. tuberosa*	Schinnerl *et al.*, 2007
tuberostemonine J	-	*S. tuberosa*	Chung *et al.*, 2003
tuberostemonine H	-	*S. tuberosa*	Chung *et al.*, 2003
tuberostemonine K	-	*S. tuberosa*	Jiang *et al.*, 2006
Benzoquinones			
parvistemin A	-	*S. japonica*	Yang *et al.*, 2007a
parvistemin B	-	*S. japonica*	Yang *et al.*, 2007a
parvistemin C	-	*S. japonica*	Yang *et al.*, 2007a
parvistemin D	-	*S. japonica*	Yang *et al.*, 2007a

Table 3. Chemical constituents of *Stemona* spp. (continued)

Chemical substance	Bioactivity	*Stemona* sp.	References
Chlorogenic acids			
ethyl 3-*O*-feruloylquinate	-	*S. japonica*	Ge *et al.*, 2007
ethyl 4-*O*-feruloylquinate	-	*S. japonica*	Ge *et al.*, 2007
3-*O*-feruloylquinic acid	-	*S. japonica*	Ge *et al.*, 2007
4-*O*-feruloylquinic acid	-	*S. japonica*	Ge *et al.*, 2007
methyl 5-*O*-caffeyolquinate	moderate inhibitory effect against AIV (H5N1)	*S. japonica*	Ge *et al.*, 2007
methyl 3-*O*-feruloylquinate	moderate inhibitory effect against AIV (H5N1)	*S. japonica*	Ge *et al.*, 2007
methyl 4-*O*-feruloylquinate	-	*S. japonica*	Ge *et al.*, 2007
Chromenols			
dehydro-α-tocopherol	scavenging activity (EC_{50}=22μM)	*S. curtisii* *S. burkillii* *S. collinsae*	Brem *et al.*, 2004
dehydro-β-tocopherol	scavenging activity (EC_{50}=21μM)	*S. collinsae*	Brem *et al.*, 2004
dehydro-γ-tocopherol	scavenging activity (EC_{50}=21μM)	*S. curtisii* *S. cochinchinensis* *S. collinsae*	Brem *et al.*, 2004
dehydro-δ-tocopherol	scavenging activity (EC_{50}=25μM)	*S. curtisii* *S. tuberosa* *S. collinsae*	Brem *et al.*, 2004
6-methoxy-3,4-dehydro-δ-tocopherol	-	*S. sessilifolia*	Zhang *et al.*, 2007
β-tocopherol	-	*S. sessilifolia*	Zhang *et al.*, 2007
γ-tocopherol	strong antibacterial activity against *Staphylococcus aureus* and *Staphylococus epidermidis*	*S. sessilifolia*	Zhang *et al.*, 2007

Table 3. Chemical constituents of *Stemona* spp. (continued)

Chemical substance	Bioactivity	*Stemona* sp.	References
Stilbenoids			
dihydropinosylvin	leukotriene biosynthesis inhibition (IC_{50}= >50μM); antifungal activity (*Pyricularia grisea*, EC_{90}=55μg/ml; *Cladosporium herbarum*, EC_{90}=209μg/ml; *Fusarium avenaceum* EC_{90}=131μg/ml)	*S. tuberosa* *S. pierrei*	Adams *et al.*, 2005 Kostecki *et al.*, 2004
3,5-dihydroxy bibenzyl	strong antibacterial activity against *S. aureus* and *S. epidermidis*	*S. sessilifolia*	Zhang *et al.*, 2007
3,5-dihydroxy-2′-methoxy bibenzyl	strong antibacterial activity against *S. aureus* and *S. epidermidis*	*S. sessilifolia*	Zhang *et al.*, 2007
stemanthrene B	leukotriene biosynthesis inhibition (IC_{50}= 25μM)	*S. pierrei*	Kostecki *et al.*, 2004; Adams *et al.*, 2005
stemanthrene C	leukotriene biosynthesis inhibition (IC_{50}= 25μM)	*S. pierrei* *S. sessilifolia*	Kostecki *et al.*, 2004; Adams *et al.*, 2005; Yang *et al.*, 2007b
stemanthrene D	leukotriene biosynthesis inhibition (IC_{50}= 4.8μM)	*S. collinsae*	Adams *et al.*, 2005
stemanthrene E	-	*S. sessilifolia*	Yang *et al.*, 2007b
stemofuran B	leukotriene biosynthesis inhibition (IC_{50}= 23.3μM)	*S. collinsae*	Adams *et al.*, 2005
stemofuran C	leukotriene biosynthesis inhibition(IC_{50}= >50μM)	*S. collinsae*	Adams *et al.*, 2005
stemofuran D	leukotriene biosynthesis inhibition (IC_{50}= 30.3μM)	*S. collinsae*	Adams *et al.*, 2005

Table 3. Chemical constituents of *Stemona* spp. (continued)

Chemical substance	Bioactivity	*Stemona* sp.	References
Stilbenoids (continued)			
stemofuran G	leukotriene biosynthesis inhibition (IC_{50}= 3.7μM)	*S. collinsae*	Adams *et al.*, 2005
stemofuran J	leukotriene biosynthesis inhibition (IC_{50}= 26.3μM)	*S. collinsae*	Adams *et al.*, 2005
stilbostemin A	leukotriene biosynthesis inhibition (IC_{50}= >50μM)	*S. collinsae*	Adams *et al.*, 2005
stilbostemin B	leukotriene biosynthesis inhibition (IC_{50}= >50μM)	*S. collinsae* *S. pierrei* *S. sessilifolia*	Kostecki *et al.*, 2004; Adams *et al.*, 2005; Yang *et al.*, 2007b
stilbostemin D	leukotriene biosynthesis inhibition (IC_{50}= >50μM)	*S. collinsae* *S. pierrei* *S. sessilifolia*	Kostecki *et al.*, 2004; Adams *et al.*, 2005; Yang *et al.*, 2007b
stilbostemin E	-	*S. pierrei*	Kostecki *et al.*, 2004
stilbostemin F	leukotriene biosynthesis inhibition (IC_{50}= >50μM)	*S. collinsae*	Adams *et al.*, 2005
stilbostemin G	leukotriene biosynthesis inhibition(IC_{50}= 25.8μM)	*S. pierrei* *S. sessilifolia*	Adams *et al.*, 2005; Yang *et al.*, 2007b
stilbostemin H	-	*S. sessilifolia*	Yang *et al.*, 2007b
stilbostemin I	-	*S. sessilifolia*	Yang *et al.*, 2007b
stilbostemin M	-	*S. sessilifolia*	Zhang *et al.*, 2007
stilbostemin N	-	*S. sessilifolia*	Zhang *et al.*, 2007
stilbostemin O	moderate antibacterial activity against *Klebsiella pneumoniae*, MICs = 50 μg/ml	*S. sessilifolia*	Zhang *et al.*, 2007
stilbostemin P	-	*S. tuberosa*	Lin *et al.*, 2007
stilbostemin Q	moderate antibacterial activity against *Klebsiella pneumoniae*, MICs = 50 μg/ml	*S. tuberosa*	Lin *et al.*, 2007

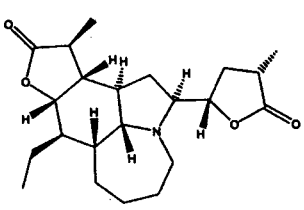
Table 3. Chemical constituents of *Stemona* spp. (continued)

Chemical substance	Bioactivity	*Stemona* sp.	References
Stilbenoids (continued)			
stilbostemin R	-	*S. tuberosa*	Lin *et al.*, 2007
stilbostemin S	-	*S. tuberosa*	Lin *et al.*, 2007
stilbostemin T	moderate antibacterial activity against *Klebsiella pneumoniae*, MICs = 50 μg/ml	*S. tuberosa*	Lin *et al.*, 2007
stilbostemin U	strong antimicrobial activitiy against *Bacillus pumilus*, MIC = 12.5-25 μg/ml	*S. tuberosa*	Lin *et al.*, 2007
stilbostemin V	-	*S. tuberosa*	Lin *et al.*, 2007
stilbostemin W	-	*S. tuberosa*	Lin *et al.*, 2007
stilbostemin X	Moderate antibacterial activity against *Cryptococcus neoformans*, MICs = 50 μg/ml	*S. tuberosa*	Lin *et al.*, 2007
stilbostemin Y	Moderate antibacterial activity against *Cryptococcus neoformans*, MICs = 50 μg/ml	*S. tuberosa*	Lin *et al.*, 2007

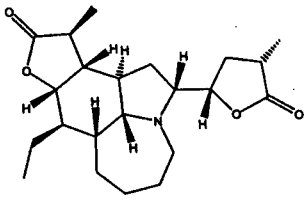
1.2.4 *Stemona* alkaloids

Bioactive principles of *Stemona* roots are alkaloids (Pham *et al.*, 2002). *Stemona* alkaloids represent a typical chemical character of the small monocotyledonous family Stemonaceae, and so far are not detected in any other plant family. They are characterized by a pyrrolo[1,2-a]azepine core usually linked with two carbon chains mostly forming terminal lactone rings (Schinnerl *et al.*, 2007). Up to now about 100 derivatives have been described, mainly isolated from the tuberous roots of various species of the genus *Stemona*. By contrast, only four alkaloids are known so far from the two other genera of

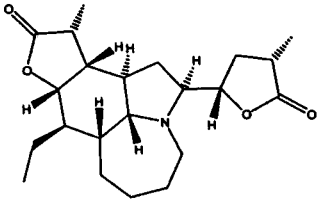
he family, *Croomia* and *Stichoneuron* (Greger, 2006; Pilli *et al.*, 2005; for new tructures not cited herein Sastraruji *et al.*, 2005; Sastraruji *et al.*, 2006; Jiang *et al.*, 2006; Lin *et al.*, 2006). *Stemona* alkaloids were recently classified into three skeletal types: the stichoneurine-, croomine-, and protostemonine-type alkaloids (Figure 2-4) (Schinnerl *et al.*, 2007).



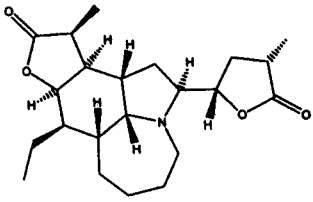
tuberostemonine



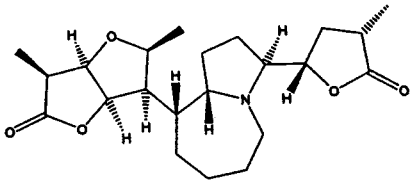
tuberostemonine A



tuberostemonine N

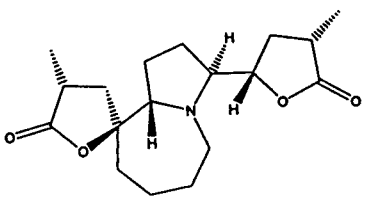


neotuberostemonine

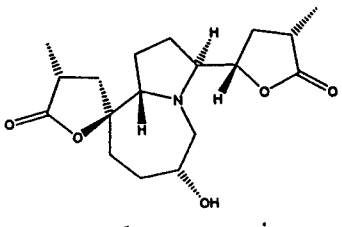


parvistemonine

Figure 2. Stichoneurine type alkaloids

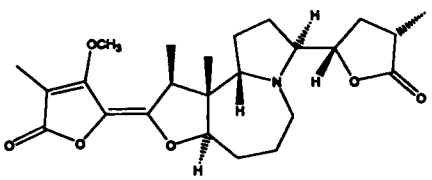


croomine

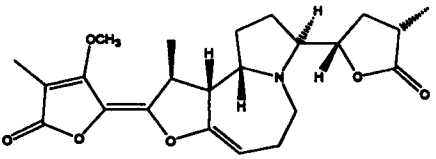


6-hydroxycroomine

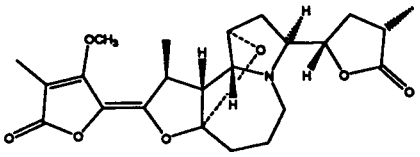
Figure 3. Croomine type alkaloids



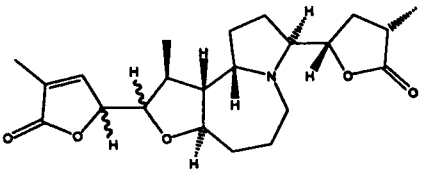
protostemonine



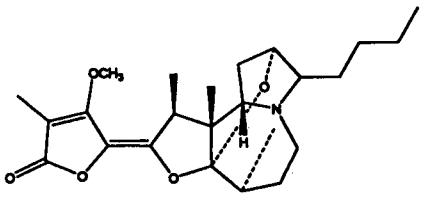
dehydroprotostemonine



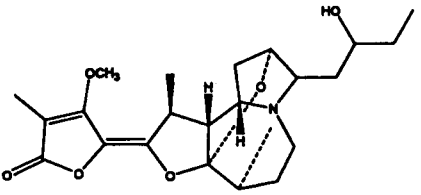
oxyprotostemonine



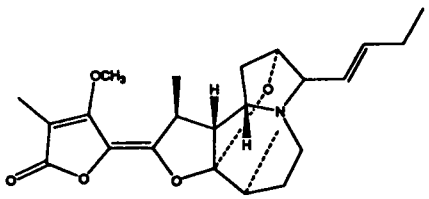
stemocochinin



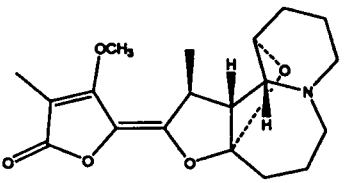
stemofoline



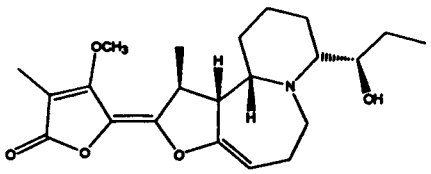
2′-hydroxystemofoline



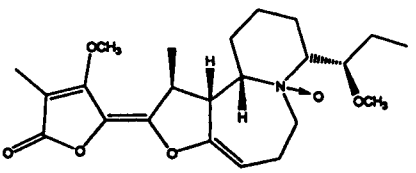
didehydrostemofoline



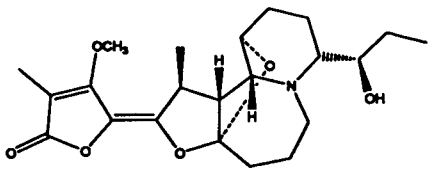
pyridostemin



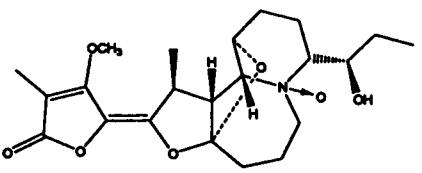
stemokerrin



methoxystemokerrin-*N*-oxide



oxystemokerrin



oxystemokerrin-*N*-oxide

Figure 4. Protostemonine type alkaloids

The chemical interest in *Stemona* spp. extracts was stimulated by their popular use in southeast Asia as insecticides and vermifuges as well as against respiratory diseases (Brem *et al.*, 2002; Chung *et al.*, 2003; Kaltenegger *et al.*, 2003; Pilli and Oliveira, 2000; Schinnerl *et al.*, 2005). Insecticidal activities have already been described (Brem *et al.*, 2002; Kaltenegger *et al.*, 2003). Kaltenegger *et al.* (2003) had compared insecticidal activities of 13 compounds listed in Table 4 in order to highlight structure-activity relationships.

Table 4. Toxicity (LC_{50}) and growth inhibition (EC_{50}) of thirteen *Stemona* alkaloids against neonate larvae of *Spodoptera littoralis* (Kaltenegger *et al.*, 2003)

Compound	LC_{50} (ppm)	EC_{50} (ppm)
Stemokerrin	58	14.1
Methoxystemokerrin-*N*-oxide	>100	16.3
Oxystemokerrin	5.9	0.7
Oxystemokerrin-*N*-oxide	12.5	0.4
Pyridostemin	149	96
Protostemonine	17.7	2.2
Dehydroprotostemonine	6.1	0.8
Oxyprotostemonine	159	47
Stemocochinin	170	61
Stemofoline	2.0	1.5
2′-Hydroxystemofoline	30	38
Parvistemonine	>200	163
Didehydrostemofoline	0.8	0.5

Kaltenegger *et al.* (2003) found that the unsaturated lactonic 4-methoxy-3-methyl-2-furanone unit also plays a crucial role. Generally, the investigated *Stemona* alkaloids displayed two different mode of action: the stemofolines were characterized by neurotoxic interactions resulting in uncontrolled hyperactivity of larvae and cause immediate death, whereas other compounds (stemokerrin, oxystemokerrin, oxystemokerrin-*N*-oxide, pyridostemin, protostemonine, dehydroprotostemonine, and oxyprotostemonine) led to paralysis and softening of the larval bodies.

In addition to contact toxicity against *S. littoralis* neonate larvae, stemofoline and didehydrostemofoline displayed very high activity (Table 5). The insect-toxic potencies of both alkaloids exceed even those of a pyrethrum extract, as yet probably the most frequently used commercial natural product in crop protection (Brem *et al.*, 2002).

Table 5. Contact toxicity against *S. littoralis* compared to commercial pyrethrum extract (Brem *et al.*, 2002)

Compound	LC_{50} ($\mu g/dm^2$)
Stemofoline	0.59
Didehydrostemofoline	1.01
Pyrethrum extract	4.21

The bioactive principle responsible for the high repellency of *S. tuberosa* proved to be tuberostemonine, showing high potency than pyrethrum extract, often described as a repellent agent in patent specifications. Tuberostemonine acts as a potent without toxic effect (Brem *et al.*, 2002). Stemofoline was already reported to be highly toxic against silkworm larvae, *Bombyx mori*, but proved to be completely inactive against larvae of the cabbage armyworm, *Mamestra brassicae*, even at concentration as high as 100 ppm (Sakata *et al.*, 1978). In the light of these results, *Stemona* species might also provide selective agricultural pesticide without harmful side effects toward nontarget mechanisms (Brem *et al.*, 2002).