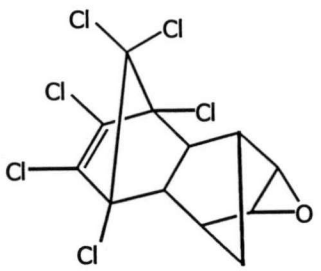
CHAPTER I

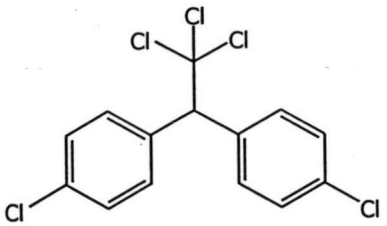
INTRODUCTION

Since Thailand is an agricultural country, the country main income almost come from a variety of agricultural products for example, corn, rice, cotton, sugar cane, soybean and others. Nevertheless, there are many problems concerning with the pests that lessened the production of crops such as weeds, fungi, insects, rodents, aphids and others.[1] Nowadays, the farmers face the new problem: *Pomacea canaliculata* Lamark or golden apple snail. *P. canaliculata* Lamark. destroyed rice after being planted to the rice field around 10-15 days. This damage is one of main factors that decrease the rice production.[2] Thus, it is a crucial need for modern agriculture to wipe off them from the field. Most farmers usually employ synthetic agrochemicals that are active across a wide range of target species and last long enough in the environment to maintain their activity for that extended period they require. However, these compounds are not generally safe for workers in the fields and economical tousle because they are toxic to users and all of them are imported from foreign countries. In addition, the synthetic pesticides are difficult to decompose and they accumulate in soil, so they make invariably environment pollute.[3]

Pesticides can be classified into five types according to chemical groups, *i.e.* inorganic, chlorinated, organophosphate, carbamate and botanical compounds. The inorganic pesticides are compounds of mineral origin and mainly include arsenic, copper, mercury, sulfur or zinc. The chlorinated hydrocarbons are a group of synthetic organic compounds containing one or more chlorine atoms, for example chlordane, dieldrin, and DDT.

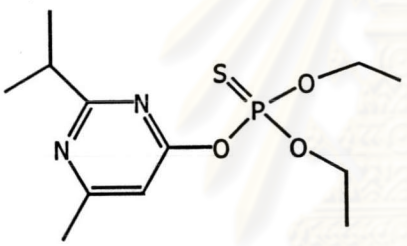


Dieldrin

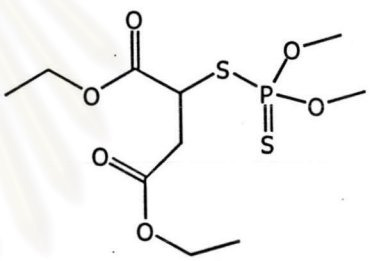


DDT

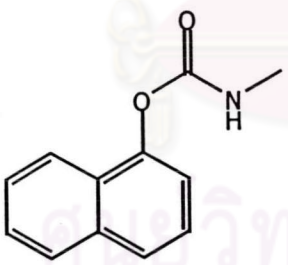
The organophosphate are synthetic compounds containing phosphorus. Some of more common examples in this group are diazinon, dichlorvos, and malathion. Other types are carbamate compounds that are synthetic compounds of salts or esters of carbamic acid. Carbaryl and propoxur are among those examples.



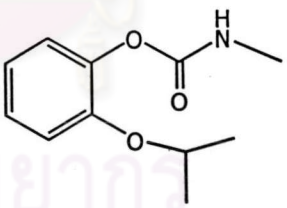
Diazinon



Malathion

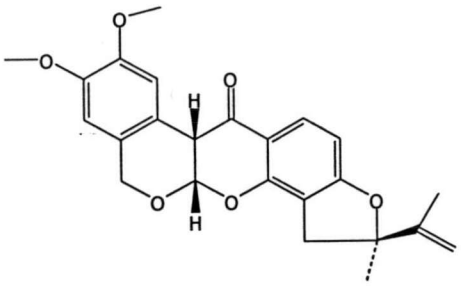


Carbaryl

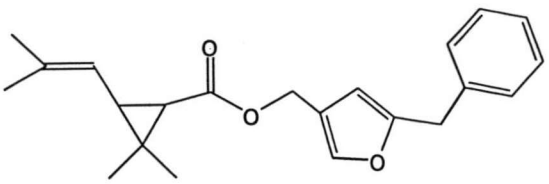


Propoxur

The last one is botanicals, which are pesticide derived from plant origin. Pyrethrums and rotenone are two examples. Synthetic pyrethroids, such as resmethrin act with similar action to pyrethrum. D-phenothrin is an example of this class.[4-6]



Rotenone



Resmethrin

In the present day, the chemistry of natural products has widely been studied. Many isolated compounds were identified as new compounds and a lot of known compounds were proved to possess agrochemical activity. This research intentionally focused on the plant in Solanaceae family, namely *Datura metel* Linn. according to the kind information from the Department of Agriculture, Ministry of Agriculture and Cooperatives in 1999. At that time various Thai plants were selected, extracted and performed preliminary screening test against *P. canalicutala*. The preliminary result displayed that the leave extract of *D. metel* showed attractively high activity.[7] In addition, from the survey based on biological activity study, *D. metel* was almost examined for pharmaceutical aspects. In this study, with the focus on the other point of view, *D. metel* was selected to investigate for the chemical constituent responsible as a good template for agricultural purpose.

1.1 General characteristic of *D.metel* L.

In Thailand, the plants belong to *Datura* genus are composed of three species: *D. metel* (its synonym is *Datura alba* Nees.), *D. fastuosa* (its synonym is *Datura metel* var Fastousa Saff), and *D. stramonium*.[8-11] The difference of the three species are shown in Table 1.1.

Table 1.1 The different characteristics of three species in *Datura* spp.[8-11]

Part of tree	*D. fastuosa*	*D. metel*	*D. stramonium*
Flower	Triple and large flower with white inside, violet and yellowish outside.	Single and large flower with white cream or yellow.	Single and large flower with white cream or lavender.
Stem, branch, leaf	Purple stem, branch and ovate leaves	Green stem, branch and ovate leaves	Greenish stem, branch and ovate leaves
Fruit	Spiny capsule	Spiny capsule	Spiny capsule
Seed	Brown	Brown	Deep brown

D. metel has been known as "Lumpong" in the middle of Thailand and as "ma-khua-ba" (Mad-egg plant) in the northern part of Thailand. This plant can be found throughout the tropical Asia and probably to the tropical Africa. Its synonym in English is "thorn apple".

D. metel or *D. alba* Nees was shrub and its life cycle was around 1-2 years. The botanical aspects of *D. metel* (Figure 1.1) can be summarized as follows:

Stem: The height is 100-150 cm, green, stem erect

Leaves: Leaves are single oval shaped, the size is about 7-12 cm wide and 12-20 cm. long

Flowers: Big, white and corolla trumpet-shaped, single, length is 9-14 cm.

Seed: Brown circular, thorn, 4-6 cm in diameter.

(a) **(b)**

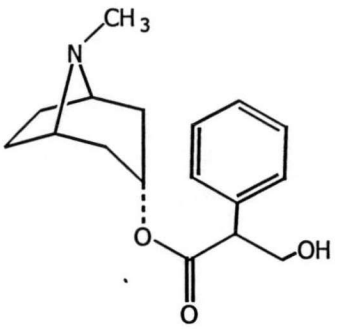
Figure 1.1 Flower and stem (a) and Flower (b) of *D.metel*

1.2 Chemical constituents studies from *D. metel* L.

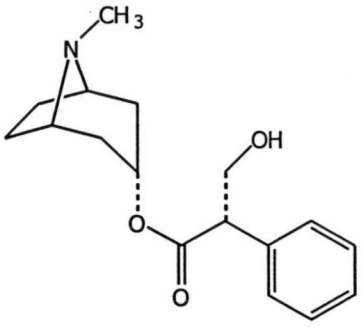
The major isolated compounds of *D. metel* were tropane alkaloids such as scopolamine (hyoscine), hyosyamine and atropine. The alkaloids found in this entire plant are presented in Table 1.2. The structures of some selected compounds are exhibited in Figure 1.2.

Table 1.2 Alkaloids found in *D. metel*

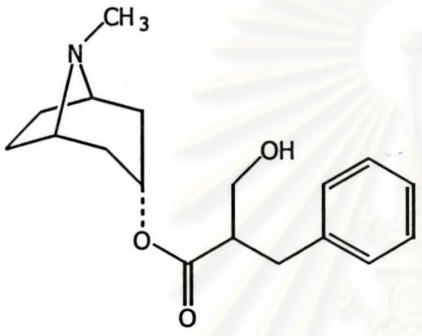
Plant part	Alkaloids	Reference
Stems	Scopolamine, hyoscyamine, meteloidine (Indian species), atropine (Iran species)	12, 13, 14, 15
Seeds	Hyoscyamine, fastudine, scopolamine, isoquinoline alkaloid, 7-hydroxy-3,6-ditigloyloxytropane, atropine	12, 16, 17
Flowers	Anisodamine, atropine (China species), isoquinoline alkaloid, hyoscyamine, tropine	12, 13, 16, 18, 19
Leaves	Scopolamine, atropine, datumitine, isoquinoline alkaloid, hyoscyamine	12,13, 16, 20
Roots	Hyoscyamine, nohyoscyamine, littorine, dopamine, scopolamine, tropine, pseudotropine, tigloidine, cuskhygrine, 3,6-ditigloyloxytropane	12, 13, 16, 19



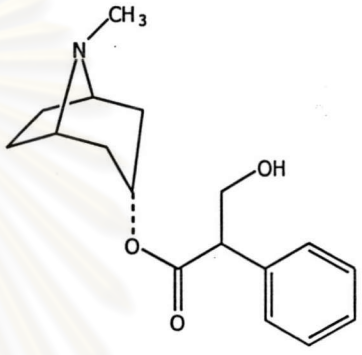
Atropine



Hyoscyamine

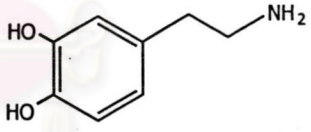


Littorine

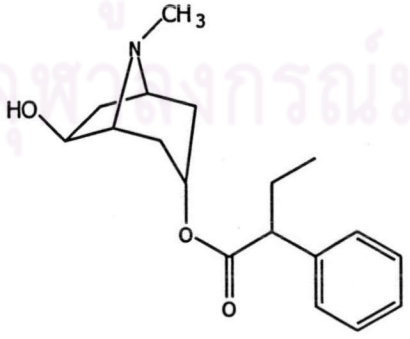


Homatropine

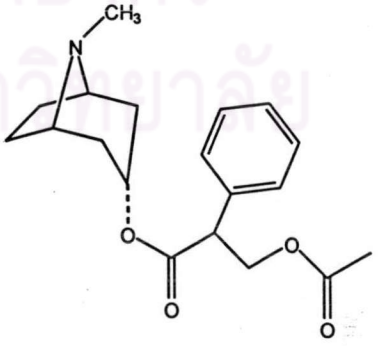
Tropine



Dopamine

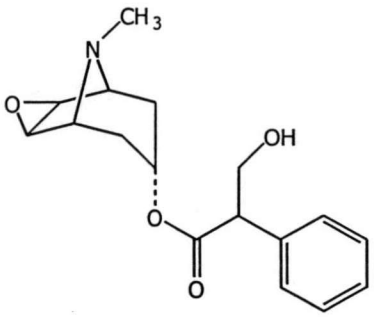


Anisodamine

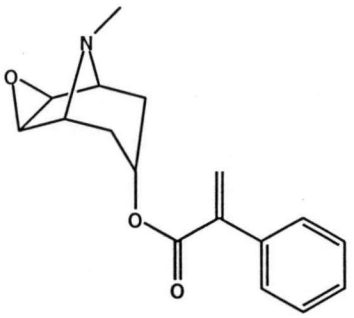


3α-Acetoxytropane

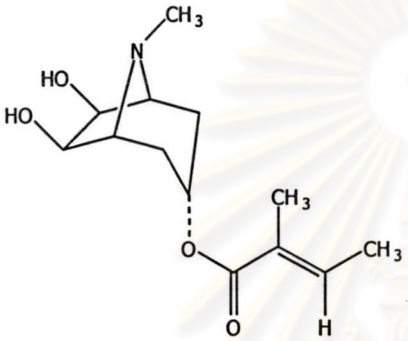
Figure 1.2 Alkaloids found in *D. metel*



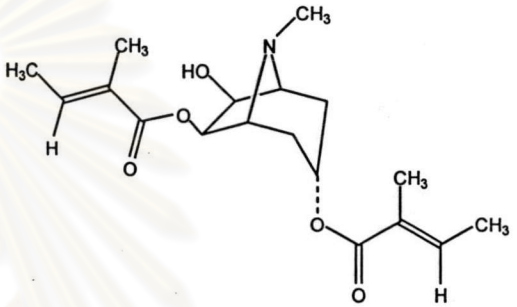
Scopolamine



Aposcopolamine



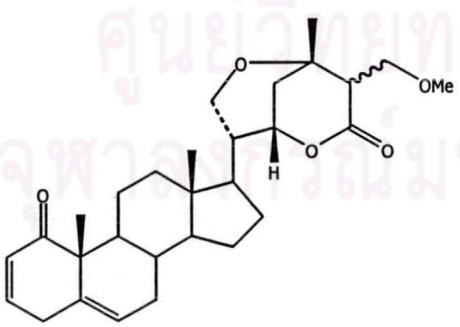
Meteloidine



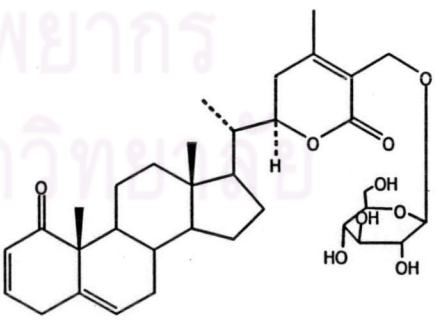
7-Hydroxy-3,6-ditigloyloxytropane

Figure 1.2 (Cont)

Besides tropane alkaloids, steroids and flavonoids were also isolated from the arial parts of *D. metel*. The structures are shown in Figures 1.3-1.4.[21-32]

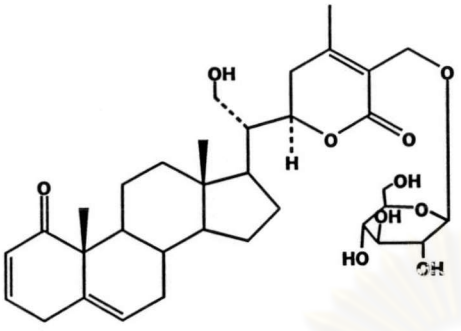


Datumetelin

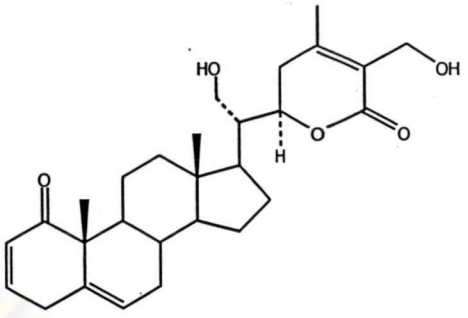


Daturametelin A

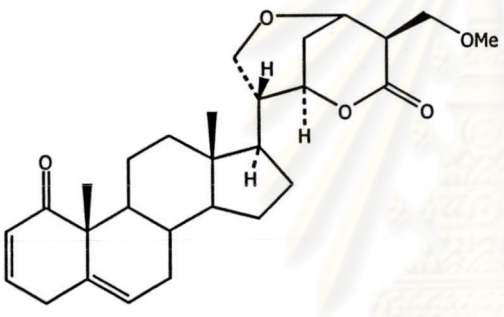
Figure 1.3 Steroids found in *D.metel*



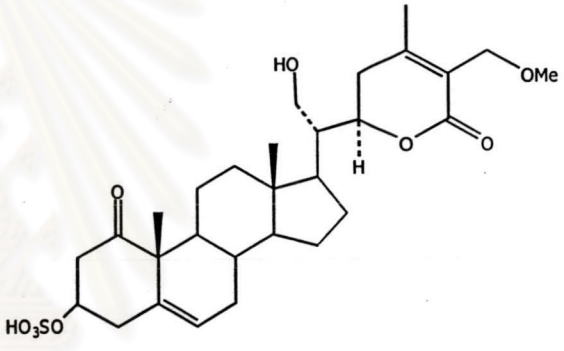
Daturametelin B



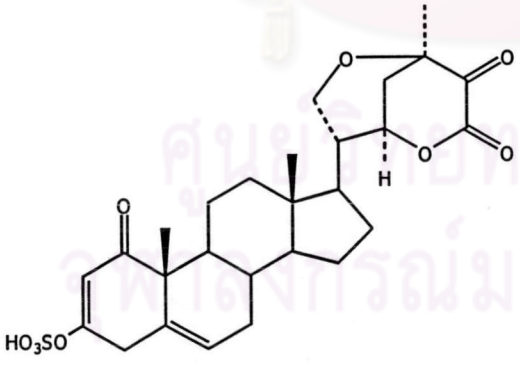
Daturametelin C



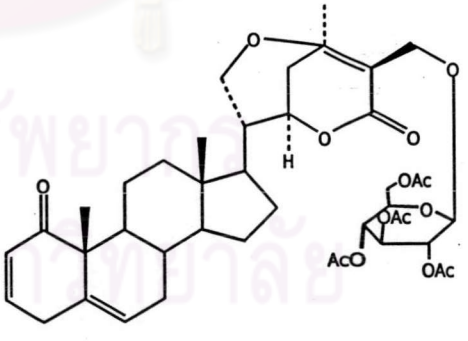
Daturametelin D



Daturametelin E

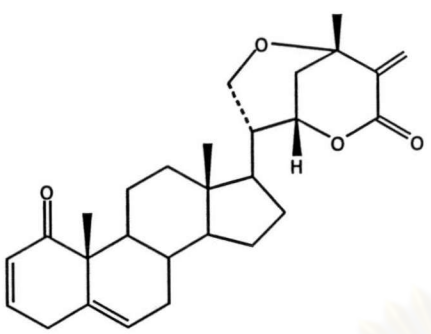


Daturametelin F

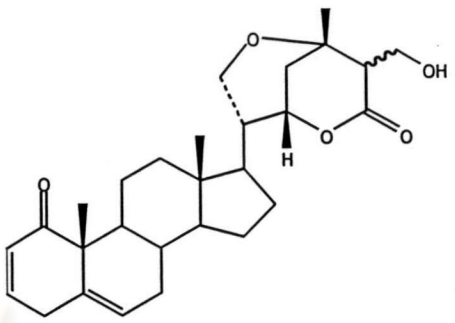


Daturameteline G-Ac

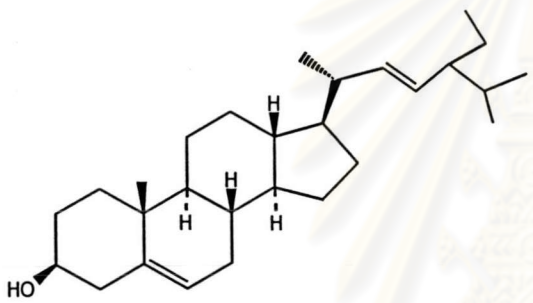
Figure 1.3 (Cont)



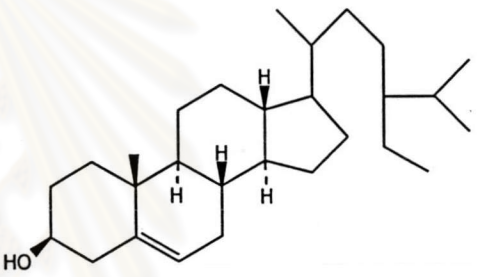
Daturiline



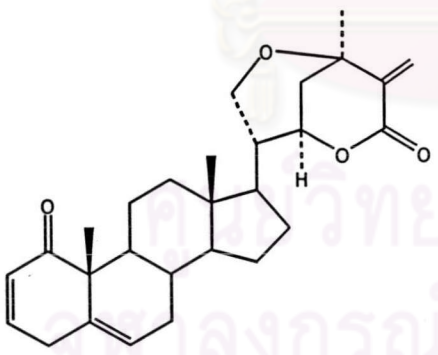
Daturilinol



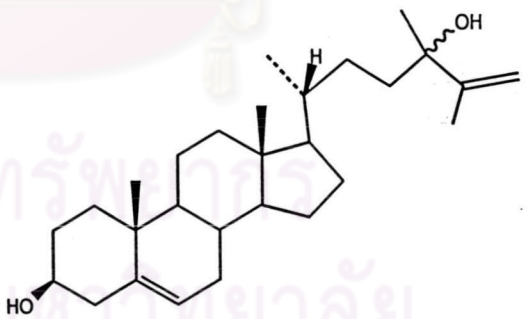
Stigmasterol



β-Sitosterol

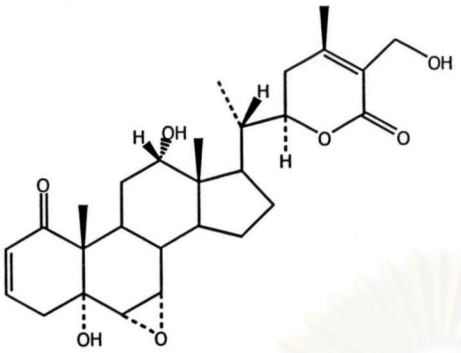


Withametelin

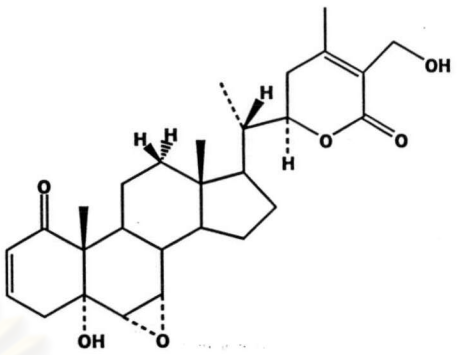


Physalindicanol A

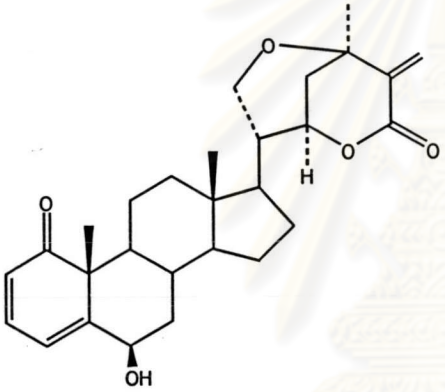
Figure 1.3 (Cont)



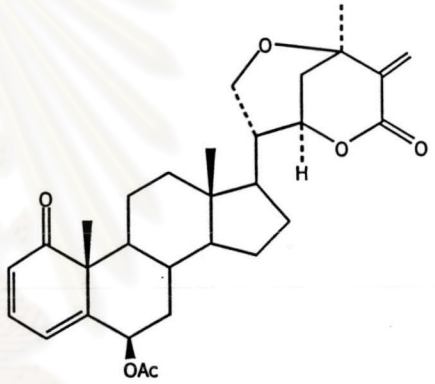
Withastramonolide



12-Deoxywithastramonolide

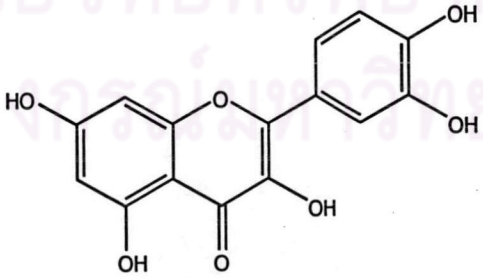


Withametelin B



Withameteline B-Ac

Figure1.3 (Cont)



Quercetine

Figure 1.4 Flavonoid found in *D. metel*

1.3 Biological activity study of *D. metel* L.

D. metel is official medicine in Indian, African and folk medicine in the Asian, and European countries. *D. metel* is utilized as a source of tropane alkaloids, which used in modern medicine such as antihistamine, anticholinegic, antispasmodic and others. The folk-lore medicine use of *D. metel*, has been reported. The roots, flowers, leaves and seeds, are used by Malaysian to treating boil, sore, hemorrhoids and ringworm. The Indian used the leaves for skin complaints. In Thailand, the cigarette derived from flowers is reputed for treatment of asthma symptoms. In addition, Thailand is the only one country that uses the flowers of *D. metel* in medicine. From the previous study of *D. metel,* the presence of tropane alkaloids leads to the suggestion that the medicine properties may be attributed to the tropane alkaloid constituents.[12,33-38] Moreover, the other biological activities study on *D. metel* has been reported including antibacterial, antiyeast[39], antifilarial[40], antinematicidal[41], antiviral[42], and antifungal.[43]

In 1992, *D. metel* has been studied on cholinesterase (ChE) inhibition properties. The extract of the leaves of *D. metel*, containing scopolamine was found to inhibit rat intestinal cholinesterase *in vitro*. Moreover, the extract of the roots of *D. metel* was found to activate cholinesterase enzyme activity at optimal or higher substrate concentrations. When the extracts of root and leaf were added together, the ChE activity level was increasing compared with that in the presence of leave extract alone. This suggests a potentiating action of the root extract on cholinesterase more than the leaf extract.[44]

In 1994, the extracts from the leaves and roots of *D. metel* were studied on isolated smooth muscle of rat uterus and rectum compared with scopolamine and acetylcholine. The results from this research found that the leave extracts and scopolamine showed antispasmodic effects, whereas those derived from the roots and acetylcholine caused contractor in isolated rat uterus and rectum whole muscle. This suggests the presence of spasmogenic factor in the root extract.[45]

In 1998 the leave extract from *D. metel* and the seed extract of *Croton tiglium* Linn were tested for molluscicidal activity against *Pomacea* Spp. and *Pomacea insularus*. The result revealed that at the sample concentration 40% and 30% of the leave extract of *D. metel* and the seed extract of *Croton tiglium* could kill the *Pomacea* Spp. and *Pomacea insularus* to 100 %.[46]

In 1999 *D. metel* was selected to investigate for preliminary screening test against *P. canalicutala*. It was reported that the leave extract and alkaloid extracts of this plant displayed high molluscicidal activity.[7]

In 2002 the hexane, chloroform, acetone and methanolic fractions of *D.metel* L. were investigated for antifungal properties using pathogenic species of *Aspergillus.* The chloroform fraction was found to be endowed with antifungal activity. The minimum inhibitory concentration (MIC) of chloroform fraction of was 625 mg/mL against all three species of *Aspergillus i.e., A. fumigatus, A. flavus* and *A. niger,* by microbroth dilution and percent spore germination inhibition assays. The MIC by disc diffusion assay was observed to be 12.5 mg/disc. The chloroform fraction of *D. metel,* when investigated for potency, turned to be 9.2 times less active than amphotericin B. However, it was important to note that cytotoxicity of chloroform fraction *in vitro* was 118 times less than amphotericin B.[47]

From the literature review, most of biological activities examined for *D. metel* were involved the pharmaceutics such as smooth muscle relaxant activity, spasmogenic activity *etc*. Only a few reports concerning agricultural activity have been addressed. Thus, this research was focused on searching for lead constituent possessing agricultural-based activity particular the mulluscicidal activity from *D. metel.*

1.4 The objective of this research

The objective of this research could be summarized as follows:

1. To extract alkaloid and non-alkaloid constituents from the flowers of *D. metel.*
2. To preliminarily screen for agricultural-based activity of alkaloid and non-alkaloid crude extracts.
3. To separate and isolate organic constituents from various crude extracts and elucidate the structures of isolated substances.
4. To perform agricultural-based activity such as molluscicidal and insecticidal activity of isolated substances.