CHAPTER I

INTRODUCTION

1. *Sapindus rarak* DC.

1.1 General characterization of *Sapindus rarak* DC.

Tree : shrubs and small trees in the Sapindaceae, native to warm temperate to tropical regions in both the old world and the new world. The genus includes both deciduous and evergreen species. Common names include soapberry and soapnut, both names referring to the use of the crushed fruits to make soap.

Leaf : The leaves are alternate, 15-40 cm. long, pinnate, with 14-30 leaflets, the terminal leaflet often absent. The flowers form in large panicles, each flower small, creamy white.

Fruit : The fruit is a small leathery-skinned drupe 1-2 cm. diameter, yellow ripening blackish, containing 1-3 seeds.

Pericarp : The pericarp contains saponin, a natural detergent used in the past to produce soap. Saponin is toxic if eaten, and can cause a severe skin rash (urticaria) in some people, so its use is not advisable [อุมาภัณฑ์และคณะ, 2539].

Sapindus is genus of trees or shrubs of the Sapindaceae family, found wild or introduced in the tropical and sub-tropical regions of the world, chiefly in the Indo-Malaysian region [อุมาภัณฑ์และคณะ, 2539]. *Sapindus rarak* DC. is the only *Sapindus* species reported in

Thailand(Thai name: Ma-kham-di-khwai) [อุมาภัณฑ์และคณะ, 2539]. This plant has long been used as a folk medicine for hair care, anti-dandruff [นันทวัน, 2542] and cure *Tinea favosa* [อุมาภัณฑ์และคณะ, 2539] as well as a source of natural surfactant [วิไลพรและคณะ, 2531] *Sapindus rarak* is a medium-sized or small tree, generally up to 15 m. in height, ascending up to an altitude of 900 m. The taxonomical description of this plant is as follows: bark grey, leathery; leaves pinnate, 8-12; flower pale, polygamo-monoecious in large terminal or axillary, pubescent panicles; drupes with fleshy saponaceous pericarp; seeds globose, with horny testa. Fruits are used like soapnuts to clean hair, clothes and jewellery. The nuts damage clothes and lead to the falling of hair due to the high concentration of saponin. An infusion of the fruits is used to remove pimples; it is also a good insecticide. The juice of the fruits causes intoxication to fish. An infusion of the seed may be employed to treat scabies. The seeds contain an oil(26%); it consists of glycerides of palmitic and stearic acids, the former being predominant.

1.2 The classification of *Sapindus rarak* DC.

Kingdom Plantae
Division Magnoliophyta
Class Magnoliopsida
Order Sapindales
Family Sapindaceae
Genus Sapindus

[Online]Available from:
http://www.thefreedictionary.com/Sapindus

2. Saponins

2.1 General introduction [Hostettmann and Marston, 1991]

Saponins are high-molecular-weight glycosides, consisting of a sugar moiety linked to a triterpene or steroid aglycone. The classical definition of saponins is based on their surface activity; many saponins have detergent properties, give stable foams in water, and show haemolytic activity. Such attributes, while not common to all saponins, have frequently been used to characterize this class of natural products, However, because of the numerous exceptions exist, saponins are now more conveniently defined on the basis of their molecular structure, namely as triterpene or steroid glycosides.

2.2 Definitions [Hostettmann, and Marston, 1991]

The aglycone or non-saccharide portion of the saponin molecule is called the genin or sapogenin. Depending on the type of genin present, the saponins can be divided into three major classes:

1. triterpene glycosides
2. steroid glycosides
3. steroid alkaloid glycosides.

The genins of these three classes can be depicted as shown in Figure 1.

The aglycones are normally hydroxylated at C-3 and certain methyl groups are frequently oxidized to hydroxymethyl, aldehyde or carboxyl

functionalities. When an acid moiety is esterified to the aglycone, the term ester saponin is often used for the respective glycosides.

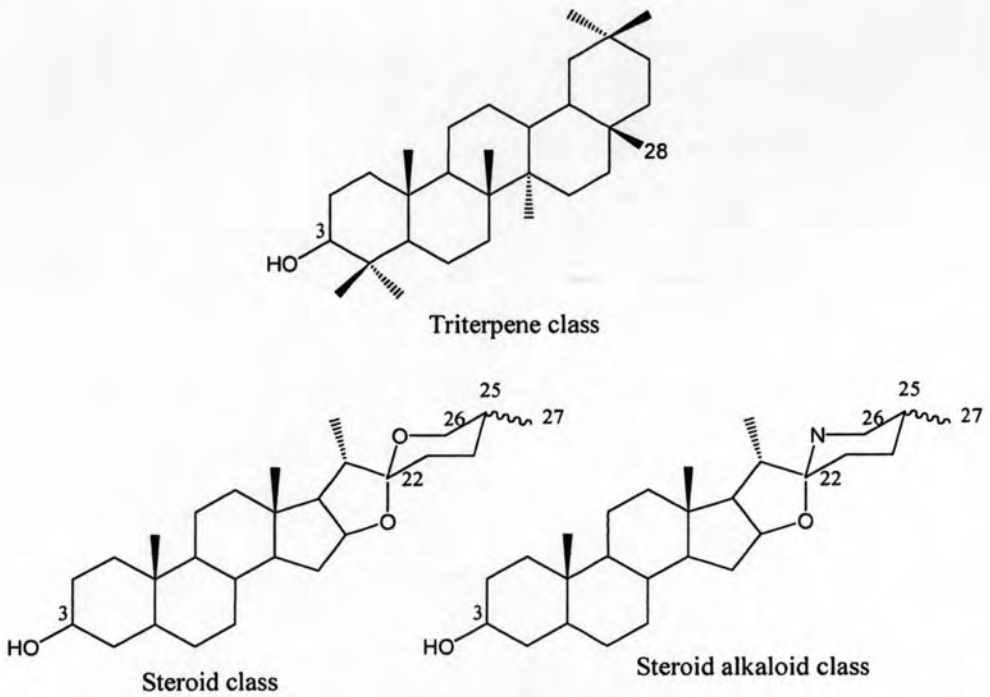
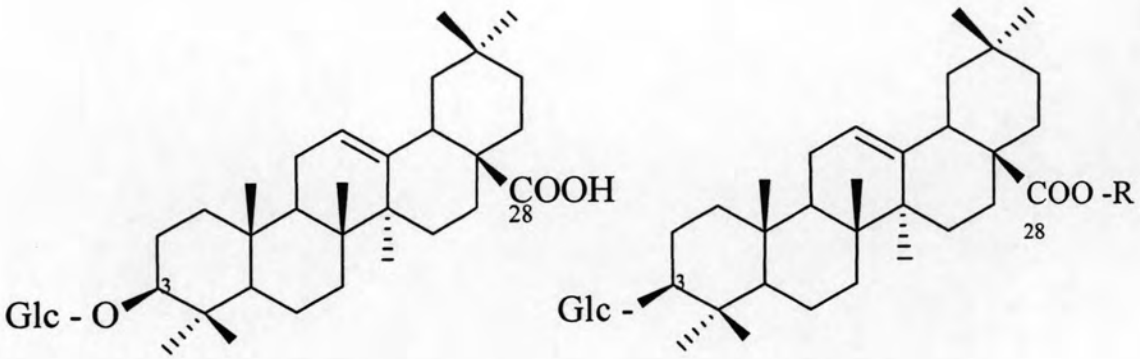


Figure 1 Skeletal types of genin found in the three principal classes of saponin.

All saponins have in common the attachment of one or more sugar chains to the aglycone. Monodesmosidic saponins have a single sugar chain, normally attached at C-3. Bidesmosidic saponins have two sugar chains often with one attached through an ether linkage at C-3 and one attached through an ester linkage (acyl glycoside) at C-28 (triterpene saponins) (Figure 2) or ether linkage at C-26 (furostanol saponins). Tridesmosidic saponins have three sugar chains and are seldom found. Bidesmosidic saponins are easily transformed into monodesmosidic saponins by, for example, hydrolysis of the esterified sugar at C-28 in triterpene saponins; they lack many of the characteristic properties and activities of bidesmosidic saponins.



Monodesmoside Bidesmoside

Figure 2 Monodesmosidic and bidesmosidic saponins

3. *Pomacea canaliculata* Lamarck.

3.1 Taxonomy of the golden apple snails.

South African apple snails are tropical and sub-tropical fresh water snails. They are commonly known as golden apple snails and the identification of taxonomy is as follows:

Phylum Mollusca

Class Gastropoda

Subclass Prosobranchia

Order Mesogastropoda

Superfamily Viviparoidea

Family Ampullariidae

[Online]Available from: http://applesnail.net

The identification of species is not difinite. There are three species of *Pomacea* that cause damage to the rice fields in Thailand as follows, *Pomacea canaliculata*, *Pomacea insularus* and *Pomacea spp.* [Chanyapate and Archawacom, 1997]

3.2 General characteristics of the golden apple snails. [Chanyapate and Archawacom, 1997]

Shell: The shell of this apple snail species is globose and relatively heavy (especially in older snails). The 5 to 6 whorls are separated by a deep, indented suture (hence the name 'canaliculata' or 'channeled'). The shell opening (aperture) is large and oval to round. Males are known to have a rounder aperture than females. The umbilicus is large and deep. The overall shell shape is similar to that of *Pomacea lineata*, except the deeper sutures and more globose shape in *canaliculata*. The size of these snails varies from 40 to 60 mm. wide and 45 to 75 mm high depending on the conditions. The colour varies completely from yellow and green (cultivated forms) to brown with or without dark spiral bands (wild form). The shell growth of this species occurs mainly in spring and summer, while it stagnates in fall and winter.

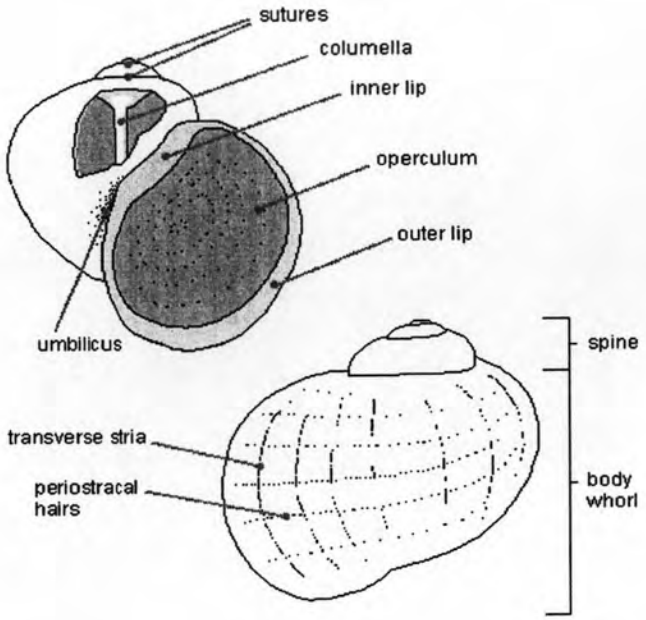


Figure 3 The structure of the shell of *P. canaliculata* (Lamarck.)

[Online]Available from: http://applesnail.net

Operculum: The operculum is moderately thick and corneous. The structure is concentric with the nucleus near the centre of the shell. The colour varies light (in young snails) to dark brown. The operculum can be retracted in the aperture (shell opening)

Figure 4 The structure of *P. canaliculata* shell and operculum

[Online]Available from : http://applesnail.net

Body: The colour of the body varies from yellow (cultivated), brown to nearly black, with yellow spots on the siphon. When at rest, the tentacles are curled under the shell.

Mantle cavity: The mantle cavity is formed by a fold of the backside of the bodywall (roof) and the head neck and body of the snail (floor). It can be considered as a sac, loosely attached to the inside of the shell and enveloping the head-neck and the gills-lung area of the body

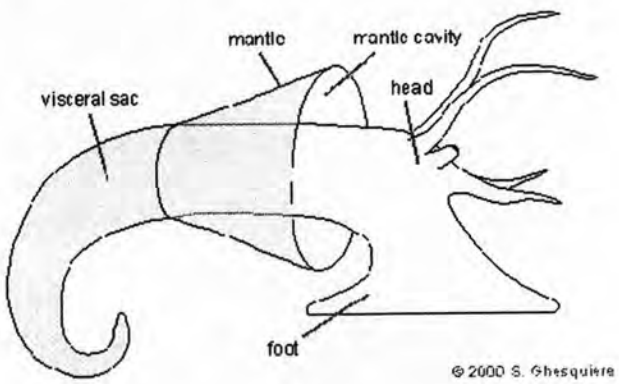


Figure 5 The structure of the mantle of *Pomacea canaliculata* [Online]Available from: http: //applesnail.net

Reproductive system: The apple snail has separated anatomy and sexes(dioecious) and is not a hermaphroditic snail. They lives for 2-6 years with high fertility. Shell is tight brown; flesh is creamy white to golden pinkish or orange. Size depends on the availability of food. Most destructive stage is when the length of the shell is from 10 mm (about the size of a corn seed) to 40 mm(about the size of a pingpong ball). Female golden apple snail operculum (a1) is concave while it is convex in male (a2).The shell of the female adult snail (b1) curves inward; the male shell (b2) curves outward. The difference of the shell of male and female *P. canaliculata* Lamarck. was shown in figure 6.

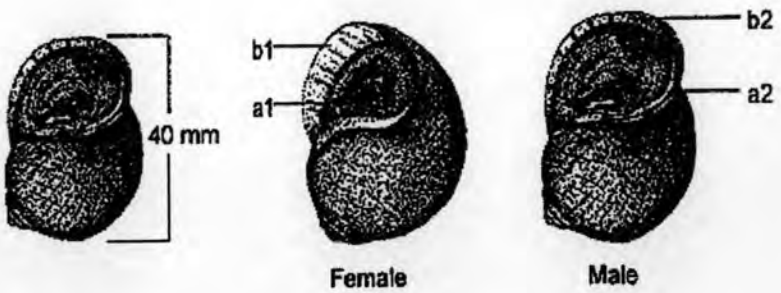


Figure 6 The difference of shell of male and female *P. canaliculata*

[Online] Available from: http://applesnail.net

The male has shell aperture rounder than the female because of the large penial complex. The male reproductive tract consist of the testis and vas deferens, the seminal vesicle, the prostate gland, the penial sac, the penis and the penis sheath. The female reproductive system consist of the egg cells, ovaria, oviduct, proximal albumen gland, distal albumen gland and egg tube *Pomacea canaliculata* is sexual mature at the size of 2.5 cm. The reproductive rate of this snail varies with the temperature and partly by the availability of food. During fall and winter, the reproduction rate is at its lowest point, while with the raising temperatures in spring their reproduction rate increases.

After copulation about few day, the female snails climbs out of the water 5-20 cm above the waterline, usually a stem of emergent aquatic vegetable or any hard object. The eggs are deposited one by one or sometime in pairs. And she drops back into the water when she finished. The total number of eggs laid at once can vary from 388-3000. The size of eggs varies 2.2-3.5 mm. diameter. The total needed to deposit a clutch of eggs 1-4 hours.

In 7-12 day, the pink color changes, becoming paler and whiter and prior to hatching the eggs are almost white with dark spots. After 2 weeks, the first snail should appear and have a weight 1.7 mg. From the day they hatch, the young snails start eating the same as their parents. After the eggs deposit for 4-10 days, the female snail can lay eggs again and lay all the year until age 2-3 years.

3.3 The life cycle of golden apple snails

The apple snail is found in the wild flood plain and swamps. Snails are well adapted to live in alternating wetland and dry land habitats such as seasonal swamp or rice fields[Chanyapate and Archawacom, 1997].

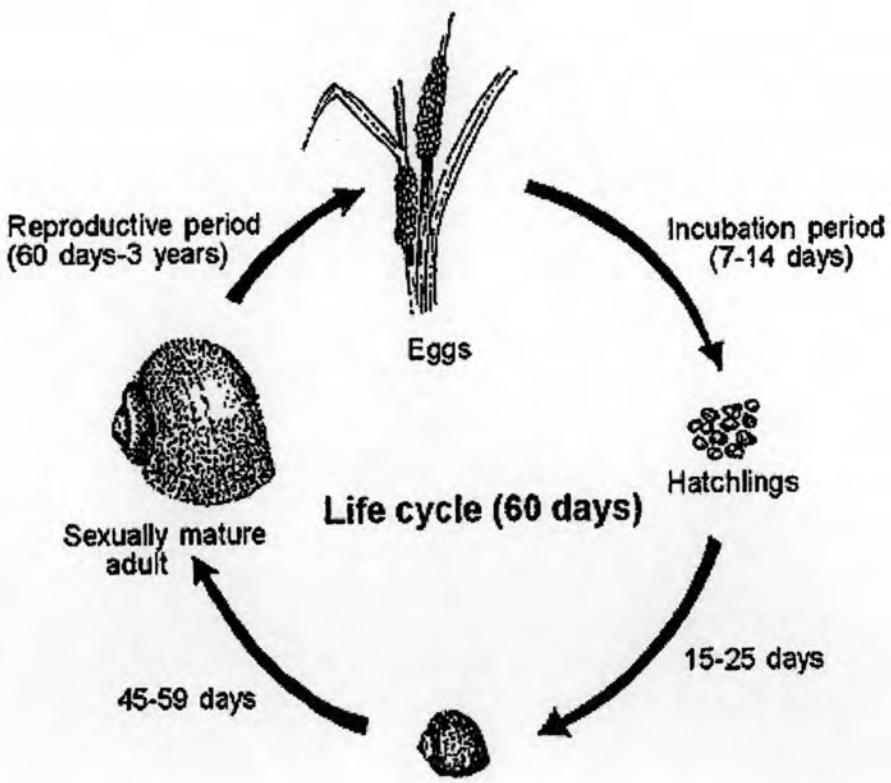


Figure 7 The life cycle of golden apple snails

[Online] Available from: http://applesnail.net

Eggs stage: To lay eggs, adult females crawl out of the water in early morning and evening and lay 25-500 eggs in bright pink batches on rice tillers, sedges, rice file dikes, or other firm substrate protruding from water. Within 1-2 weeks after deposition, the eggs in the egg masses gradually lighten and hatching commences. Hatch ability was highly variable, ranking from 7 % to 90 %

Pre-adult stage: The newly hatched neonated snail drops into the water and soon starts moving about, feeding on algae and detritional aggregates. The abundance of juvenile snails is negatively correlated with the density of adult snails. When grown to shell height of about 1.5 cm., juveniles start consuming plant material.

Adult stage: The golden apple snail has separated sexes, which can be morphologically distinguished by curve of the operculum. Under favorable conditions, females reach maturity within 60-85 days after hatching and may spawn at weekly intervals throughout the year.

4. State of problem

Thailand, as a tropical country, is rich in various economic plants. Rice is one of the most important crops that cover the most cultivated area in Thailand. Thai farmers have two major agricultural problems which are weeds and pets such as crabs, birds, rats and snails especially golden apple snail (*Pomacea canaliculata*) [Chanyapate and Archawacom, 1997].

The golden apple snail (*Pomacea canaliculata*) is found in the new world; South America and Central America, which were introduced into Thailand from Japan and the Philippines. The golden apple snail was first discovered in Thailand in 1984 [Keawjam, 1990] and have become major pests of rice and other aquatic crops throughout the whole island [Huang, 2003]. These snails are also spread out and distributed in the Philippines, Malaysia, Thailand, Indonesia, China, and the other Asian countries [Naylor, 1996]. In addition, the snail may also serve as a vector for diseases and parasites, as it may play a potential role as an intermediate host for *Angiostrongylus cantonensis* [Chao *et al.*, 1987]. Several techniques have been used to control snails, including biological, cultural and chemical controls such as Niclosamide, Metaldehyde, Copper Sulfate and Endosalfan [กองกีฏและสัตววิทยา, 2543].These chemicals are toxic to human and other species such as fish, shrimp and are hazard to public health including skin problems, blurring vision and blindness. The snails can be controlled by chemical pesticides such as niclosamide and metaldehyde. However, synthetic molluscicides are expensive and generally toxic to other organisms and may produce damage to the environment [Calumpang, 1995]. Natural plant molluscicides are gradually gaining attention, because they are considered ecologically

more appropriate for snail control. In the search for suitable molluscicidal agents from natural sources, it was the extracts of the soapnut, *Sapindus rarak* (Sapindaceae) exhibited the molluscicidal effects against golden apple snails. The fruits of *Sapindus rarak* DC. have been traditionally used as an expectorant, as well as a source of natural surfactants. This genus has been reported to contain water-soluble monodesmosidic saponins. These saponins are known to possess molluscicidal activity and are present in various plants. However, there is not reported concerning the isolated compounds that showed molluscicidal activity to the golden apple snails. Bioassay directed isolation method from *Sapindus rarak* DC. fruits for searching the substances that showed the molluscicidal effect against *Pomacea canaliculata.*

5. Objectives of study

5.1 To study the chemical constituents of saponins from *Sapindus rarak* DC. fruits.

5.2 To study the molluscicidal activity of saponins from *Sapindus rarak* DC. fruits against golden apple snails (*Pomacea canaliculata* Lamarck).

6. Scope of study

6.1 Extraction and isolation of saponins with molluscicidal activity from *Sapindus rarak* DC. fruits.

6.2 Structure determination of saponins by spectroscopic method.

6.3 Molluscicidal activity of saponins on golden apple snail.