CHAPTER II
LITERATURE REVIEWS

2.1 The phytochemistry of the plants

The *Annona reticulata* Linn. (Annonaceae)

The *A. reticulata* , commonly know as Custard apple or Bullock Heart, is an imported plant which wildely cultivated in tropical and subtropical region. Flowering during April to August and fruiting in November to March. In the indigenous doctor recorded, the barks are used in curing diarrhea; the fruits eliminate infectious disease in early childhood, edema, intestinal parasite and gas pain in the stomach; the leaves take away the edema and skin parasite (Bunyaprapatsorn *et al.*, 1996). The chemical constituents are reviewed by Duke (2004). The bark contains 14-hydroxy-25-desoxyrollinicin, anonaine and tannin.

Chang *et al.* (1993) reported the 5 formazan annonaceous acetogenins; annoreticuin-9-one, squamone, solamin, annomonicin and rollinastatin from leaves. The other acetogenins are purified from the seed extracts; annoreticulin, annoreticuin-9-one, bullatacin, isoannonareticin, squamocin, squamone, cis-/trans-bullatacinone and cis-/trans-murisolinone (Yu *et al.*,1997 and Chang *et al.*, 1998). The annonacin, mono-tetrahydrofuran acetogenin from the seeds shew cytotoxic activity against the T24 (bladder cancer cell line) with LC_{50} = 26.1 nM (Yuan *et al.*, 2003).

The *Annona squamosa* Linn. (Annonaceae)

The Custard apple, Sugar apple or Sweetsop is the common name of the *A. squamosa*. The plant is widely cultivated in the subtropical and the tropical regions. It is frequently used as foodstuff and medicinal plant in Southeast Asia. The plant is flowering between May and July and fruiting in June to November. The bark is used in traditional medicine as astringent, antidote to snake bites, wound healing and diarrhea; the fruits are used to treat the skin parasite, intestinal parasite, ringworm, chloasma, tuberculosis, antidote to snake bites and infectious disease in early childhood; the leaves cure edema, ringworm, chloasma, louse, crab louse, bedbug, chicken mites, wound healing, antidote to snake bites and intestinal parasite; the seeds

eliminate the louse, crab louse, roundworm and trichina (Bunyaprapatsorn *et al.*, 1996). The barks contains (-)-kaur-16-en-19-oic-acid, bullatacin, bullatacinone and liriodinine; the leaf contains (+)-o-methylarmepavine, (-)-xylopine, beta-caryophyllene, borneol, corydine, demethylcoclaurine, friedelin, glaucine, higenamine, isocorydine, lanugiosine, norcorydine, norisocorydine, norlaureline and roemerine; the roots contain liriodenine, michelalbine and squamolone; the seeds contain annonacin, annonacin-A, annonastatin, annonin-I, annonin-VI, annonaine, asimicin, neoannonin and squamocin (Duke, 2004).

Saxena *et al.* (1993) reported the larvicidal and chemosterilant activity of *A. squamosa* alkaloids. These agents act to *Anopheles stephensi* in reducing the female fecundity and fetality. The larvae, pupae and adult affect 52-92% of mortality at 50-200 ppm. Wu *et al.* (1996) isolated the two kaurane diterpenoids such as annosquamosin A (16 beta-hydroxy-17-acetoxy-ent-kauran-19-al) and annosquamosin B (19-nor-ent-kaurane-4 alpha,16 beta,-17-triol) from the fruit of *A. squamosa.* These compounds inhibited the HIV replication in H9 cell line (lymphocytes) with the ED_{50}- 0.8 μg/ml. El *et al.* (1999) reported that the methanolic extract from the leaves and the stem bark of the plant shew antiplasmodial activity at 2 and 8.5 μg respectively. These concentrations had no effect on the lymphocyte proliferation. Furthermore, Shirwaikar *et al.* (2004) reported the antidiabitic activity of aqueous leaf extract in streptozotocin-nicotinamide induced diabetic rats. The plasma glucose level, serum insulin level, serum lipid profiles and body weight of the rats were found normal and the liver glycogen and pancreatic TBRS were not different to diabetic rats. The recent study by Singh *et al.* (2004 and 2005) found that the acetogenins from the seed shew molluscidal effect by causing significant reduction in the fecundity, hatchability, and survival of the young snail, *Lymnaea acuminata.*

The *Cananga odorata* (Lam.) Hook. f. & Thomson var. *odorata* (Annonaceae)

The *C. odorata* var. *odorata* is also known as Ilang-Ilang or Ylang-Ylang or Kradangnga-Thai. The plant is distributed in the subtropical and tropical region especially Southeast Asia. The volatile oil from flower is frequently used in aromatherapy. The plant flowers in April to June and fruiting in October to March. In

the indigenous records, several parts of the plant have been used (Bunyaprapatsorn *et al.*, 1996). The bark is used in the treatment of infected abscess. The flower is used to treat vertigo, gastric pain, stomach pain, fever and used as cardiac stimulant, cardiotonic, blood tonic, element tonic and improving of cardiovascular function. The leaf is used for skin disease, diuresis, dysuria, ringworm and chloasma. The roots are used in contraception. In the phytochemical study, the flower contain 2-methyl-but-3-en-2-ol, 3-methyl-but-2-en-l-ol, 3-methyl-but-3-en-1-ol, benzaldehyde, benzoic-acid, benzylacetate, benzylalcohol, benzylbenzoate, benzylsalicylate, butylacetate, cadinene, canangine, caryophyllene, creosol, D-alpha-pinene, delta-cadinene, epsilon-cadinene, eugenol, euginalmethylether, farnesol, formic acid, furfural, gamma-cadinene, geraniol, geranylacetate, isoeugenal, isosafrole, L-cadinol, L-linalal, methylanthralinate, methyl-benzoate, methyl-salicylate, nerol, nerolidiol, p-cresol, p-cresolmethylether, p-tolylmethylether, phenylethylalcohol, salicylic acid, sesquiterpene and terpenes (Duke, 2004).

In 1997, Woo *et al.* reported the cytotoxicity of the alkaloid extracted from the fruits, lireodinine. This alkaloid acts as topoisomerase II inhibitor to SV40 infected CV-1 cells in the karyokinesis. Chu *et al.* (1998) found the amebicidal activity from the polar and non-polar extracts of the leaves against *Acanthamoeba culbertsoni*, *A. castellanii* and *A. polyphaga* but not against macrophage. Orabai *et al.* (2000) described the antibacteria activity of sampangine alkaloid from the barks against *Crytococcus neofarman.*

The *Cananga odorata* (Lam.) Hook. f. & Thomson var. *fruticosa* (Annonaceae)

The *C. odorata* var. *fruticosa* or *Canangium fruticosum* Craib also known as Kradanga-Songkla, cultivated in Southeast Asia. The plant flowers between November to February and fruiting in March to June. The phytochemical and pharmacological reports are not available.

The *Merodorum fruticosum* Lour. (Annonaceae)

The *M. fruticosum* (*Popowia aberrans* Pierre ex Finet et Gagnep), also known in Thailand as Lumduan and Homnuan, distributes in subtropical and tropical regions. The plant flowers during February to March and fruiting in May to July. Only the

flowers are used in the traditional medicine for fever, vertigo and as cardiotonic (Bunyaprapatsorn *et al.*, 1996). The chemical constituents of the flower are 7-benzoyloxy-6-oxo-2,4Z-heptadiene-1,4-olide, 7-benzoyloxy-4-hydroxy-1-methoxy-2E,4Z-heptadiene-1,6-dione, 7-benzoyloxy-6-oxo-2,4E-heptadiene-1,4-olide, acetylmelodorinal, benzoic acid, benzylbenzoate, chrysin, dichamanetin, melorodinone, melodoriol and polycarpol.

In 1991, Jung *et al.* isolated some heptenes (melodorinol, homomelodienone, 7-hydroxy-6-hydromelodienone and homoisomelodienone) from the flower. These agents are significantly cytotoxic to human tumor cell lines. Recently, a group of Thai scientists reported bioactive compounds from the ethanolic flower extract (Chaichantiyuth *et al.*, 2001). The 7-benzoyloxy-6-oxo-2,4Z-heptadiene-1,4-olide, 7-benzoyloxy-4-hydroxy-1-methoxy-2E,4Z-heptadiene-1,6-dione and 7-benzoyloxy-6-oxo-2,4E-heptadiene-1,4-olide shew the cytotoxic activities to BT474 (human breast ductal carcinoma), HEP-G2 (liver hepatocarcinona), KATO3 (gastric carcinoma) and SW620 (colon adenocarcinoma) with ED_{50}, 3, 3.7, 3.3 and 2.6 μg/ml, respectively.

2.2 The human amniotic fluid cell line (AMC-K46)

The human amniocyte primary cells are routinely used in the prenatal diagnosis with the overall composition of the amniotic fluid changes predictably throughout the pregnancy. The extra-embryonic coelom develops during the 4^{th} week of gestation and the fluid could be aspirated from the 5^{th} week (Fauza, 2004). Kaviani *et al.* (2001 and 2003) reported that human and sheep amniotic fluid cells consist of the mesenchymal, fibroblasts and myofibroblast linage by using immunofluorescent staining techniques. The fetal cells grow faster than the newborns and adults origin. In contrast to the stem cell criteria, Samuel (2001) reported the amniotic fluid cells seeded on the scaffold grew into the connective tissue much faster than the cells directly from the fetus. The cells were used to repair the lambs with defected body wall. Moreover, Fauza (2004) reported that human amniotic fluid contains the amniotic progenitor and stem cells; the human amniotic epithelial cells, the mesenchymal stem cells and the embryogenic-like stem cells. The human amniotic fluid cells shew multipotent potential that can differentiated into neural cells, glial cells and hepatocytes precursors. The amniotic-fluid-derived mesenchymal cells

expressed the ability to differentiate into the multiple mesenchymal linages (i.e. fibroblasts, adipocytes and osteocytes). The embryonic-like stem cells were unusually found (<1%) in the amniotic fluid and the cells could be observed by Oct-4 nuclear marker labeling. This kind of cells can be differentiated into muscle, adipocytes, osteogenic, neurogenic, neural and endothelial cells. The recent study of human amniotic fluid primary cells was reported by Sangngam (2005). The 59 samples of the Thai amniotic primary cells were studied for the laboratory application. Only one sample could be developed as a cell lines. The chromosome pattern of the cells exhibited aberration evidences with the translocation between 15^{th} and 18^{th} chromosome (46, XX, t(15;18)). The spontaneous mutation in marsupials, snails and human amniocytes was reported by Walen (2002). This phenomenon occured in the polyploid cells and indicated in the senescing crisis during the replication. By that time, the cells had abnormal DNA replication as amitotic nuclear division or endoreduplication which related to the abnormal nuclear fragments. The nuclear fragments were then excluded from the donors by cytoplasmic budding. The cells may drive themselves passthough the death from the abnormal chromosome distribution and survived under the abnomal condition. In the recent review by Kaviani (2001), amniocytes was reported to be used in not only the *in vitro* toxicology, but also in other studies such as stem cells technology, cells and tissue engineering etc.

2.3 The human cervical adenocarcinoma cell line (HeLa)

HeLa is the first human cancerous cell line which established in 1951 from the cancerous of cervix of 31 year old Negroid woman named Henrietta Lacks. HeLa is the instrumental in creating the polio vaccine (Potier, 2001). For decade, HeLa is a famous model in the cancer research such as signal transduction, cytology, especially, the cytotoxicity assay. The cell has been transformed by human papilliomavirus 18 (HPV18). The HeLa has a hypertriploid chromosome number (3n+) with 20 clonally abnormal chromosomes that contain multiple copies of HPV type 18 integrated specific sites in which supported the cell stable character (Macville *et al.*, 1999). Chiang *et al.* (1992) reported the antitumor activity of Phyllasin F from the ethanolic extract of the plant in family Solanaceae (*Physalis angulata*) against 8 cancerous cell

lines including HeLa. Bullatacin, annonaceous acetogenin from the seeds of *Annona atemoya* could inhibit HeLa growth at 10,000 times of adriamycin (the potent anticancer drug). The mechanism of action of this compound is the inhibition of NADH oxidase in the plasma membrane and the electron transport chain (Morre *et al.*, 1995).