CHAPTER II

LITRATURE REVIEWS

2.1 Silkworm excreta and their traditional uses

Silkworm excreta or silkworm excreta are the fecal matter excreted by silkworm in various growth stages (Figure 2a - c).The know-how of silkworm excreta utilization has been transferred for next generation people for hundreds year. In the name of "Can Sha" which means silkworm sand, silkworm excreta have a long history of use as a folk medicine. Because of their properties which are sweet, pungent, and warm, instant tea of silkworm excreta are trusted for its efficacy for headache and abdominal pain. Furthermore, silkworm excreta are considered as effective medicine in the treatment of gastric disorders, hepatitis, acute pancreatitis, chronic nephritis, blood cholesterol, various leukocytopenia as well as infectious diseases (Key, 1976; Singh and Jayasomu, 2002; Tulp & Bohlin, 2004).

Nowadays, different kinds of silkworm excreta products have been sold widely in Asian countries. Silkworm excreta instant tea, pillow stuffed with silkworm excreta, and chlorophyll paste, and coloring substances are the products that regularly claim for the biological activity and beneficial effects for health.

2.2 Chemical constituents of silkworm excreta

Although the silkworm excreta have been used in medicinal purposes for long time, the published reports of the isolation of chemical compounds and their structure elucidation are rare.

Chlorophyll and chlorophyll degradation products seem to be the most attractive group due to their biological activities. Chlorophyll derivatives (Table 1a-h)

were reported on specifically cytotoxic activity to tumor cells *in vitro* (Lee *et al.*, 1990). 13^2S, *R*-hydroxypheophytin a (10- hydroxy pheophytin a) and pheophorbide a isolated from silkworm excreta were reported for their cytotoxic activity against mice and human tumor cells (Nakatani *et al.*, 1981; Lim *et al.*, 2004). These chlorophyll degradation products were suggested to be a potential drug for photodynamic therapy (PDT). Hirayama and others (Hirayama *et al.*, 2002) reported on L4-1, the chlorophyll-like substances, from an extract of silkworm excreta which have inhibitory activity against HVJ (Sendai virus). Moreover, vesicular stomatitis virus (VSV) could be inactivated by chlorophyll derivatives used as photodynamic agent in photodynamic antimicrobial chemotherapy (PACT) (Lim *et al.*, 2002).

Apart from chlorophyll derivatives, xanthophyll pigments (Table 1i-l) such as canthaxanthin, cryptoxanthin, neoxanthin, and echinenone were found in fresh silkworm excreta (Park *et al.*, 2003).

Lectin-like glycoprotein was successively extracted from silkworm excreta by using hot buffer (Hirayama, 1993). Several studies indicated the inhibitory effect of protein extracted from silkworm excreta against enveloped virus such as HVJ (Sendai virus), HSV (herpes simplex virus type-1), and HIV (human immunodeficiency virus type-1) (Hiraki *et al.*, 1996; Hiraki *et al.*, 2000). Recent study reported on antiviral protein from silkworm fecal matter on nuclear polyhydrosis virus (NPV) *in vitro* and *in vivo* (Neelagul *et al.*, 2007).

The excreta have been found to contain polyisoprenoid alcohol named solanesol (Table1m) which is a highly value precursor for cardiac drugs (Babu, 1994), coenzyme Q10, and vitamin K analogue. Besides, the recent study suggested that solanesol derivatives could be used as wound healing agents (Srivastrava *et al.*, 2009)

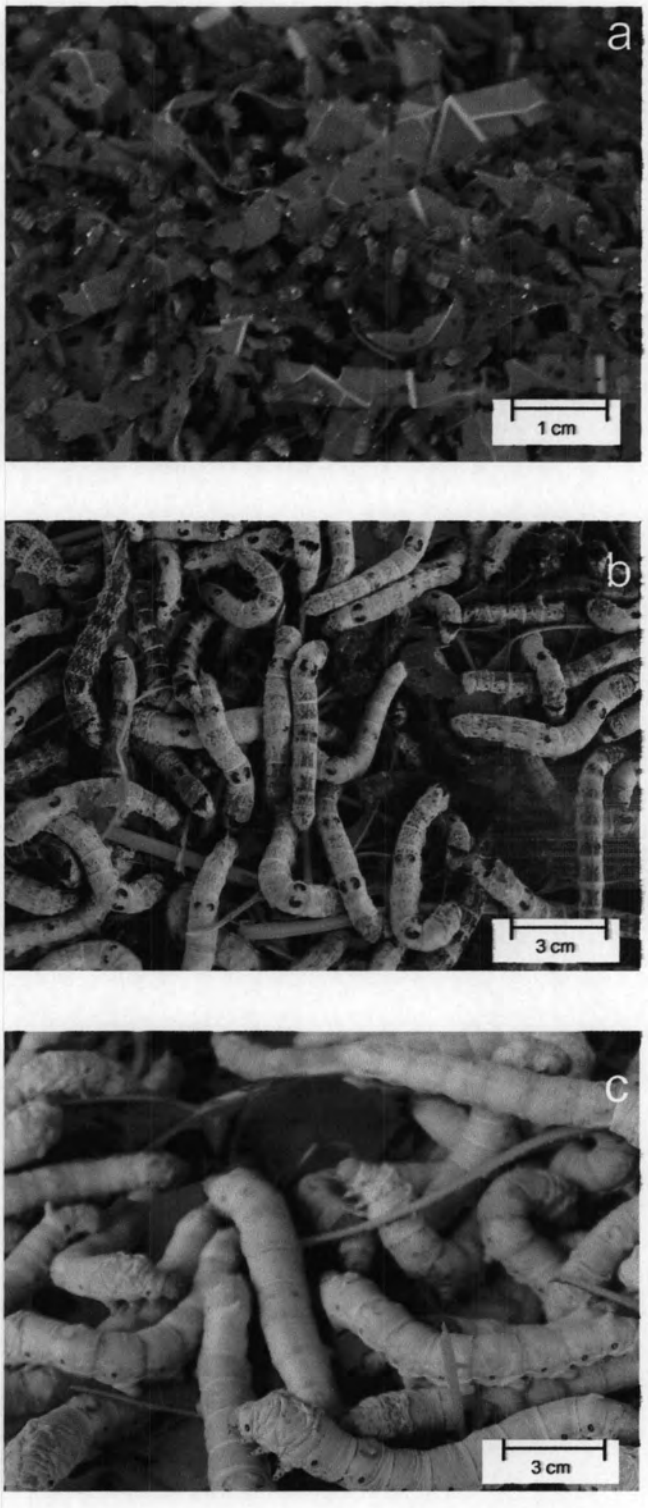


Figure 2 silkworm larva in various growth stages. a) first instar larva, b) third instar larva, and c) fifth instar larva

Table 1 Chemical constituents found in silkworm excreta.

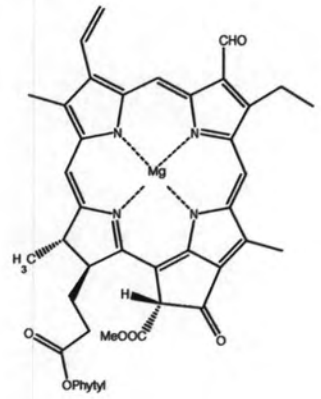
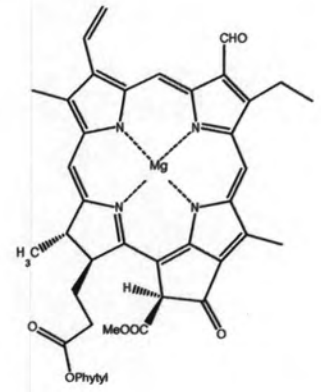
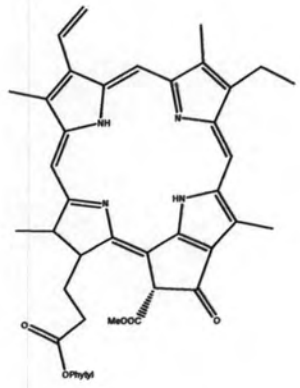
Compound	**Category**	**Reference**
a) Chlorophyll b 	Chlorophyll derivatives	Park *et al.*, 2003
b) Chlorophyll b′ 	Chlorophyll derivatives	Park *et al.*, 2003
c) Pheophytin a 	Chlorophyll derivatives	Park *et al.*, 2003

Table 1 (continued)

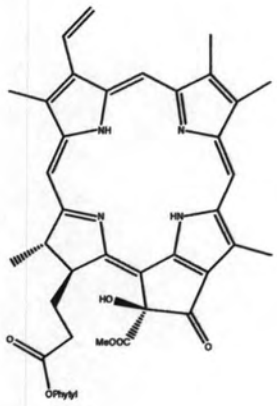
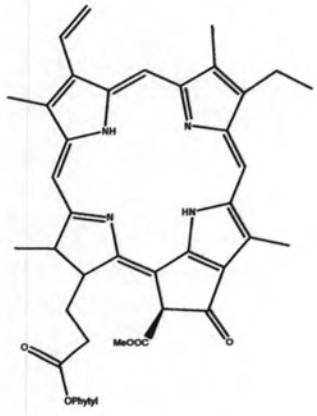
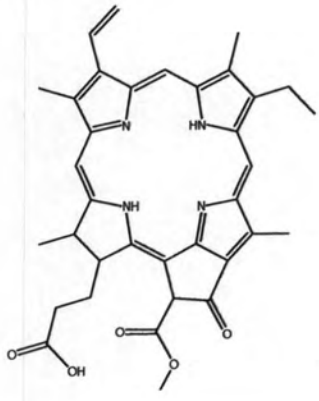
Compound	**Category**	**Reference**
d) 10-hydroxypheophytin a (13^2S-hydroxypheophytin a) 	Chlorophyll derivatives	Dai *et al.*, 1992; Ma and Dolphin, 1999; Nakatani *et al.*, 1981
e) Pheophytin a 	Chlorophyll derivatives	Park *et al.*, 2003
f) Pheophorbide a 	Chlorophyll derivatives	Lim *et al.*, 2004

Table 1 (continued)

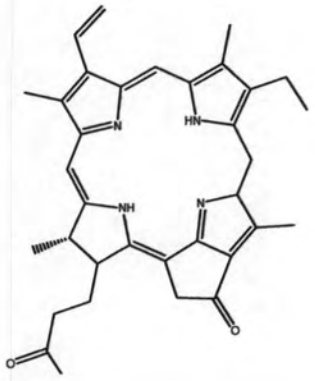
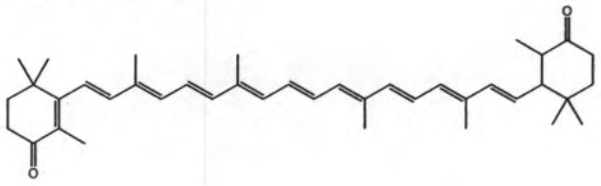
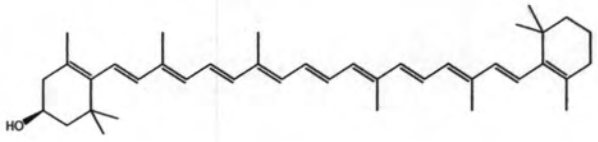
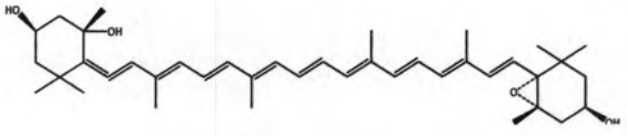
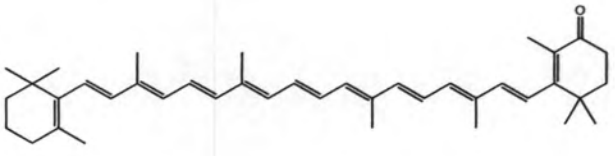
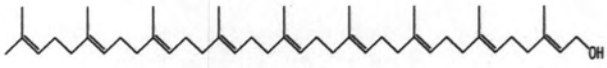
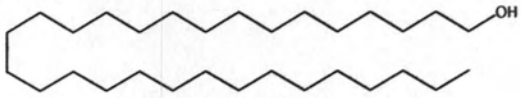
Compound	Category	Reference
g) Pyropheophorbide a 	Chlorophyll derivatives	Park *et al.*, 2003
i) Canthaxanthin 	Xanthophylls	Park *et al.*, 2003
j) Cryptoxanthin 	Xanthophylls	Park *et al.*, 2003
k) Neoxanthin 	Xanthophylls	Park *et al.*, 2003
l) Echinenone 	carotenoids	Park *et al.*, 2003

Table 1 (continued)

Compound	**Category**	**Reference**
m) Solanesol 	Polyisoprenoid alcohol	Babu, 1994; Chen *et al.*, 2007
n) Triacontanol 	Fatty alcohol	Singh and Jayasomu, 2002

2.3 Mulberry leaves and its traditional uses

Mulberry (*Morus alba* L.) leaves containing many nutritional components are the best feed for silkworms (*Bombyx mori* L.) (Kim *et al.*,1999). In traditional medicine, mulberry leaves are used to treat fever, headache, and use as an eye wash for painful eye (Bensky and Gamble, 1993). Also, mulberry leaves have functions in dispersing wind-heat, clearing lung-heat, moistening dryness and removing liver fire for improving eyesight as well as in lowering blood pressure and reducing dieresis (Wang and Zhou, 2008; Sun and Lu, 1999). Leaves were used to treat diabetes since ancient times in China (Wang and Zhou, 2008). Recently, methanolic extract of mulberry leaves showed a promising anxiolytic effect (Yadav *et al.*, 2008).

2.4 Chemical constituents of mulberry leaves

The compounds found in mulberry leaves have been reported to have biological activities. Flavonoids (Table 2a-d) from leaves are toxic to some cancer cells (Butt *et al.*, 2008).The coumarin named 5, 7-dihydroxycoumarin-7-methyl ether (Table 2e) was reported from mulberry leaves. Phenolic compounds and anthocyanins (Table 2f-g) found in leaves exhibited antioxidant and neuroprotective activity (Katsube *et al.*, 2006). Mulberroside F and oxyresveratrol (Table 2f and h) have been reported to have an inhibitory effect on mushroom tyrosinase, in vitro (Shin *et al.*, 1998; Baurin *et al.*, 2002). Moreover, A piperidine alkaloid named 1-deoxynojirimycin (Table 2i) has been reported for its inhibitory activity to alpha-glucosidase enzyme (Asano *et al.*, 1995). Other alkaloid types were also reported such as 2-arylbenzofuran derivatives and Polyhydroxylated alkaloids (Table 2j-k). Fagomine (Table 2l) isolated from mulberry leaves extract exhibited induction activity to insulin secretion (Tanaguchi *et al.*, 1998).

Table 2 Chemical constituents found in mulberry leaves.

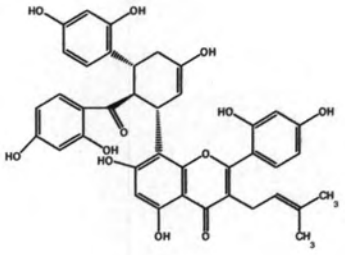
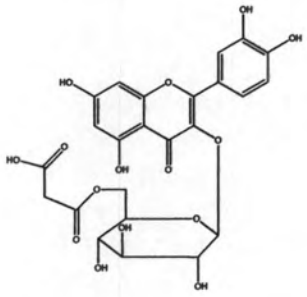
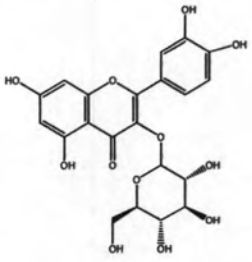
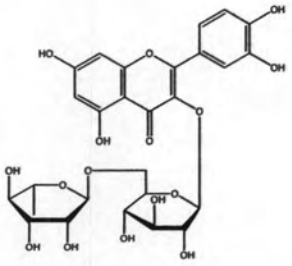
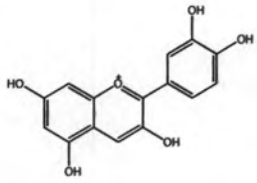
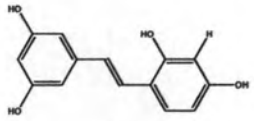
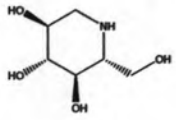
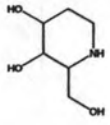
Compound	Category	Reference
a) Kuwanon G 	flavonoids	Butt *et al.*, 2008
b) Quercetin 3-(6-malonylglucoside) 	flavonoids	Enkmaa *et al.*, 2005
c) Isoquercetin 	flavonoids	Butt *et al.*, 2008
d) Rutin 	flavonoids	Butt *et al.*, 2008
e) 5, 7-dihydroxycoumarin-7-methyl ether	coumarin	Butt *et al.*, 2008

Table 2 (continued)

Compound	Category	Reference
f) Mulberroside F	Phenolic compounds	Shin *et al.*, 1998
g) Cyanidin 	anthocyanins	Katsube *et al.*, 2006
h) Oxyresveratrol 	stilbenoids	Butt *et al.*, 2008
i) 1-deoxynojirimycin (DNJ) 	alkaloids	Asano *et al.*, 1995
j) 2-arylbenzofuran derivatives	alkaloids	Asano *et al.*, 1995
k) Polyhydroxylated alkaloids	alkaloids	Asano *et al.*, 1995
l) Fagomine 	alkaloids	Tanaguchi *et al.*, 1998

2.5 Antityrosinase and antioxidant

Silkworm is normally fed on mulberry leaves which have been reported for their antityrosinase and antioxidant activity (Butt *et al.*, 2008). Thus it is interesting to investigate whether the compounds attribute to the activity are still remained in the silkworm excreta after digestion and excretion process of the silkworm or not.

Tyrosinase (monophenol monooxygenase or polyphenol oxidases E.C. 1.14.18.1) is a key enzyme involved in melanin synthesis distributed widely in plants and animals. Tyrosinase catalyzes two distinct reactions of melanin synthesis: The hydroxylation of tyrosine by monophenolase action and the oxidation of 3, 4-dihydroxyphenylalanin (L-DOPA) to *o*-dopaquinone by diphenolase action. In food, browning generally results from both enzymatic and nonenzymatic oxidation (Yu *et al.*, 2007). Tyrosinase catalyzes the oxidation of phenolic compounds to the quinones and is responsible for the enzymatic browning (Kim and Uyama, 2005). In animals, tyrosinase is responsible for skin and hair pigment. Excess of this enzyme can cause the abnormal melanin accumulation results in dermatological disorder in human such as melasma, freckles, ephelide, senile lentigines etc. (Solano *et al.*, 2006) which would be an aesthetic problem.

Therefore, the use of tyrosinase inhibitors is becoming increasingly important in the cosmetic industry due to their skin-whitening effects (Momtez *et al.*, 2008). Using of topical hypopigmenting agent is possibly the least invasive procedure (Kadekaro *et al.*, 2003; Rendon *et al.*, 2006). And, as antibrowning agent, tyrosinase inhibitors are very important substances to control the quality and economic of fruits and vegetables.

Free radicals such as reactive oxygen species were produced during basic cellular oxidation process. In food, reactive oxygen species involved in nonenzymatic browning by trigger several reactions such as caramelization and the Maillard reaction. In animals, when there is an imbalance in the generation and removal of these radical species within an organism, oxidative stress occurs (Kelly *et al.*, 1998), resulting cell

membrane and tissue damage (Seifried *et al.*, 2007, Syvacy and Sökmen, 2004). Oxidative damage can cause initiation and progression of the diseases (Kelly *et al.*, 1998, Jacob and Burri, 1996). Moreover, oxidative stress caused by free radicals was proposed for hyperpigmentation (Butt *et al.*, 2008).

For this reason, many research focused on determination if supplementation of antioxidant, the compound that can inhibit the initiation or propagation of these oxidative chain reactions (Velioglu *et al*, 1998), can be used in prevention or treatment various diseases (Seifried *et al.*, 2007) including hyperpigmentation.