CHAPTER 1

INTRODUCTION

1.1 Background and Hypothesis

Thailand has several biological assets especially herbal plants which have been used for treatment of diseases and health-enhancement since ancient time. The appropriate usages of herbal plant are conducted in the sufficiency economic theory and play important roles in the development and competition with foreign trade. Thus the Royal Thai government has been supporting and applying this as one of its major policies. However, the development of herbal products has not integrated enough of the science and technology knowledges, causing the product development to progress at a slow rate and not meeting the international standard for export. In addition, herbal products suffered from many problems, such as no active compounds standard control, microbial contamination, instability of active compounds, etc.

Houttuynia cordata Thunb. (HC), an important traditional medicine of East and South East Asia, especially China, Japan and Thailand, where the plant is known as Kao-Tong or Plu-Kao (Bansiddhi *et al*., 2003), contains numerous active compounds including essential oils, flavonoids, alkaloids, sterols, fatty acid and other compounds (Bansiddhi *et al*., 2003). Three major components are commonly listed, including essential oil, phenol and alkaloids (Kim *et al.*, 2001; Meng *et al.,* 2005). HC consists of essential oil, flavonoids, alkaloids, fatty acid, sterol and the others such as polyphenolic acid (Bansiddhi *et al*., 2003), however in this study focuses on

flavonoids which HC leaves had more flavonoids up to 1.14% than other plants (Ling-Shang *et al*., 2009).The plant were report to possess many pharmacological properties such as anti-inflammatory, antibacterial, antitumor, antimicrobial, immunodulatory, anti-SARS, anti-leukemic, anti-cancer and anti-allergic activities (Bansiddhi *et al*., 2003 and Ling-Shang *et al*., 2009). HC is currently applied in many kinds of products, including as food supplement, drug, beverage and cosmetics. HC was prepared in the form of injection (Lau *et al*., 2008; Lu *et al*., 2006). HC was also combined with other herbal plants in capsule dosage form (Li, 2003) and injection (Yu, 2007). Extracts of HC and other medicinal plants were prepared as buccal tablet for acute and chronic pharyngitis and stomatitis (Xuan and Lui, 2004). In Thailand, the Government Pharmaceutical Organization (GPO) manufactures a capsule formulation containing HC and other plant extracts for use as food supplement to improve immune response (Sriwanthana *et al*., 2007). Preparations of sole HC are commercialized in the forms of powdered plant capsule, fermented drinks and wines. The form of tablet, both of HCP or HCE, has not been found.

Tablet is generally the most desirable dosage form as it has advantages over other forms in terms of consistency and accuracy of active compound(s) for a unit dose, tampered-proof, low cost, convenience of taking and carrying and storage. However, several reports suggested that rheological property and compressibility were two main obstacles for tabletting of plant extract. Proposed solutions to overcome these problems included the wet granulation, the preparation of spray-dried extract powder, or the use of pharmaceutical excipients to enable direct compression (Plazier-Vercamen and Bruwier, 1986; Díaz *et al*., 1996; Renoux *et al*., 1996; Palma *et al*., 2002). Tablets of plant extract contained higher amounts of active components

than those in capsules of herbal powder. In addition, herbal extract formulation minimized the rate of microbial contamination often associated with the herbal powder preparation. The quality control of active compounds can also be facilitated in the extract formulation which leads to the higher quality and safety of medicinal plant products for modern medicines. This study reports the preparation and quality control of standardized HCE and the formulation of stable tablets containing consistent HCE as a food supplement product.

1.2 Objectives

1. To prepare the standardized HC extract
2. To investigate the appropriate analytical methods for quality control of HC extract using chromatographic technique.
3. To improve the formulation of HC standardized extract tablets as a good property and stability also to apply the standard control according to tablet requirement for food supplements

1.3 Literature Review

Houttuynia cordata Thunb. Plant

Houttuynia cordata Thunb. (HC) (Saururaceae) is a traditional plant in East Asia and south-east Asia especially Korea, India (Bansiddhi *et al*., 2003) China, Japan (Meng *et al*., 2005 and Bansiddhi *et al*., 2003). The synonymous specific science names of HC are *Polypara cochinchinensis* and *Polypara cordata* O.K. moreover the other names dependent on the locations see table 1.1 (Bansiddhi *et al*., 2003).

Table 1.1 Names of HC depended on origin (Bansiddhi *et al*., 2003)

Origin	Names
General	Chinese lizard tail, fishwort, heartleaf, Houttuynia, chameleon Plant
Thailand	pak-kan-tong, pak-khau-tong, pak-cao-tong, plu-kea, plu-cao
Kampuchea	chil yaab kaa
China	chi, chu tsai, yu hsing tsao, yu xing cao
Japan	chung-yao, doku-dami, gyoseiso, shih-yao, zyuyaku
Nepal	ukur paile
Vietnam	cay la giap, diep ca, giap ca, ngu tinh thao
Germany	buntblatt, chinesischer, eidechsenschwanz, chamaleonpflanze
Lao	khaaw thong
Indonesia	jukut hanyir

In 1783, HC was truly named by Carl Peter Thunberg, Swiss botanist according to international naming standard to exhibit respect Maarten Houttuyn, the Dutch naturalist. Next in 1784, it was named *Houttynia cordata* Thunb. and classified as a master type of this species (Bansiddhi *et al*., 2003).

Family Saururaceae consisted of 4 genera (Mabberley, 1997; Walter *et al*., 2002; Bansiddhi *et al*., 2003), including *Anemopsis, Gymnotheca, Houttuynia and Saururus*. There are 2 genuses and 1 species (Ohwi, 1965; Bansiddhi *et al*., 2003) for each in Asia as *Gymnotheca* and *Houttuynia*. However, in Thailand has only 1 genus and 1 species as *Houttuynia cordata* Thunb. (Larsen, 2000).

Classification of *Houttuynia cordata* Thunb. plant shown below (Bansiddhi *et al*., 2003)

Kingdom Plate (Plants)

Subkingdom Tracheobionta (Vascular plants)

Superdivision Spermatophyta (Seed plants)

Division Magnoliophyta (Flowering plants)

Class Magnoliopsida Dicotyledons (Dicotyledons)

Subclass Magnoliidae

Order Piperales

Family Saururaceae (Lizard's-tail family)

Genus Houttuynia Thunb (houttuynia)

Species *Houttuynia cordata* Thunb.

Botanic character of HC

HC is an annual plant, short-lived, with fishy smell (Bansiddhi *et al*., 2003). Bunchy flowers bloom the top or upper leaves like spiked flowers (Nilsamranchit *et al*., 2000). The bunchy flowers without corolla were grown in 4 white petals that around. There are composed of small flowers tightly arrange and bloom in May and August. The underground stems were equipped with short internodes of cellar roots (Bansiddhi *et al*., 2003) nevertheless aerial stems were both short and long internodes base on the species and light red, light green, purplish red or purplish green (Ling-Shang *et al*., 2009). The aerial stems are 10-30 centimeters tall (Bansiddhi *et al*., 2003). It is alternated singer heart leaves, acuminated end leave, wavy or nearly smooth leaf edge, green or purplish red colors (Bansiddhi *et al*., 2003).

Figure 1.1 *Houttuynia cordata* Thunb. Plant

Genetics of HC

The genetic of HC has not much reported, a number of chromosomes were 2n=96 in Japan (Kurosawa, 1996; Bansiddhi *et al*., 2003) while HC from Japan that study in Thai was found 2n=90 of HC chromosomes (Nilsamranchit *et al*., 1999; Bansiddhi *et al*., 2003). There was studied chromosome in HC of Chiang Mai and Lampoon for 10 source found that 2n=74-94 especially 2n=74 was the most identify (Nilsamranchit *et al*., 1999).

Chemical components and Pharmaceutical properties of HC

Herbal plants were natural so their origin and habitat were affected to identity property, chemistry compounds and pharmacological properties. The chemistry compound of HC depended on three factors for example biochemical variation, determination and adulteration/substitution which effect to various quality and bioequivalent (Bansiddhi *et al*., 2003). So those could be controlled through the herbal standardization

Major components of HC were separated to six groups that as essential oils, flavonoids, alkaloids, fatty acid, sterol and others (Bansiddhi *et al*., 2003) but others was suggested that these composed of three major types such as essential oils, phenols and alkaloids (Baure *et al*., 1996; Meng *et al*, 2005). However this study was focused on flavonoids that a group of polyphenolic compounds, the components of HC were provided to six groups that explained below.

1. Flavonoids

Flavonoids are polyphenolic compounds (Formica and Regelson, 1995) and pigments in nature compounds of green plant cells, generally found in the second plant metabolisms (Havsteen, 2002) as glycoside form (Formica and Regelson, 1995), chiefly obtained from bezo-γ-pyrone and formed of diphenyl propane structure (C6-C3-C6) (Havsteen, 2002); Ring A, B and C such as flavan-3,4-diols; quercetin, myricetin and kaemferol and regularly appeared in glycoside term; aglycone, quercetin league with 3-O-rhamnose (quercetrin) or 3-O-rutinose (rutin) (Formica and Regelson, 1995).

There were reported several flavonoids in HC such as afzelin, isoquercetin, rutin (Bansiddhi *et al*., 2003), hyperin, isoquercitrin and quercertin, (Benskey, 1993). Additional Xu *et al*. (2005) found four flavonoids (rutin, hyperoside, quercitrin and quercetin) in *Houttuynia cordata* Thunb. and *Saururus chinensis* (Lour.) Bail.

Flavonoids, which occur entirely in medicinal herbal plant, display a large of biological and pharmaceutical properties, such as anti-inflammatory, anti-tumor, anti-cancer, anti-allergic, anti-virus, anti-bacteria, anti-oxidation, anti-hepatoxic, platelet and mast cell stabilization (Formica and Regelson, 1995) and cells protection (Matsui *et al.*, 2006).

Nine flavonoids: hyperoside, rutin, aristolactam, quercitrin, apigenin, luteolin, quercetin, acacetin and acaciin that were obtained from Herba Houttuyniae and four Chinese medicinal plants exhibited for treatment and prevention of serve acute respiration syndrome (SARS) (Zhang and Chen, 2008). Single or coalescent Rutin is enhanced the symptoms of venous and lymphatic vessel insufficiency, In case of the symptomatic remedy for functional signs of capillary fragility, to treat the functional expression of the acute attack of piles, and for detriment of visual acuity and alterations of the field of vision probable of vascular origin (Bruneton, 1999). Quercitrin that was flavonol glycoside in HC was suggested to urinate (Somnapan, 1998). Also quercetin could block activity of protein kinase and lactate transport so tumor was inhibited. In addition quercetin could improve interferon in antiviral activity (Formica and Regelson 1995).

(a) (b)

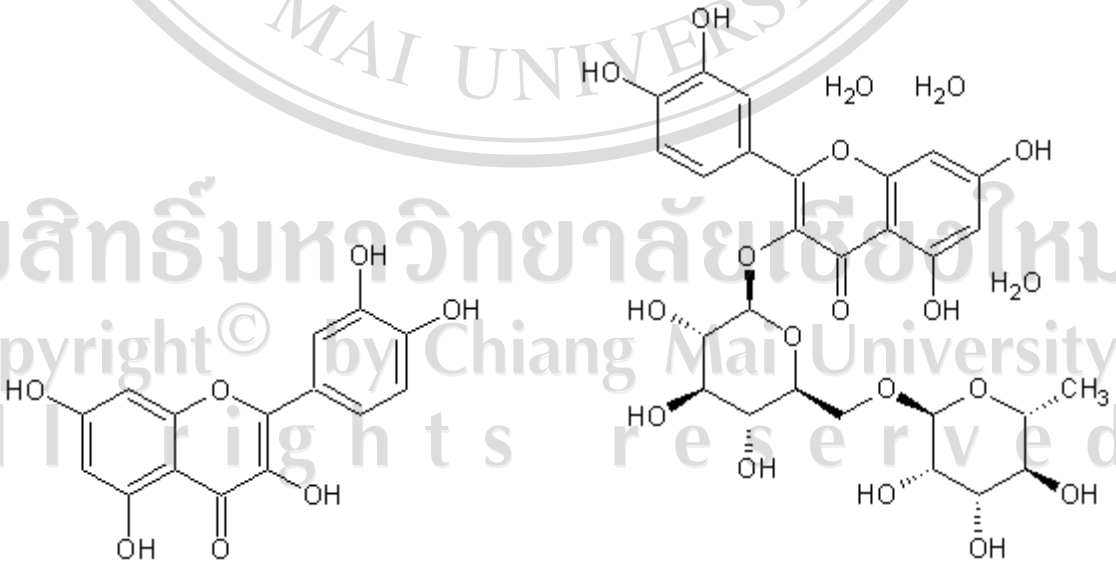

Figure 1.2 Structures of flavonoids compounds in *H. cordata* Thunb.; (a) quercetin and (b) rutin

2. Essential Oils

Volatile and essential oils from steam distillation were volatile compounds at normal temperature (Huy *et al*., 2004). In China, the essential oils of HC were found major compounds such as methyl-n-nonylketone, capric aldehyde, 1-decanol and others. (Lu *et al*., 2006). In Japan, Kameoka *et al.* (1972) found essential oils such as pinene, camphene, limonene and etc. Also they suggested that 3-keto-decanal, methyl-n-nonyl ketone and methyl lauryl sulfide might be odor.

3. Alkaloids

Alkaloids were obtained from secondary metabolism of some multi-cellular plants that were composed of nitrogen atom in alkaline form (Bansiddhi *et al*., 2003). There were in HC for 2 groups, first pyridine derivative and 1, 4-dihydropyridine derivative for example 3,5-didecanoyl pyridine and 3-decanoyl-6-nonylpyridene and second aporphine derivative such as cepharanone B, cepharadione B, aristolactam A, norcepharacdione, aristolactam A II (Somnapan, 1998).

4. Fatty acids

Fatty acid in HC was a group of fixed oils such as capric acid, lauric acid, linolenic acid (Bansiddhi *et al*., 2003) and capric aldehyde (Benskey, 1993)

5. Sterol

HC was composed of sterols for instance phytol; b-sitosterol, spinasterol, stigmasterol (Bansiddhi *et al*., 2003)

6. Others

The others were found in HC as polyphenolic acid and many mineral such as fluoride, potassium chloride, potassium sulfate (Bansiddhi *et al*., 2003), calcium sulphate and calcium chloride (Benskey, 1993)

Toxicology

In case of toxicities, mice were feed 1.6 g of HC extract/kg body weight/day every 1, 3 and 6 hours that found all mice can survive all 7 days (Lau *et al*., 2008). The female patients 63 years old were intake oral liquid HC and dried HC powder every day for 20 day shown violet spot around both of cheeks, burn in skin, forehead, neck, chin, palm and lower arm including in the patients for patch test and photopatch test, water extraction of dried leave CH manifest a few responded patch test and more in photopatch (UV 320 nm and 340 nm) (Bansiddhi *et al*., 2003)

Extractions

Phytomedicines were different from synthesis drug because there were distinctive attributions which were more than one active component (Shahoo *et al*., 2010) similarly Houttuynia cordata composed of 32 essential oil compounds (Kameoka *et al*., 1972), 44 volatile oils such as terpenoids, aldehyde, ketone, acid and others (Kang *et al*., 1997) and 9 flavonoids in 3 herbal medicines containing HC (Zhang and Chen, 2008). Conditions of cultivation, manufacturing, marketing and distribution was influent to chemical patterns of medicinal herbal plant while the biochemical profiles and secondary metabolite production in herb were depended on physical properties, genetic and surrounding variables (Shahoo *et al*., 2010).

There were several methods of extraction which objective of extraction were presented as separation of active constituents from medicinal plants, high concentration of active constituents and decreasing of suitable dosage of plant drug (Intranupakorn, 2004).

Method of extraction (Intranupakorn, 2004)

- Macerations

Medicinal plants were macerated in closed container with solvents that permeated to dissolve and removed off the active compounds. The benefits of this method are safe cost in low solvent using and suitable for thermal sensible extraction.

Factors of selecting extraction

- Nature of medicinal plants
    - Characteristics and structures of plant tissues
    - Solubility of active components in extracting solvent
    - Stability of active compounds; heated sensitivity
- Efficiency and cost of extraction
- Required concentration of exhausted extraction.

Quality control and standardization

Quality control of herbal medicines was directly effect to their safety and efficacy. The important factors such as environmental and agricultural were influenced to collections of herbal materials. A series of technical guidelines and documents relating to the safety and quality assurance of medicinal plants and herbal materials was improved by WHO that had published the 'Quality Control Methods for Medicinal Plant Materials', a collection of recommended test procedures for assessing the identity, purity, and content of medicinal plant materials to assist national laboratories engaged in drug quality control (WHO, 1998; Shahoo *et al*., 2010).

Botanical and herbal preparations are provided prerequisite clinical trials for quality assurance. Parameters such as identification, water content, chemical assay of active ingredients, inorganic impurities (toxic metals), microbial limits, mycotoxins,

pesticides and others were applied to improve their certification. For herbal preparations, in addition to these tests, disintegration, dissolution, hardness/friability and uniformity of dosage unit should be reported (Ong, 2004; Shahoo *et al*., 2010). The chemistry, manufacturing and control documentation that should be performed for botanical drugs is often different from that for synthetic or highly purified drugs, whose active constituents can be more readily chemically identified and quantified. For industrial process in USA, botanical drugs was not necessarily identified the active components at the measurement of new drug scenes (Shahoo *et al*., 2010).

The quality control standards of differential medicinal plant employed in indigenous of medicine are becoming more relevant today for the commercial issues of medicinal plant formulations (Yadav and Dixit, 2008). The therapeutic use of herbal medicines is obtaining important motivation in the world pending the ancient decade. The World Health Organization (WHO) suggested that herbal medicine is still popularity up to 75-80% in the world, especially in the developing countries (Kamboj, 2000; Yadav and Dixit, 2008).

Standardization refers to the body of information and controls necessary to produce material of reasonable consistency. This is achieved through minimizing the inherent variation of natural product composition through quality assurance practices applied to agricultural and manufacturing process (Waldesch *et al*., 2003; Yadav and Dixit, 2008)

The focuses of Standardization were served below (Yadav and Dixit, 2008).

- Batch to batch consistency
- Conformation of correct amount of extract per dosage unit
- Positive control to indicate possible loss or degradation during processing.

Quality control of raw material medicine plants (Intranupakorn, 2004)

The quality controls of plant medicines were important for manufacturing according to requirement and efficiency quality that were included physical and chemical property.

There was not inadequate for single method to evaluate medicinal plant so the appropriated procedures were complicate which depended on suitability. The popularity methods were:

- Herbarium specimen
    - Identification of external characteristic and internal structure with microscopy
    - Chemical Identification through chromo reaction and/or Thin Layer Chromatography (TLC).
- Purity
    - Contaminations
    - Ash contents
    - Crude extract in specific solvent
    - Moisture content or loss on drying
    - Microbial contamination and pesticide contents
- Active constituents
    - Ultraviolet spectroscopy: alkaloids and vitamins
    - Spectrofluorometry: quinine
    - Chromatography: others

Quality control of tablet formulations (Tourtip, 1991)

The quality controls of tablet formulation were depended on tablet formula requirement both in-process control and finished product control which were:

- In-process controls
    - Weight variations
    - Disintegration time, dissolution, hardness, friability
    - Separation of defected tablets before packing
    - Cleanness, precision and accuracy of manufacture equipments
    - Sanitation and hygiene of premises
- Finished product controls
    - Weight variations
    - Disintegration time, dissolution, hardness, friability
    - Fingerprints and consistency of the active compounds in tablets

Chromatographic Technique

Thin Layer Chromatography (Wagner *et al*., 1984)

TLC was capacious chromatographic method to rapidly and positively analyse for drug and drug preparations.

This popular method was admired for enormous reasons:

- TLC was spent very short time to exhibit the most of attribute components of drug.
- TLC supplies semi-quantitative information on the major active constituents of a drug or drug preparation, hence it is availed an appraisal of drug quality for quantitative investigations.

- TLC is consequently appropriated for monitoring the identity and purity of drug also detecting contamination and replacements including it can contribute a chromatographic drug fingerprint.
- TLC can apply to analyze drug combinations and phytochemical preparations follow the aid of appropriate separation procedures,
- TLC can be documented.

TLC was the habitual method for herbal analysis before instrumental chromatography methods like GC and HPLC were set up. In recent century TLC is still regularly employed to herbal medicines measurements inasmuch as several pharmacopoeias for instance American Herbal Pharmacopoeia (AHP) (Upton, 2002; Liang *et al*., 2004), Chinese drug monographs and analysis (Wagner *et al*, 1997; Liang *et al.*, 2004), Pharmacopoeia of the People's Republic of China, etc. still applied TLC to furnish first characteristic fingerprints of herbal medicines because of it is simple method to prescreening and often lesser vary than other chorography method (Liang *et al.*, 2004). The advantages of TLC application to construct the fingerprints of herbal medicines are its simplicity, versatility, high velocity, specific sensitivity and simple sample preparation. Thus, TLC is a suitable method of determining the quality and possible adulteration of herbal products (Wagner *et al*., 1984)

High Performance Liquid Chromatography (HPLC)

HPLC has been the most widely using and popular application for previous decennium because it is simple to study and use. There were also several sample compounds for volatility or stability with unlimited. The most of products analysis

can be employed HPLC to measure the major component of herbal medicines (Liang *et al*., 2004).

Hong *et al* (2001) analyzed baicalin in HC tablets via HPLC with Hypersil ODS C18 column (250 mm×4.6 mm, 5µm), Methanol:0.4% H_3PO_4 (53:47) as mobile phases and 277 nm of wave length, then results was shown 100.6% and 1.60% of average recovery and RSD respectively while others was applied Hypersil C18 anal. Column (250 mm×4.6 mm, 5µm), Methanol:Water:Glacial acetic acid (50:50:1) as mobile phases with 1.0 ml/min and wave length at 278 nm thence average recovery and RSD were 100.5% and 0.68% consecutively (Li *et al*., 2005). Hyperoside and quercitrin in HC were identified through Shimadzu C18 column (150 mm×4.6 mm, 5 µm), Methanol:0.2% Phosphoric acid (45:55) as mobile phases with 1.0 ml/min at room temperature and 350 nm of wave length so the rate of recovery and relative standard deviation were presented 102.47% and 1.04% respectively for hyperoside beside 97.92% and 2.13% for quercitrin (Zheng *et al*., 2005).

Lui *et al.* (2006) measured rutinoside and quercetin in HC via reverse phase HPLC, Diamonsil C18 (4.6 mm×150 mm, 5µm), Methanol:Water:Acetic acid (48:50:2) as mobile phase with 1.0 ml/min, wave length 214 nm, temperature 30°C and 5 µm for injection volume when recovery value and RSD was 98.9% and 1.8% for rutinoside also 98.3% and 3.2% for quercetin respectively that was expressed high simple and accuracy method to quality control (Lui *et al*., 2006) in the other hand quercetin in HC that was obtained 90% ethanol extraction by the way of Dimonsil C18 column (4.6%×150 mm, 5 µm), mobile phase for Methanol:Water:HAc (48:49:3) and 245 nm of wave length shown rate of recovery and RSD were 98.3% and 3.2% (Bian *et al*., 2005). In additional flavonoids in extraction of HC which was removed

by solid phase extraction with 80% ethanol as mobile phase were isolated in Sep-Pak-C18 chromatog column (3.9×150 mm, 5 µm), Methanol:0.05 M KH_2PO_4 buffer solution (60:40) using as mobile phase and photodiode array 360 nm afterward recovery values were 98-101% (Yang *et al*., 2006). Above mention were presented available and confidential HPLC method to excellent accuracy and precision also the advantage of HPLC which was simple control and able application to appropriate for this study.

Tablets

Tablet formulation

Tablet is a solid dosage form that composes of active compounds and their excipients (Sirithunyalug *et al*., 2008). The selections of excipients are depended on their chemical/physical compatibility with drugs, regulatory acceptance, and processability (Teng *et al*., 2009). These can create to identify for difference of appearance, size, weight thickness and disintegration. There was prepared through compression of bulk powder or granules with suitable pressure force of compressor when tablets were measured the pre-controls such as appropriate hardness, rare friableness and requiring disintegration time (Sirithunyalug *et al*., 2008).

Advantages and disadvantages of tablets

- The advantage of tablet were (Sirithunyalug *et al*., 2008):
    - Tablets were constancy for the active components in unit dosage.
    - Tablets were comfortable for keep, carry and transportation.
    - Tablets were stable in chemical and physical properties; less growing of microorganism.

- Tablets were tamper-proof for safety.
- Tablets can create and identify on drug character.
- Tablet formulations did not need preservative.
- Tablets can improve to coated-tablets for immoral flavor and/or order drug problems.

- The disadvantage of tablets were:
  - Tablets formulations were not suitable for fluffy bulk powder, high dosage drug and drug with dissolution problem
  - For coated-tablets may higher cost for coating.
  - The other forms may better efficient using for some kinds of drugs.

Direct compression manufacture (Jivraj, 2000)

For thermolabile and moisture sensitive tablet, direct compression was a technique of ways for manufacturing.

Advantages and disadvantages of direct compression

- Advantages
  - Unit operations of direct compression were less than wet granulation (shorter processing time and lower energy consumption)
  - Sensitive to heat or moisture of active components were applied for stability issues
  - Direct compression may construct to faster dissolution of reliable tablet compared than wet granulation.
  - Some excipients may require a direct compression formula.

- Disadvantages
    - Active compounds of drugs containing excipients can be decreased in particle size and density in segregation state.
    - There is limited to roughly 30% or 50 mg of drug components.
    - Substances with a low bulk density may not be suitable because finished tablets may be slim of thickness.
    - There is not appropriate for worse flowability of drug components.
    - Bulk or excipients with poor mixing property may agglomerate during mixing process cause of static charges.