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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
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FORMULATION DEVELOPMENT OF CHEWABLE TABLETS CONTAINING *Phyllanthus emblica* Linn. FRUIT EXTRACT BY DIRECT COMPRESSION

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy Program in Industrial Pharmacy
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Faculty of Pharmaceutical Sciences
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อาคม สมบัติ : การพัฒนาสูตรตำรับยาเม็ดเคี้ยวผสมสารสกัดผลมะขามป้อมโดยการตอกอัดโดยตรง (FORMULATION DEVELOPMENT OF CHEWABLE TABLETS CONTAINING *Phyllanthus emblica* Linn. FRUIT EXTRACT BY DIRECT COMPRESSION) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: ดร.พรรณเพ็ญ วัฒนาอาษากิจ, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม: ดร.จิตติมา ชัชวาลย์สายสินธ์, รศ.อารีรัตน์ ลออปักษา, 168 หน้า.

มะขามป้อม (*Phyllanthus emblica* Linn.) เป็นพืชในวงศ์ Euphobiaceae ขึ้นอยู่ในเขตร้อนชื้นของเอเชียตะวันออกเฉียงใต้ ส่วนของผลนำมาใช้เป็นยาพื้นบ้านอย่างกว้างขวาง ประโยชน์ของมะขามป้อมเกี่ยวข้องกับฤทธิ์ทางเภสัชวิทยาที่หลากหลาย เช่น ต้านออกซิเดชัน ปกป้องตับ ลดระดับคอเลสเตอรอล ต้านอักเสบ ระงับปวด ลดไข้ และบรรเทาอาการไอ เป็นต้น วัตถุประสงค์ของการศึกษานี้คือ เพื่อพัฒนาสูตรตำรับยาเม็ดเคี้ยวผสมสารสกัดผลมะขามป้อมพ่นแห้งโดยการตอกอัดโดยตรงและศึกษาคุณสมบัติทางเคมีกายภาพรวมถึงความคงตัวของผลิตภัณฑ์ ยาเม็ดเคี้ยวเตรียมโดยกระบวนการตอกอัดโดยตรงด้วยเครื่องตอกเม็ดยาแบบสากเดี่ยว ในบางกรณีสารสกัดผลมะขามป้อมพ่นแห้งผ่านการเคลือบด้วยเอธิลเซลลูโลสก่อนที่จะผสมลงในตำรับ ศึกษาผลของสารช่วยยึด สารหล่อลื่น และสารแต่งรสชาติ ต่อคุณสมบัติของยาเม็ด เช่น ลักษณะภายนอก ความแข็ง ปริมาณแทนนิน ปริมาณกรดแกลลิก การปลดปล่อยแทนนิน เป็นต้น ศึกษาความคงตัวของยาเม็ดเคี้ยวซึ่งเก็บในตู้เย็น ในสภาวะแวดล้อมที่ไม่ได้ควบคุมความชื้นและที่ควบคุมความชื้นสัมพัทธ์ 75 เปอร์เซ็นต์ รวมทั้งในสภาวะที่ควบคุมอุณหภูมิ 40 องศาเซลเซียสและความชื้นสัมพัทธ์ 75 เปอร์เซ็นต์ เป็นระยะเวลา 3 เดือน และทดสอบการยอมรับของผู้บริโภคต่อลักษณะภายนอก กลิ่น ความรู้สึกในช่องปากของยาเม็ด ทั้งนี้ได้ทำการทดสอบการปนเปื้อนจุลชีพในสารสกัดผลมะขามป้อมพ่นแห้งก่อนที่จะนำมาเตรียมเป็นยาเม็ด และในยาเม็ดที่เตรียมได้ก่อนนำไปทดสอบการยอมรับของผู้บริโภค ผลการทดลองพบว่ายาเม็ดเคี้ยวที่มีสารสกัดผลมะขามป้อมพ่นแห้ง 30 เปอร์เซ็นต์สามารถตอกอัดเป็นเม็ดได้ด้วยกระบวนการตอกอัดโดยตรง ซึ่งในกระบวนการผลิตต้องใช้สารหล่อลื่นที่เพียงพอ ในการเก็บยาเม็ดที่อุณหภูมิ 40 องศาเซลเซียส และความชื้นสัมพัทธ์ 75 เปอร์เซ็นต์ พบว่าเม็ดยาเปลี่ยนเป็นสีน้ำตาล แต่ความแข็งของเม็ดยามีการเปลี่ยนแปลงแตกต่างกันในสูตรตำรับที่ผสมสารสกัดที่ไม่ผ่านการเคลือบและในสูตรตำรับที่ผสมสารสกัดที่ผ่านการเคลือบ โดยความแข็งเม็ดยาที่ผสมสารสกัดที่ไม่ผ่านการเคลือบจะมีค่าเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) ในขณะที่ความแข็งของเม็ดยาที่ผสมสารสกัดที่ผ่านการเคลือบจะมีค่าลดลงอย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) ปริมาณแทนนินและปริมาณกรดแกลลิกจะมีความคงตัวมากขึ้นเมื่อยาเม็ดเคี้ยวเตรียมโดยใช้ไมโครคริสตัลลีนเซลลูโลส พีเอช 102 เป็นส่วนประกอบและเก็บที่อุณหภูมิแวดล้อม การปลดปล่อยแทนนินจากเม็ดยาที่ตัดเป็นชิ้นแล้วขึ้นอยู่กับชนิดของสารช่วยยึด โดยในสูตรตำรับที่มีโคโพวิโดนเป็นส่วนประกอบจะชะลอการปลดปล่อยแทนนินจากเม็ดยา สูตรตำรับที่มีความคงตัวและได้รับการยอมรับจากอาสาสมัครโดยเปรียบเทียบกับสูตรตำรับอื่นที่ศึกษา ประกอบด้วย สารสกัดผลมะขามป้อมพ่นแห้ง 30 เปอร์เซ็นต์ แมนนิทอล 20.35 เปอร์เซ็นต์ ไซลิทอล 20.35 เปอร์เซ็นต์ ไมโครคริสตัลลีนเซลลูโลส พีเอช 102 10 เปอร์เซ็นต์ ทัลคัม 10 เปอร์เซ็นต์ กรดซิตริก 5 เปอร์เซ็นต์ แอสปาร์แตม 1 เปอร์เซ็นต์ โซเดียมคลอไรด์ 2 เปอร์เซ็นต์ แมกนีเซียมสเตียเรต 1 เปอร์เซ็นต์ และ ซิลิคอนไดออกไซด์ 0.3 เปอร์เซ็นต์

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ARKOM SOMBAT : FORMULATION DEVELOPMENT OF CHEWABLE TABLETS CONTAINING *Phyllanthus emblica* Linn. FRUIT EXTRACT BY DIRECT COMPRESSION. ADVISOR : PHANPHEN WATTANAARSAKIT, Ph.D., CO-ADVISOR : JITTIMA CHATCHAWALSAISIN, Ph.D., ASSOC. PROF. AREERAT LAORPAKSA, M.Sc. in Pharm., 168 pp.

Indian gooseberry (*Phyllanthus emblica* Linn.), a plant in family Euphobiaceae, is a tree growing in tropical parts of Southeast Asia. Its fruits have been widely used in folk medicine. The uses of this plant are associated with various pharmacological activities such as antioxidant, hepatoprotective, cholesterol-lowering, anti-inflammatory, analgesic, antipyretic and antitussive. The purposes of this study were to develop chewable tablets containing *Phyllanthus emblica* Linn. spray dried fruit extract and to evaluate physicochemical properties of the products and their stability. The chewable tablets were prepared by direct compression using a single punch tableting machine. In some cases, spray dried extract was coated with ethylcellulose before incorporating into the formulations. The effects of binders, lubricants and flavoring agents on the tablet properties, such as appearance, hardness, total tannins and gallic acid contents, as well as the release of total tannins were investigated. The stability studies of the chewable tablets were carried out under refrigeration, at ambient temperature and 75%RH or uncontrolled humidity, also at 40°C and 75%RH for 3 months. Consumer acceptance of tablet appearance, flavor and mouth feel was tested for selected formulations. The microbial limit test was carried out for the spray dried extract before tablet preparation, and for the products prior to the consumer acceptance test. The results showed that the chewable tablets containing 30% spray dried *Phyllanthus emblica* Linn. fruit extract could be prepared by direct compression. The process required sufficient lubrication. Under storage condition at 40°C and 75%RH, the color of the tablets was changed to brownish. The hardness of tablets, however, behaved variably for the formulations containing uncoated or coated extract. It was significantly increased when the tablets contained uncoated extract, while decreased when the tablets contained coated extract ($p < 0.05$). The total tannins and gallic acid contents were more stable when the chewable tablets were prepared with microcrystalline cellulose PH 102 and stored at ambient temperature. The release of total tannins content from the tablets cut into pieces was dependent on the type of binder used. With copovidone, the release was retarded. The formulation which was relatively stable and accepted by volunteers contained 30% *Phyllanthus emblica* Linn. spray dried fruit extract, 20.35% mannitol, 20.35% xylitol, 10% microcrystalline cellulose PH 102, 10% talc, 5% citric acid, 1% aspartame, 2% sodium chloride, 1% magnesium stearate and 0.3% silicon dioxide.

Department :	Pharmaceutics and Industrial Pharmacy	Student's Signature…….....
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LIST OF ABBREVIATIONS

%	=	Percentage
°C	=	Degree Celsius
CFU	=	Colony Forming Unit
cm	=	Centimeter
et al	=	*et alii* , ' and others'
g	=	Gram
GAE	=	Gallic acid equivalence
HPLC	=	High performance liquid chromatographic
Kg	=	Kilogram
KP	=	Kilopound
Kv	=	Kilovolt
LOD	=	Loss on drying
µg	=	Microgram
µl	=	Microliter
MPa	=	Megapascal
mg	=	Milligram
min	=	Minute
ml	=	Milliliter
mm	=	Millimeter
nm	=	Nanometer
pH	=	The negative logarithm of the hydrogen concentration
RH	=	Relative humidity
rpm	=	Revolution per minute
RSD	=	Relative standard deviation
SD	=	Standard deviation
SEM	=	Scanning electron microscopy
UV	=	Ultraviolet
w/v	=	Weight by volume
w/w	=	Weight by weight

CHAPTER I

INTRODUCTION

Phyllanthus emblica Linn. (*Emblica officinalis* Gaertn., Amla, Aovla, Amalaki, Dhatriphata, Indian gooseberry, Emblic myrobalan) or ‘‘Ma-khaam-Pom’’ in Thai, a plant in the Euphobiaceae family, is a tree growing in subtropical and tropical parts of China, India, Indonesia , Malaysia and Thailand. It is widely used in folk medicine. The tree is small or medium-sized, up to 20 m high, deciduous, with crooked trunk and spreading branches. It has a greenish gray bark, peeling off in conchoidal flakes and 10 to 20 cm long glabrous or finely pubescent branchlets. The leaves are light green, digitate compound, sunsessile, 10-15 mm long and 3-8 mm wide, closely set on the branchlets. Flowers are greenish-yellow in auxiliary fascicles. Its fruits are freshly, globrous, shiny yellowish-green when ripe, and contain three loculi each containing two trigonous seeds (Department of Medical sciences, 2000; Elizabeth, 2002; Khare, 2004).

All parts of the plant are used for medicinal purpose such as fruits, leaves, seeds, roots, barks and flowers. In traditional medicine the fruits are used as a general tonic, particularly in the winter, and for constipation, urinary problems, headache, anxiety, diabetes mellitus, vomiting and burning sensation. It is considered to improve memory and intelligence. A paste of the dried fruit powder is also applied to hair and skin as a substitute for soap. The leaves are used in conjunctivitis and bronchitis and the powdered root bark is mixed with honey and applied to mouth ulcers (Department of Medical sciences, 2000; Elizabeth, 2002; Khare, 2004).

The medicinal and pharmacological activities of *Phyllanthus emblica* Linn. have been reported in many publications. Dhir et al. (1990) investigated modification of clastogenicity of lead and aluminium in mouse bone marrow cells by dietary ingestion of *Phyllanthus emblica* Linn. fruit extract. Jose et al. (2001) studied antitumour activity of *Phyllanthus emblica* Linn. Jose and Ramadasan (2000) examined hepatoprotective activity of *Phyllanthus emblica* Linn. Pramyothin et al. (2006) found the protective effects of *Phyllanthus emblica* Linn. extract on ethanol

induced rat hepatic injury. Al-Rehaily et al. (2002) studied gastroprotective effects of *Phyllanthus emblica* Linn. on *in vivo* test models in rats. Bafna and Balaraman (2005) studied antiulcer and antioxidant activity of *Phyllanthus emblica* Linn. Sandip et al. (2000) examined antioxidant activity of *Phyllanthus emblica* Linn. fruits on prevention from indomethacin induced gastric ulcer. Anila and Vijayalakshmi (2003) studied antioxidant action of flavonoids from *Phyllanthus emblica* Linn. in hypercholesterolemic rats. Mathur et al. (1996) studied hypolipidaemic effect of the *Phyllanthus emblica* Linn. fruit juice in cholesterol-fed rabbits. Bhattacharya et al. (2002) studied effect of bioactive tannoid principles of *Phyllanthus emblica* Linn. on ischemia-reperfusion-induced oxidative stress in rat heart. Perianayagam et al. (2004) investigated anti-pyretic and analgesic activity of *Phyllanthus emblica* Linn. Nosal'ova et al. (2003) studied antitussive activity of the fruit extract of *Phyllanthus emblica* Linn. Mayachiew and Devahastin (2008) tested antimicrobial and antioxidant activities of Indian gooseberry extract.

According to Khare, the composition of *Phyllanthus emblica* Linn. fruits is following: moisture content 81.2%, protein 0.5%, fat 0.1%, mineral matter 0.7%, fiber 3.4%, carbohydrate 14.1%, calcium 0.05%, phosphate 0.02%, iron 1.2% and vitamin C 600 mg/100 g (Khare, 2004). They have ascorbic acid content approximately 20 times that in orange and major chemical constituents containing polyphenols such as gallic acid, phyllembin, phyllemblic acid, emblicol, ellagic acid, chebulagic acid, glucogallin, corillagin, 3,6-digalloyl glucose, putranjivin A, emblicanin A and B, punigluconin, pedunculagin and quercetin (Elizabeth, 2002).

Phyllanthus emblica Linn. distributes in all parts of Thailand. In ancient time, it was used in traditional Thai medicine as an antitussive, expectorant or for reducing thirst. However, it was administered as easy such as eating the fresh fruits or dried fruits. The pharmacological properties of this plant become more recognized as the utilization of *Phyllanthus emblica* Linn. extract medicinal products. Many patented medicines are currently available in the maket place such as Shawkat® (for treatment of hepatitis B and C, treatment of hyperlipidemia, increasing lymph node function, stimulation of immune system), Livzon® (for prevention and treatment of AIDS, influenza, tuberculosis, hepatitis), cough syrup of Chowphaya Abhaibhubejhr

Hospital, Ma-Khaam-Pom sachet of Thanyaporn Samunplai (Thanyaporn, Online). Currently, chewable tablets containing *Phyllanthus emblica* Linn. fruit extract such as Capros® have been in the market (Shibnath, 2001). Furthermore, *Phyllanthus emblica* Linn. is incorporated in cosmetic products such as the shampoos and conditioners of Chowphaya Abhaibhubejhr Hospital or the acne whitening of Pan Cosmetic (Thaitambol, Online ; Pan cosmetics, Online).

Antitussive activity of *Phyllanthus emblica* Linn. fruit extract was reported in traditional medicine. The cough is a protective reflex mechanism that removes foreign material and secretion from bronchi and bronchioles of the airways. There are two kinds of cough: productive and non-productive. A productive cough, also known as chesty cough, is usually due to a viral or bacterial infection. This type of cough expels the phlegm which has formed in the respiratory passages, so that abnormal mucus and germs are eliminated from the respiratory tract and breathing becomes easier. A non-productive cough is dry, tickling and irritating. This cough can for example be caused by phlegm that is so viscous that it is not loosened and expectorated. It can also have an allergic or neurotic origin or can be caused by other perhaps more severe diseases (Nosal'ova, 2003). Currently, products containing *Phyllanthus emblica* Linn. fruit extract for the relief of cough symptom have astringent taste such as cough syrup creating a consumer acceptance problem. Chewable tablets containing *Phyllanthus emblica* Linn. fruit extract offer a wide range of potential formulation therefore an opportunity to solve this problem.

To prepare chewable tablets, many formulation factors must be taken into consideration, such as taste, flavor, aroma, mouth-fell, after taste effects and adequate hardness and friability (Robert, 1989). The selection of excipients in a formulation must take in account the compatibility between drug and excipients and the physicochemical properties of the excipients such as moisture content, particle size distribution, flow and compressibility, sweetness, chewability, mouthfeel, taste, and cost. In general, the use of low-calorie and non-sugar-based excipients may represent a marketing advantage, especially with consumers concerned about calorie intake and dental caries (Bowe, 1998; Mullarney, 2003). The polyols, xylitol and mannitol also called sugar alcohols, were selected as fillers for the formulation of

chewable tablets containing *Phyllanthus emblica* Linn. fruit extract because these substances provide energy 2.4 cal/g, 100 % sweetness relative to sucrose and 1.6 cal/g, 60 % sweetness relative to sucrose, respectively; and both provide a cooling effect or cool taste (Saulo, 2005). Furthermore, xylitol has been shown to have a protective effect and to reduce tooth decay, in part by reducing the levels of *Streptococcus mutans* in plaque and saliva and by reducing the level of lactic acid produced by these bacteria (Amaechi, 1998; Hietala, 1995).

The preparation of chewable tablets can be performed by wet granulation, dry granulation and direct compression processes. In this study, direct compression process was used to prepare chewable tablets because *Phyllanthus emblica* Linn. spray dried fruit extract is hygroscopic, and its chemical composition will degrade when expose to high temperature and moisture. Tablet fabrication by direct compression has increased steadily over the years as it can provide high efficacy. It offers advantages over other manufacturing processes, such as fewer processing stages, the elimination of heat and moisture effects, increase productivity and a reduction in manufacturing cost of the product. Furthermore, direct compression is considered an appropriate process for hygroscopic and thermo-sensitive substances, more directly promoting disintegration in primary particles (Shangraw, 1989; Jivraj et al., 2000). A serious limitation of this technique is the use of more than 30% of the drug in the formulation, mainly for drugs that present low flowability and segregation (Jivraj et al., 2000). Regarding manufacturability, a good flowability of the blend, i.e., the dry mixture of excipients and drug, is critical for the compression of the tablets in terms of dissolution, friability and content uniformity. However, the disadvantages of this process are the high cost of filler-binders, the problem of content uniformity for low dose drug. The fact that it is not practical for large dose, poorly compact or poorly flowing drugs requires tight controls over the physical properties of filler-binders. In addition, the problem of dust and the softness of tablet when stearate-salts are used as lubricant in the formula were found (Van der Watt et al., 1997). When formulating direct compression tablets, the choice of direct compression filler-binders is extremely critical. It must fulfill certain requirements: good binding functionality and powder flowability, a well-designed particle size distribution providing favorable mixing conditions, having a good compatibility with other excipients or drugs; as is

the ability to carry high amounts of active ingredient (Prescott, 1997; Jivraj et al., 2000; Bolhuis and Armstrong, 2006; Martinello et al., 2006).

As mentioned previously, the development of chewable tablets containing *Phyllanthus emblica* Linn. fruit extract by direct compression was of interest. It is a part of Thai herbal medicine development which is involved the control of quality to universal standard. The results from this study will give guidance for further development of products containing *Phyllanthus emblica* Linn. fruit extract and enhance the capability of using Thai herbal medicines.

The objectives of this study:

- To develop formulations of chewable tablets containing *Phyllanthus emblica* Linn. fruit extract by direct compression
- To evaluate the physicochemical properties of chewable tablets containing *Phyllanthus emblica* Linn. fruit extract
- To study the stability of chewable tablets containing *Phyllanthus emblica* Linn. fruit extract

CHAPTER II

LITERATURE REVIEW

1. *Phyllanthus emblica* Linn. fruit extract

Phyllanthus emblica Linn. (synonym: *Emblica officinalis* Gaertn.), belongs to the plant Euphorbiaceae family. It is also called Indian gooseberry , Amalaki , Emblic Myrobalan , Nelli , Amalakam , Sriphalam , Gebrau chilicher , Amlabaum , Amla , Ziphiyu-si , Shabju , Amlaj , Amulch , Toppinelli , Ngop , An moLe , Yeowkan Tse and Ma-khaam-Pom (Department of Medical sciences, 2000; Elizabeth, 2002; Khare, 2004).

1.1 Botanical description

Phyllanthus emblica Linn. is small or medium-sized deciduous tree, up to 20 m high, with a crooked trunk and spreading branches; with greenish gray bark, peeling off in conchoidal flakes; glabrous or finely pubescent branchlets of 10 to 20 cm long. Its leaves are light green, digitate compound, sunsessile, 10-15 mm long and 3-8 mm wide and closely set on the branchlets. Its flowers are greenish-yellow in auxiliary fascicles, and its fruit, depressed globose with six vertical furrows of 1.5 to 2 cm of diameter, start developing by the middle of spring and ripening towards the beginning of autumn. The color of the fruit is pale yellow and its taste is astringent-sour (Department of Medical sciences, 2000; Elizabeth, 2002; Khare, 2004).

(A)

(B)

(B)

(D)

Figure 1 Characteristic of *Phyllanthus emblica* Linn. (A) Tree, (B) Bark, (C) Flowers and (D) Fruits (Elizabeth, 2002; Khare, 2004)

1.2 Distribution

Phyllanthus emblica Linn. is found growing in tropical South-Eastern Asia, in countries such as India, Sri Lanka, Nepal, Burma, Vietnam, Laos and Thailand. It is found in the mixed deciduous forests and open hill evergreen forests. This tree is generally propagated through seeds, but seed propagated trees bear inferior quality fruits and have a long gestation period. For commercial purposes, propagation is done through budding, done on one year old seedlings with buds collected from superior strains yielding big size fruits (Department of Medical sciences, 2000; Elizabeth, 2002; Khare, 2004).

1.3 Chemistry

The fruits of the plant are most commonly used in Traditional Thai Medicine. They contain a high concentration of ascorbic acid, which degrades with heating or cooking (Nisha et al, 2004). In addition, the fruits contain phenols, including ellagic acid, gallic acid, quercetin, kaempferol, corilagin, geraniin, furosin, gallotanins, emblicanins, flavonoids, glycosides, and proanthocyanidins (Al-Rehaily et al, 2002; Anila and Vijayalakshmi, 2000 and 2002; Bhattacharya et al, 2002; Scartezzini and Speroni, 2000; Zhang et al, 2001).The leaves contain phenols similar to those found in the fruits. The roots contain glycosides and tannins (Zhang et al, 2000 and 2001).

The study reported that *Phyllanthus emblica* Linn. fruits are one of the richest source of ascorbic acid, containing up to 720 mg of ascorbic acid per 100g of fresh pulp and 921 mg per 100 ml of pressed juice. That content is approximately 20 times higher than what is found in orange pulp or juice (Khopde et al, 2001). The major constituents of *Phyllanthus emblica* Linn. fruits are compounds of low molecular weight in the hydrolysable tannin group (molecular weight < 1000), such as emblicanin A, emblicanin B, pedunculagin and punigluconin as shown in figure 2. These compounds have the potent vitamin C like activity (Ghosal et al, 1996).

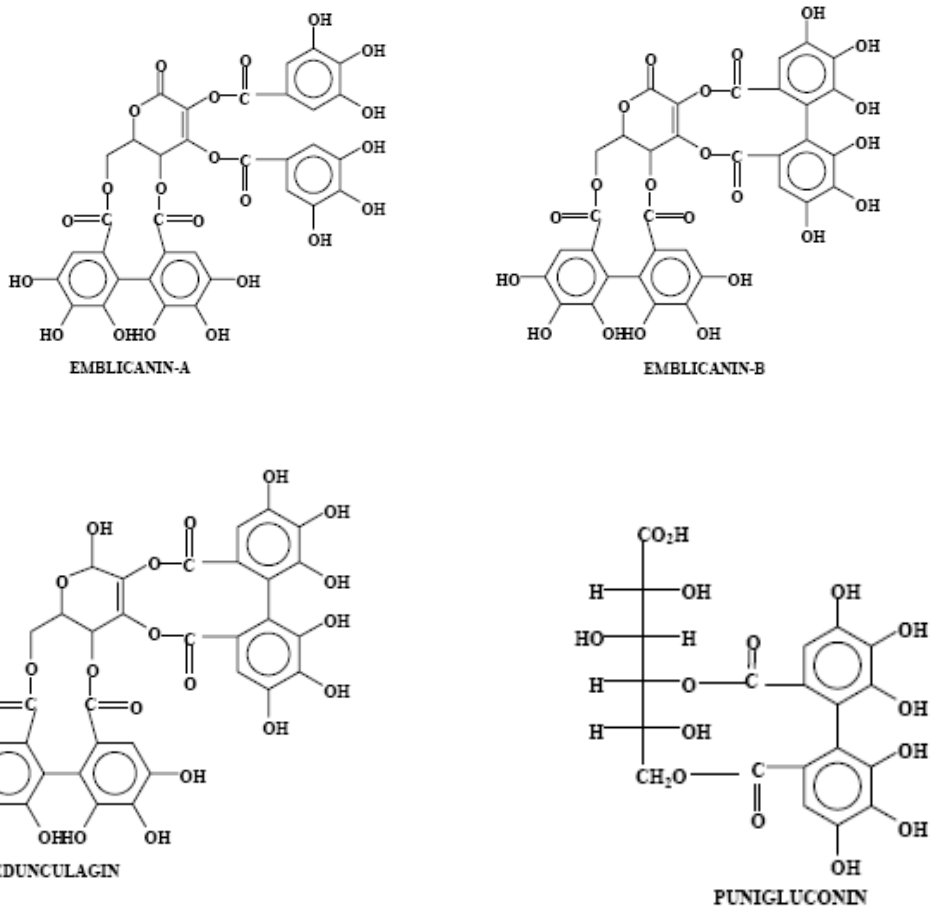
Emblicanin A is a yellow-gray amorphous hygroscopic powder. Its chemical name is 2,3-di-O-galloyl-4,6-(S)-hexahydroxydiphenoyl-2-keto-glucono-δ-lactone and its molecular formula is $C_{34}H_{22}O_{22}.2H_2O$. Partial hydrolysis of emblicanin A with tannase produces two moles of gallic acids (Ghosal et al, 1996), shown in figure 3A.

Emblicanin B is a tan color hygroscopic powder. Its chemical name is 2,3,4,6-bis-(S)- hexahydroxydiphenoyl-2-keto-glucono-δ-lactone and its molecular formula is $C_{34}H_{20}O_{22}.2H_2O$. With boiling water, the emblicanin B becomes hydrolyzed and produces one mole hexahydroxydiphenic acid per mole of Emblicanin B and haxahydroxydiphenic acid changes its structure to ellagic acid (Ghosal et al, 1996).

Punigluconin is a grey amorphous hygroscopic powder. Its chemical name is 2,3-di-O-galloyl-4,6-(S)- hexahydroxydiphenic acid gluconic acid and its molecular

formula is $C_{34}H_{26}O_{23}.2H_2O$ (Ghosal, 1996). Punigluconin (22 mg) in 1 N H_2SO_4 solution (5 ml) gives gallic acid (4 mg) and elegic acid (3 mg).

Pedunculagin is a tan hydroscopic powder. Its chemical name 2,3,4,6-bis-(S)-hexahydroxydiphenoyl-D-glucose and its molecular formula is $C_{34}H_{24}O_{23}.2H_2O$ (Ghosal et al, 1996). The acid hydrolysis of this compound produces a mixture of ellagic acid and hexahydoxydiphenic acid.



Figures 2 Structure of emblicanin A, emblicanin B, pedunculagin and punigluconin (Anthony, 2008 Online)

1.4 Uses and Pharmacology

Few human trials have been documented. Despite the widespread use of *Phyllanthus emblica* Linn. in the traditional health systems,

1.4.1 Antioxidant action

Most of the properties assigned to *Phyllanthus emblica* Linn. are attributed to its strong antioxidant action (Babu and Stanely, 2004; Bajpai et al, 2005; Naik et al, 2005; Rao et al, 2005). The ascorbic acid content of the fruit has been assayed at approximately 1 g per 100 mL of fresh fruit juice and accounted for 45% to 70% of the antioxidant activity (Scartezzini and Speroni, 2000). The Ayurvedic process for preparing the *Phyllanthus emblica* Linn. berry resulted in a 3-fold increase of ascorbic acid and an increase in the concentration of polyphenols. This procedure mixes dried fruit powder with fresh juice for a few hours, and then the mix is dried and powdered again. This process was repeated up to 21 times, making this method of fruit processing nutritionally beneficial. Other compounds having antioxidant properties include the emblicanins, gallic acid, methyl gallate, corilagin, furosin, and geraniin (Scartezzini and Speroni, 2000; Kumaran and Karunakaran, 2006).

1.4.2 Cardiac effects

The fruits of *Phyllanthus emblica* Linn. showed a protective effect against ischemic reperfusion injury in rats (Rajak et al, 2004). In another study, the emblicanins demonstrated an oxidative stress preventive (Bhattacharya et al, 2002).

1.4.3 Gastrointestinal action

Alcoholic and aqueous extracts from the *Phyllanthus emblica* Linn. fruits have consistently shown protective and healing effects in alcohol-induced gastric ulcers in animal experiments. Gastric sections are decreased, acidity reduced, and mucosal injury lessened. On the other hand mucus secretions are increased as well as the life span of mucosal cells. Similar results have been shown in hypothermic stress ulcers, indomethacin-induced ulceers, and pyloris ligation-induced ulcers.

Mixed results have been obtained for aspirin-induced ulcers (Al-Rehaily et al, 2002; Bafna and Balaraman, 2005; Bandyopadhyay et al, 2000; Rege et al, 1999; Sairam et al, 2002).

1.4.4 Hepatoprotective effects

Alcoholic and aqueous extracts of the *Phyllanthus emblica* Linn. fruits have shown hepatoprotective properties from experiments on rats. Hepatic insults include antituberculosis drugs, ethanol, thiacetamide, carbon tetrachloride, and cyclophosphamide. The experiments have demonstrated histological and/or enzymatic protective and restorative effects. A decreased severity of hepatic fibrosis has also been demonstrated (Haque et al, 2001; Jose and Kuttan, 2000; Pramyothin et al, 2006; Sultana et al, 2004; Tasduq et al, 2005).

1.4.5 Cholesterol - lowering effects

A number of animal experiments reported a reduction in serum and tissue cholesterol (Anila and Vijayalakshmi, 2000; Mishra et al, 1981; Thakur and Mandal, 1984; Thakur, 1985; Thakur et al, 1988; Mathur et al, 1996; Kumar and Muller, 1999). Flavonoid extracts from the fruits of *Phyllanthus emblica* Linn. inhibited synthesis and enhanced degradation of cholesterol via increased hepatic HMG-CoA reductase (Anila and Vijayalakshmi, 2000). Low-density lipoprotein and very low-density lipoprotein have been reduced while high-density lipoprotein remained unchanged in experiment. Fresh *Phyllanthus emblica* Linn. fruit juice administered to rabbits have resulted in induced aortic plaques regressing to near normal, while serum and tissue lipids have decreased (Mathur et al, 1996).

In a human clinical trial, healthy and hypercholesterolemic men (35 to 55 years of age) given *Phyllanthus emblica* Linn. supplementation for 28 days experienced a decrease in serum cholesterol levels. The condition became reversible upon discontinuation of the supplement (Jacob et al, 1988).

1.4.6 Diabetes

In singledose and multidose experiments of *Phyllanthus emblica* Linn. there was a decreased blood glucose levels in rats with an induced diabetic state (Anila and Vijayalakshmi, 2000; Babu et al, 2004); Rao et al, 2005). However, Triphala (mixed preparation containing *Phyllanthus emblica* Linn.) showed a stronger effect than *Phyllanthus emblica* Linn. alone (Sabu and Kuttan, 2002), and Hyponidd (another mixed preparation) reduced blood glucose in a manner similar to glibenclamide. Serum creatinine was reduced, and serum albumin increased within 20 days in rats fed with emblica (Rao et al, 2005).

1.4.7 Anticancer effects

Much interest surrounds the potential of *Phyllanthus emblica* Linn. in treating cancer; however, there are no published clinical trials or epidemiological data on the subject. *Phyllanthus emblica* Linn. has inhibited induced mutagenesis in Salmonella strains (Grover and Kaur, 1989). A dose-dependent effect was demonstrated in one experiment (Sharma et al, 2000), while *Phyllanthus emblica* Linn. was the least active amoung 5 plants tested (Arora et al, 2003). Another study reported that an aqueous extract of *Phyllanthus emblica* Linn. inhibited dose-dependent hepatocarcinogenesis as measured by parameters such as tumor incidence, enzyme measurements, and other liver injury markers (Jeena et al, 1999; Jose et al, 2001).

In response to heavy metal carcinogens (arsenic, chromium, nickel), rats given emblica extracts have shown a reduction in the number of chromosomal aberrations, a reduction in the number of damaged cells, lower frequency of micronuclei in bone marrow cells, free radical production, and increased cell survival (Biswas et al, 1999; Sai Ram et al, 2002 and 2003; Dhir et al, 1991). *Phyllanthus emblica* Linn. extracts have protected irradiated mice from radiation sickness, increased the 30-day survival rate, and decreased total mortality (Singh et al, 2005; Jagetia et al, 2002).

In response to tumor cells (lymphoma and mammary carcinoma), mice fed with emblica extracts showed an increase activity of the natural killer cells,

antibody-dependent cellular cytotoxicity, and survival (Suresh and Vasudevan, 1994; Veena et al, 2006). In one of these experiments, there was no effect on the development of tumors, but it showed a decrease in the tumor volume (Biswas et al, 1999). Cytotoxicity in tumor cells has been demonstrated by organic acid gallates and hydrolysable tannins. One report has been published in which emblica had no effect in reducing lung cancer parameters in mice (Menon et al, 1997).

1.4.8 Analgesic and antipyretic

Alcohol and aqueous extracts of *Phyllanthus emblica* Linn. fruit were tested for analgesic and antipyretic activity in mice. Results were similar to aspirin, except for the response to heat pain in which model *Phyllanthus emblica* Linn. had no activity (Perianayagam et al, 2004).

1.4.9 Antimicrobial

Alcoholic and aqueous extracts of *Phyllanthus emblica* Linn. showed positive results in *in vit*ro activity against certain dermatophytes (Ahmad et al, 1998) and against common human pathogens, although no comparisons were made with standard antibiotics (Rani and Khullar, 2004)**.**

1.4.10 Antitussive

An alcoholic extract of the fruits showed a dose-dependent effect similar to dropropizine in cats but was less active than codeine (Nosal'ova et al, 2003).

1.4.11 Antivenom

An alcoholic extract of the roots showed neutralizing capacity against the hemorrhagic action of snake venom in mice (Alam and Gomes, 2003).

1.4.12 Immune

Rats exposed to noise stress for 15 days and given Triphala, containing *Phyllanthus emblica* Linn., *Terminalia chebula* Retz. and *Terminalia bellerica* Roxb., showed an increased neutrophil function and lowered cortisone release (Srikumar et al, 2006). However, in another experiment, an aqueous extract of *Phyllanthus emblica* Linn. fruits had no effect on cold stress-induced cortisone release (Rege et al, 1999).

1.4.13 Ophthalmic

In a clinical study, emblica is one of the components of a mixed herbal eye drop formulation (Ophthacare) that showed activity in mild infections and inflammatory eye conditions; however, the study methodology was poor (Biswas, 2001).

1.5 Dosage

There are no published clinical studies to support the dosing of emblica. A dose of 3 to 6 g/day of powdered emblica has been extrapolated from a vitamin C 1 g/day dose (Dhir et al, 1991). An LD_{50} in rats has been suggested at 1 g/kg body weight, but 2.5 g/kg has been used in cancer studies in animals (Rege et al, 1999; Biswas et al, 1999).

1.6 Toxicology

No major reported toxicities have been associated with the fruit. In toxicity studies with rats, no toxicity was observed in single-dose or chronic-dose administration. Additionally, no detrimental effect was noted on liver or renal function (Rege et al, 1999). No chromosomal aberrations were found following 7-days and 14-days treatment regimens in rats with crude fruit extract (Jose et al, 2001). In another experiment, no toxicity or mutagenicity were observed in rats even at the highest administered doses (Sharma et al, 2000).

1.7 Extraction, specification and analysis of *Phyllanthus emblica* Linn. fruits

The *Phyllanthus emblica* Linn. fruits are offered fresh, dry or in extract form. For traditional medicine, *Phyllanthus emblica* Linn. fruits were usually used in the form of crude powder made from dried fruit. In Thai Herbal Pharmacopoeia (2000) the specifications of the crude drug of *Phyllanthus emblica* Linn. fruits powder is described as no more than 9% w/w after drying at 105°C to constant weight loss on drying, no more than 1% w/w of acid-insoluble ash, no more than 4% w/w of total ash, no less than 16% w/w of ethanol-soluble extractive, no less than 26% w/w of water-soluble extractive and no less than 20% w/w of tannins content.

Ghosal (1996) isolated hydrolysable tannins by comprehensive chromatographic such as column, TLC, HPLC and HPTLC. Low molecular weight hydrolysable tannins group (molecular weight < 1000) such as emblicanin A, emblicanin B, pedunculagin and punigluconin were found.

Many groups of researchers studied the quantity of ascorbic acid and tannins in *Phyllanthus emblica* Linn. fruits. Kumar et al. (2006) analyzed the free and bound phenolic by HPLC method. Raghu et al. (2007) compared different analysis method of ascorbic acid content of *Phyllanthus emblica* Linn. fruits i.e. 2,4-Dinitrophenylhydrazine method, Indophenol–xylene method, Enzymatic method and Liquid chromatography and fluorescence detection.

For Enzymatic method, ascorbic acid in the sample was determined after treatment with ascorbic acid oxidase in phosphate buffer (pH 5.6). Scartezzini et al. (2006) analyzed the ascorbic acid content by HPLC method and total polyphenol content by the Folin-Ciocalteu colorimetric method.

The Folin-Ciocalteu colorimetric method was the best suitable method for determination of the total polyphenol content. Folin-Ciocalteu's phenol reagent did not not contain phenol. Rather, the reagent reacted with phenols and nonphenolic reducing substances to form chromogens that could be detected spectrophotometrically. The color development was due to the transfer of electrons at basic pH reducing the phosphomolybdic/phosphotungstic acid complexes to form chromogens in which the metals had lower valence (Slinkard and Singleton, 1977). Addition to Folin & Ciocalteu's phenol reagent generated chromogens that gave

increasing absorbance between 550 nm and 750 nm. Normally, absorbance at the peak 750 nm (Fluka, 2008 Online).

Leewongpan and Laoruangsinchai (2004) investigated validation of HPLC determination of gallic acid in *Phyllanthus emblica* Linn. fruit extract. They found that the best condition for the HPLC method was C18 (150x4.6 mm, 5 μm) with mobile phase containing 8:92 (methanol per 0.3%trifluoroacetic acid) at a flow rate of 1 ml/min. The gallic acid was analyzed at wavelength 270 nm.

1.8 Stability

Ghosal et al. (1996) described that low molecular hydrolysable tannin group such as emblicanin A, emblicanin B, punigluconin and pedunculagin were degraded by hydrolysis process.Partial hydrolysis of emblicanin A with tannase produced two moles of gallic acids per one mole of emblicanin A. With boiling water, emblicanin B was partially hydrolyzed and produces one mole hexahydroxydiphenic acid per mole of Emblicanin B and haxahydroxydiphenic acid changed its structure to ellagic acid. Punigluconin (22 mg) in 1 N H_2SO_4 solution (5 ml) gave gallic acid (4 mg) and elegic acid (3 mg). Acid hydrolysis of pedunculagin produced a mixture of ellagic acid and hexahydoxydiphenic acid.

Zhang You-lin and Zhang Run-guang found that tannins were destroyed at both low and high temperature because phenolic compounds were oxidized into quinone compounds under aerobic conditions by polyphenoloxidase, and the quinine compounds underwent polymerization forming brown polymeric pigments, leading to browning.

Nisha et al. studied the kinetics of ascorbic acid degradation in *Phyllanthus emblica* Linn. fruits as well as in pure ascorbic acid solutions at initial concentrations present in *Phyllanthus emblica* Linn. fruits over a temperature range of 50-120°C. They suggested that the ascorbic acid degradation followed first-order reaction kinetics where the rate constantly increased as the temperature increased. The temperature dependence of the degradation was adequately modeled by the Arrhenius equation**.**

1.9 Product containing *Phyllanthus emblica* Linn. fruits

The utilization of this plant became recognized as medicinal products containing *Phyllanthus emblica* Linn. extract have been invented. For example, Shawkat®, is a tablets that contain 40% from the dried fruits of *Phyllathus emblica* Linn.; 10% from the dried fruit of *Terminalia Chebiola* Retz.; 7% from the plant *Cichorium Intyus* Linn., excluding the roots; 10% from the flower of the plant *Carthamus Tincorius* Linn.; 3% from the plant *Solenostemma Argel* Hayne, excluding the roots; 10% from the seeds of *Nigella Sativa* L.; 5% from the plant *Erythraea Centaurium* Pers, excluding the roots; 10% from the stem and leaves of the plant *Cynara Cardunculus* Var. Scoly; and 5% from the rhisome (stems) of *Rheuon officinale* Baill. These indications were stimulation of liver cells to regenerate, blood cholesterol reducing effect, increasing Bilirubin secretion and excretion and stimulation of lymphocytic system of the body and increasing immune response which results in clearance of vital DNA. In patients who suffer from active viral Hepatitis-B and Hepatitis-C. It has been noted by testing patient's sera for viral DNA by PCR technique, that the treatment of hepatitis B and C, hyperlipidemia, increase functional of lymph node, stimulation of immune system (Shawkat, 1997).

Livzon®, a capsule, consists of *Phyllanthus niruri* (292-310 mg), *Tinospora cordifolia* (190-210 mg), *Phyllanthus emblica* Linn. (90-110 mg), *Terminalia belerica* (90-110 mg) and *Terminalia chebula* (290-310 mg) for the prophylaxis and treatment of AIDS, flu, TB and other immuno-deficiencies (Surendra, 1996).

Pisansalhidikam (2000) investigated the development of a vitamin C pill from *Phyllanthus emblica* Linn. fruits. The fruits were first processed into three products : *Phyllanthus emblica* Linn. powder, *Phyllanthus emblica* Linn. pickle and *Phyllanthus emblica* Linn. juice, which were then used as raw materials for the pills. Other ingredients: icing sugar, brown sugar, palm sugar and honey were added at various concentrations yielding nine formulas. The result was that the pills containing *Phyllanthus emblica* Linn. pickle, icing, sugar and honey were the most accepted and contained 44.9 mg of vitamin C per 100 g. The pills stored in air-tight containers for one month at 5-100°C and at room temperature changed in physical properties. After

storing pills at room temperature for 1 month, the color was changed to a much daker hue, there was a noted decreased of vitamin C content and the tablets became sticky.

In Thailand, dosage forms containing *Phyllanthus emblica* Linn. extract were developed as liquid, powder, tablet and capsule for oral intake, such as the cough syrup of Chowphaya Abhaibhubejhr Hospital. It contains 60 ml of concentration of 40% of *Phyllanthus emblica* Linn. extract in 100 ml of cough syrup. Other examples are the herbal pill of Ouayun containing platycodon grand florum, licorice, *Phyllanthus emblic*, menthol and honey, or the Ma-Khaam-Pom sachet of Thanyaporn Samunplai (Thanyaporn, Online). *Phyllanthus emblica* Linn. is also used in the composition of cosmetic products, such as the shampoo and conditioner of Chowphaya Abhaibhubejhr Hospital, or the acne whitening cream and melasma whitening cream of Pan cosmetic (Thaitambol, Online ; Pan cosmetics, Online).

In addition, Temdee et al. (2007) investigated the development of *Phyllanthus emblica* Linn. fruits and maltitol syrup lozenge products that reduce tooth decay. Lozenge products are prepared by process of hard candy and pastilles. The hard candy consists of maltitol syrup, glucose syrup, *Phyllanthus emblica* Linn. extract (fresh fruits or pickle fruits), licorice extract, salt and ascorbic acid. The pastille consists of maltitol syrup, glucose syrup, *Phyllanthus emblica* Linn. extract (fresh fruits or pickle fruits), licorice extract, gelatin, arabic gum, salt, orange flavor and ascorbic acid. The study compare the effect of sweetening agent between maltitol syrup and mixture of maltitaol syrup and glucose syrup and found that consumer acceptance of maltitol syrup was higher than the mixture of maltitol and glucose syrup and that the consumer acceptance of *Phyllanthus emblica* Linn. extract from pickle fruits was higher than *Phyllanthus emblica* Linn. extract from fresh fruits. Some chewable tablets containing *Phyllanthus emblica* Linn. fruit extract, such as Capros® is already available in the market (Shibnath, 2001).

Figure 3 Products containing *Phyllanthus emblica* Linn. (Adfac Labs the probiotic company, Online; Chophaya abhaibhubejhr hospital, Online; ayursiddhainc.com, Online; Pan cosmetics, Online)

2. Chewable tablets

Chewable tablet is a type of tablets dosage forms and part of the pharmacist's armamentarium for a very long time. Compared to solid dosage forms intended to be swallowed, their possible advantages include: better bioavailability through bypassing the disintegration step and perhaps enhancing the dissolution steps, patient convenience through the elimination of the need of water for swallowing, possible use as a substitute for liquid dosage forms where rapid onset of action is needed, improved patient acceptance especially in pediatrics through pleasant taste, and product distinctiveness from a marketing perspective. The use of chewable tablets, however has some limitations specialy with bad-tasting drugs and those where an extremely high dosage levels present to the formulator with significant obstacles to be overcome. Powder coating with polymer is a possible choice to mask the bad-tasting drugs (Hiroyuki, 2003; Hiroyuki, 2004). So, the formulation of chewable tablets requires additional considerations beyond those necessary for conventional tablets.

Factors such as color, flavor, taste, aftertaste, particle size, grittiness, compressibility, moisture content, stability of drugs, chewability and gumminess must be taken in account. The chewable tablets should have a pleasant taste without a bitter aftertaste, an unobstructive flavor but preferably a cooling mouth-feel, good chewability, non gritty, absence of gumminess to preclude sticking to teeth or dentures, usually an attractive and uniform color distribution to match the flavor, and adequate hardness and friability (Robert, 1989). The active drug profile would eliminate potential incompatible excipients, flavors, and like at the outset, leading to the use of excipients that would best compliment the drug chemically, physically, and organolepticcally. The choice of excipients and other product modifiers would involve judgment, balancing their cost with their functionality. Many of the excipients commonly used in tablet formulation are especially applicable for use in chewable tablets due to their ability to provide the necessary properties of sweetness and chewability.

The formulation factors are consideration to prepare chewable tablets, these can describe:

2.1 Taste and Flavor

Physiologically, taste is a sensory response resulting from a chemical stimulation of the taste buds on the tongue. There are four basic of the tastes: salty, sour, sweet and bitter. Salty or sour tastes are derived from substances capable of ionizing in solution. Many organic medicinal compounds stimulate a bitter response even though they may not be capable of ionizing in an aqueous medium. Substances incapable of producing a sensory stimulation of the buds are referred to as bland or tasteless.

The term flavor generally refers to a specific combined sensation of taste and smell. For example, sugar has a sweet taste but no flavor but honey has a sweet taste and a characteristic smell. The combination of the two was known as honey flavor (Robert, 1989).

2.2 Aroma

Pleasant smells are generally referred to as aromas. For examples, a well formulated, orange-flavored, chewable tablet should have the characteristic sweet and sour taste and the aroma of fresh orange (Robert, 1989).

2.3 Mouth-feel

The term mouth-feel is related to the type of sensation or touch that a tablet produces in the mouth upon chewing. As such, it has nothing to do with chemical stimulation of olfactory nerves or taste buds. However, for a formulation to be successful, the overall effect in the mouth is important. In general, gritty or gummy textures are undesirable but a soothing and cooling sensation with smooth texture is preferred (Robert, 1989).

2.4 After effects

The most common after effect of many compounds is aftertaste. For example, some iron salts leave a rusty aftertaste; saccharin in high amounts tents to leave a bitter aftertaste.

Another common after effect is a numbing sensation of a portion of the whole surface of the tongue and mouth (Robert, 1989).

2.5 Assessment of the formulation problems

The first step in the formulation of a chewable tablet is to obtain a complete profile of the active drug. This usually leads to the most efficient formulation of a stable and quality product as the drug usually dictates the choice of fillers, carriers, sweeteners, flavor compounds and other product modifiers. The drug ideally should contain information mainly its physical properties, chemical properties, drug dose and any limit on final dosage size. This active drug profile would eliminate potentially incompatible excipients, flavors and in favor of those that would best compliment the drug chemically, physically and organoleptically.

The choice of excipients and other product modifiers would involve judgment in order to obtain a good balancing their functionality. Many of the excipients commonly used in tablet formulation are especially applicable for use in chewable tablets due to their ability to provide the necessary properties of sweetness and chewability. The selection of excipients in the formulation must study the compatibility between drug and excipients and also the physicochemical properties of excipients for example the moisture content, particle size distribution, flow and compressibility, sweetness, chewability, mouthfeel, taste, and cost. In general, the use of low-calorie and non-sugar-based excipients may represent a marketing advantage, especially with consumers concerned about calorie intake and dental caries (Bowe, 1998; Mullarney, 2003). The polyols, also called sugar alcohols, xylitol and mannitol were selected as filler to formulation of chewable tablets containing *Phyllanthus emblica* Linn. fruit extract because these provide energy 2.4 cal per g, 100 % sweetness relative compare to sucrose at 1.6 cal/g, 60 % sweetness relative to sucrose respectively and both of these provide a cooling effect or cool taste (Saulo, 2005). Furthermore, xylitol has been shown to have a protective effect and to reduce tooth decay in part by reducing the levels of *Streptococcus mutans* in plaque and saliva and by reducing the level of lactic acid produced by these bacteria (Amaechi, 1998; Hietala, 1995).

Therefore, the formulation of chewable tablets requires additional considerations beyond those necessary for conventional tablets such as color, flavor, taste, particle size, compressibility, moisture content, stability of drugs, including pleasant taste without a bitter aftertaste, unobstructive flavor, good chewability, non gritty, preferably cooling mouthfeel, absence of gumminess to preclude sticking to teeth or dentures, usually an attractive and uniform color distribution to match the flavor, and adequate hardness and friability (Robert, 1989).

3. Direct compression process

The preparation of chewable tablets can perform in wet granulation, dry granulation and direct compression process. In this study direct compression process was used to prepare chewable tablets because *Phyllanthus emblica* Linn. spray dry fruit extract is very hygroscopic when expose with high temperature and moisture the

chemical compositions of *Phyllanthus emblica* Linn. spray dry fruit extract will degrade. Tablet manufacturing by direct compression has increased steadily over the years. The term direct compression was long used to define the process by which tablets are compressed directly from powder blends of the active ingredient and suitable excipients (including fillers, disintegrants and lubricants) which will flow uniformly into a die cavity and form into a firm compact. No pretreatment of the powder blends by wet or dry granulation procedures is necessary. Occasionally, potent drugs will be sprayed out of solution onto one of the excipients (Robert, 1989).

The advent of direct compression was made possible by the commercial availability of direct compression tablet vehicles that possess both fluidity and compressibility. It offers advantages over other manufacturing processes, such as wet granulation, and provides high efficiency. The advantages of direct compression are well known: fewer processing stages, elimination of heat and moisture effects, disintegration more directly in primary particles, faster dissolution rate when compared with wet granulation, fewer excipients may be needed, increase of productivity and reduction of the final cost of the product. Furthermore, direct compression is considered an appropriate process for hygroscopic and thermo-sensitive substances (Shangraw, 1989; Jivraj et al., 2000). A serious limitation of this technique is the use of more than 30% of the drug in the formulation, mainly for drugs that present low flowability and segregation (Jivraj et al., 2000). Regarding to the manufacturability, a good flowability of the blend, i.e., the dry mixture of excipients and drug, is critical for the compression of the tablets in terms of dissolution, friability and content uniformity. However, the disadvantages of this process are consist of: high cost of filler-binders, problem of content uniformity for low dose drug, not practical for large dose poorly compact or poorly flowing drugs, required tight control over physical properties of filler-binders, problem of dust, segregation may occur in mass transport hopper and feed frame and softness of tablet when use stearate-salts for lubricant in formula. The process is not applicable for materials possessing a low bulk density because after compression the tablets produced may be too thin and static charges may develop on the drug particles or excipients during mixing, which may lead to agglomeration of particles producing poor mixing (Van der Watt et al., 1997).

When formulating direct compression tablets, direct compression excipients, particularly filler-binders, are specialty excipients. In most cases they are common materials that have been modified in the chemical manufacturing process to impart to them greater fluidity and compressibility. The physical and chemical properties of these specialty products are extremely important. Many factors influence the choice of the optimum direct compression filler to be used in a tablet formulation. These factors vary from primary properties of powders i.e. particle size, shape, bulk density and solubility to characteristics needed for making compacts. Therefore, the choice of direct compression filler-binders is extremely critical. It must fulfill certain requirements: good binding functionality and powder flowability are essential; a well-designed particle size distribution provides favorable mixing conditions; compatibility with other excipients or drugs is also essential, as is the ability to carry high amounts of active ingredient (Prescott, 1997; Jivraj et al., 2000; Bolhuis and Armstrong, 2006; Martinello et al., 2006).

4. Chewable tablets additives

4.1 Xylitol

The chemical name of xylitol is *xylo* -Pentane-1,2,3,4,5-pentol ($C_5H_{12}O_5$). It occurs as a white, granular solid comprising crystalline, odorless and a sweet taste that imparts a cooling sensation. It is also commercially available in powdered form, granular form and directly compressible form. It is a sweetener found in natural sources, such as many fruits and vegetables, even produced by the human body during normal carbohydrate metabolism, and typically manufactured from birch trees or other natural xylan-rich sources. In scientific studies over more than 30 years, dental researchers have pointed to xylitol, as a key ingredient in the fight against tooth decay. Xylitol is a familiar sweetener in sugar-free products such as chewing gum. It has the same sweetness and bulk as sucrose providing 2.4 cal/g of energy. It has no aftertaste and a very pleasant cooling sensation when it dissolves in the mouth. It is widely approved for use in foods, pharmaceuticals and cosmetics in many countries around the world. Current xylitol products include chewing gum and other confectionery, pharmaceuticals (syrups and chewable tablets), oral hygiene products

such as toothpastes and mouthwashes, and dietetic and diabetic foods. Because of, xylitol is a low-glycaemic sweetener and metabolize independently of insulin, so does not cause the sharp increase in blood sugar levels or the associated serum insulin response. It is usually seen following consumption of other carbohydrates. Thus, xylitol can be recommended as a sugar-free sweetener suitable for diabetics as well as for the general population seeking a healthier lifestyle (Rowe et al., 2003).

4.2 Mannitol

The chemical name of mannitol is D-mannitol ($C_6H_{14}O_6$). It is a hexahydric alcohol related to mannose and is isomeric with sorbitol. It occurs as a white, odorless, crystalline powder, or free-flowing granules, a sweet taste, approximately as sweet as glucose and half as sweet as sucrose and imparts a cooling sensation in the mouth. Microscopically, it appears as orthorhombic needles when crystallized from alcohol, and shows polymorphism.

Mannitol is widely used in pharmaceutical formulations and food products. In pharmaceutical preparations it is primarily used as a diluent (10–90% w/w) in tablet formulations, where it is of particular value since it is not hygroscopic and may thus be used with moisture-sensitive active ingredients. It may be used in direct-compression tablet applications, for which the granular and spray-dried forms are available, or in wet granulations. Granulations containing mannitol have the advantage of being dried easily. Mannitol is commonly used as an excipient in the manufacture of chewable tablet formulations because of its negative heat of solution, sweetness and mouth feel (Rowe et al., 2003).

4.3 Microcrystalline cellulose PH 102

Microcrystalline cellulose is purified, partially depolymerized cellulose that occurs as a white, odorless, tasteless, crystalline powder composed of porous particles. It is commercially available in different particle sizes and moisture grades that have different properties and applications, and widely use in pharmaceuticals i.e. primarily as a binder/diluent in oral tablet and capsule formulations where it is used in both wet-granulation and direct-compression processes. In addition to its use as a

binder/diluent, microcrystalline cellulose has also some lubricant and disintegrant properties that make it useful in tabletting. It is widely used in oral pharmaceutical formulations and food products and is generally regarded as a relatively nontoxic and nonirritant material (Rowe et al., 2003).

4.4 Copovidone

The chemical name of copovidone is vinylpyrrolidone vinyl acetate copolymer. That consists of 60:40 copolymer of vinylpyrrolidone and vinyl acetate. It occurs as white to creamy white and free-flowing powder. Its properties are soluble in water and most pharmaceutically acceptable solvents. Another property of copovidone is lower glass transition temperature (Tg) than vinylpyrrolidone homopolymers (106°C vs.165°C), helping to form hard, glossy, transparent, air-permeable and water removable films. It is also help forming better film former than vinylpyrrolidone homopolymers due to the increasing of hydrophobicity and lower the Tg. Its applications are seen as a binder in wet and dry granulation and direct compression tabletting processes, also improve the compressibility of other binders and fillers in direct compression tablet process, improve solubility and enhances bioavailability of drug actives, give excellent performance as binder in moisture sensitive formulations, enhance appearance and color stability of tablet film coatings, increase productivity of film coating operations, improve adhesion of tablet coatings to hydrophobic cores, and possess excellent film forming properties and high substantivity to skin (Rowe et al., 2003).

4.5 Colloidal silicon dioxide

The chemical name is silica. Colloidal silicon dioxide is submicroscopic fumed silica with a particle size of about 15 nm. It is a light, loose, bluish-white-colored, odorless, tasteless and non-gritty amorphous powder. It is widely used in pharmaceuticals, cosmetics, and food products. Its small particle size and large specific surface area give it desirable flow characteristics that are exploited to improve the flow properties of dry powders in a number of processes such as tableting. Colloidal silicon dioxide is also used to stabilize emulsions and as a

thixotropic thickening and suspending agent in gels and semisolid preparations. With other ingredients of similar refractive index, transparent gels may be formed. The degree of increase viscosity depends on the polarity of the liquid (polar liquids generally require a greater concentration of it than non-polar liquids). Viscosity is largely independent of temperature. However, changes to the pH of a system may affect the viscosity. It also used as a tablet disintegrant and as an adsorbent dispersing agent for liquids in powders. Colloidal silicon dioxide is frequently added to suppository formulations containing lipophilic excipients to increase viscosity, prevent sedimentation during molding, and decrease the release rate (Rowe et al., 2003).

4.6 Magnesium stearate

The chemical name of magnesium stearate is octadecanoic acid magnesium salt. It occurs as a fine, white, precipitated or milled, impalpable powder of low bulk density, having a faint odor of stearic acid and a bad taste. The powder is greasy to the touch and adheres easily to the skin. That is widely used in cosmetics, foods and pharmaceutical formulations. It is primarily used as a lubricant in capsule and tablet fabrication at concentrations between 0.25% and 5.0% w/w. It is also used in barrier creams.

4.7 Talc

Talc is a very fine, white to grayish-white, odorless, impalpable, unctuous, crystalline powder. It adheres easily to the skin and is soft to the touch and free from grittiness. It was once widely used in oral solid dosage formulations as a lubricant and diluent, although today it is less commonly used. However, it is widely used as a dissolution retardant in the development of controlled-release products. In topical preparations, talc is used as a dusting powder, although it should not be used to dust surgical gloves. Talc is a natural material; it may therefore frequently contain microorganisms and should be sterilized when used as a dusting powder. The application of talc is additionally used to clarify liquids and is also used in cosmetics and food products, mainly for its lubricant properties (Rowe et al., 2003).

4.8 Sodium chloride

Sodium chloride occurs as a white crystalline powder or colorless crystals; it has a saline taste. The crystal lattice is a face-centered cubic structure. Solid sodium chloride contains no water, although, below 0°C sodium chloride may crystallize as a dihydrate. It is widely used in a variety of parenteral and nonparenteral pharmaceutical formulations, where its primary use is to produce isotonic solutions. In the past, sodium chloride has been used as a lubricant and diluent in capsules and direct-compression tablet formulations, although this practice is no longer common. In addition, sodium chloride has also been used as a channeling agent and as an osmotic agent in the cores of controlled-release tablets. It has been used as a porosity modifier in tablet coatings, and to control drug release from microcapsules. The addition of sodium chloride to aqueous spray-coating solutions containing hydroxypropyl cellulose or hypromellose suppresses the agglomeration of crystalline cellulose particles. Sodium chloride can also be used to modify drug release from gels and from multiple emulsions. It can be used to control the size of micelle, and to adjust the viscosity of polymer dispersions by altering the ionic character of a formulation (Rowe et al., 2003).

4.9 Citric acid anhydrous

The chemical name of citric acid is 2-hydroxypropane 1,2,3-tricarboxylic acid. Citric acid anhydrous occurs as odorless or almost odorless, colorless crystals or as a white crystalline powder. Crystal structure is monoclinic holohedra. It is widely used in pharmaceutical formulations and food products, primarily to adjust the pH of solutions. Citiric acid anhydrous is widely used in the preparation of effervescent tablets. In food products, citric acid is used as a flavor enhancer, acidic taste. It is also a component of anticoagulant citrate solutions. Therapeutically, preparations containing citric acid have been used to dissolve renal calculi (Rowe et al., 2003).

4.10 Sucralose

The chemical name of sucralose is 1,6-Dichloro-1,6-dideoxy-ß-D-fructofuranosyl-4-chloro-4-deoxy-alpha-D-galactopyranoside. Sucralose is white to

off-white, practically odourless crystalline powder and freely soluble in water, methanol and ethanol; slightly soluble in ethyl acetate. It is used as sweetening agent (Rowe et al., 2003).

CHAPTER III

MATERIALS AND METHODS

1. Materials

The following materials were used

1.1 Active ingredient and diluents

- *Phyllanthus emblica* Linn. spray dried fruit extract batch no. 08122006 and batch no. 28122006 was used for chewable tablets formulation was supplied by the Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand
- Xylitol granulated (Xylitab®) (Lot No. H125A6F24, Forbest chemical CO., Ltd., Beijing, China)
- Mannitol powder (Lot No. H100509006, Forbest chemical CO., Ltd., Beijing, China)
- Copovidone (Plasdone S-630®) (Lot No. 05500140787, ISP Technologies, Inc., Beijing, China)
- Microcrystalline cellulose PH 102 (Comprecel M102®) (Lot No. 61216, Mingtai Chemical CO., Ltd., Taoyuan Hsien, Taiwan)
- Silicon dioxide (Aerosil HDK N 20®) (Lot No. ZB56966, Wacker Chemie AG, Munchen, Germany)
- Sodium chloride (Lot No. 500408 and Lot No. 0709156, Ajax Finechem, Taren Point 2229, Australia)
- Citric acid (Lot No. 587465 Ajax Finechem, Taren Point 2229, Australia)

- Aspartame (Lot No. A61004, The Nutrasweet Company, Chicago, USA)
- Magnesium Stearate (Lot No. N5687, Hangzhou Starshine Pharmaceutical Co., Ltd., Zhejiang, China)
- Sucralose (Lot No. 071203, CCS.CHEM. CO.,LTD., Zhejiang, China)
- Ethyl cellulose (Ethocel 10CPS) (Lot No. QD20013T01, Shandong Head Co.,Ltd, Shandong**,** China)
- Poly(ethyl acrylate, methyl methacrylate, trimethylammonioethyl methacrylate chloride) (1:2:0.1) (Eudragit® RS PO) (Lot No. 0461238135, Rohm GmbH Chemische Fabrik, Munchen, Germany)

1.2 Materials for microbial contamination test

1.2.1 Specific microbial organisms

- *Escherichia coli* ATCC 25922
- *Salmonella* spp. Laboratory isolation
- *Staphylococcus aureus* ATCC 6538P
- *Pseudomonas aeruginosa* ATCC 27853

1.2.2 Microbial contamination test mediums

- Triple Soy Agar (Lot no. VM091758 326, Merck GaA, 64271 Dammstadt, Germany)
- Triple Soy Broth (Lot no. 8177623, Becton Dickinson and company Sparks, MD 21152, USA)
- Xylose-Lysine-Desoxycholate Agar (Lot no. 8118380, Dickinson and company Cockcysville, MD 21030, USA)
- Cetrimide Agar (Lot no. 4268698, Becton Dickinson and company Sparks, MD 21152, USA)
- Bismuth Sulfite Agar (Lot no. V744718 607, Merck GaA, 64271 Dammstadt, Germany)

- Lactose Broth (Lot no. V189561 820, Merck GaA, 64271 Dammstadt, Germany)
- Cooked Meat Medium (Lot no. 887988, Oxoid Ltd., Basingstoke, Hampshire, England)
- Selenite-Cystine Enrichment Broth (Lot no. V138009 806, Merck GaA, 64271 Dammstadt, Germany)
- Triptose Blood Agar (Lot no. 72455JB, Difco Laboratories, Michican, USA)
- Mannitol Salt Agar (Lot no. 458.3, Laboratories Britania, Los Patos, Argentina)
- Brilliant Green Agar (Lot no. 12907, Difco Laboratories, Michican, USA)
- Triple Sugar Iron Agar (Lot no. 789089, Difco Laboratories, Michican, USA)
- Levine Eosin-Methylene Blue Agar (Lot no. 799632, Difco Laboratories, Michican, USA)
- Pseudomonas Agar Medium for Detection of Pyocyanin (Lot no. 765226, Difco Laboratories, Michican, USA)
- Pseudomonas Agar Medium for Detection of Fluorescin (Lot no. 8198341, Becton Dickinson and company Sparks, MD 21152, USA)
- MacConkey Agar (Lot no. V960465 735, Merck GaA, 64271 Dammstadt, Germany)
- Enterobacteria Enrichment Medium (Lot no. 8161081, Becton Dickinson and company Sparks, MD 21152, USA)
- Crystal Violet Neutral Red-Bile-Dextrose-Agar (Lot no. 6194920, Becton Dickinson and company Sparks, MD 21152, USA)
- Sabouraud Dextrose Agar (Lot no. 6038536, Becton Dickinson and company Sparks, MD 21152, USA)
- GasPack^{TM} EZ (Lot no. 8165983, Becton Dickinson and company Sparks, MD 21152, USA)
- Liquid paraffin (Lot no. 623587, Srichand United Dispensary Co.,LTD, Thailand)

- Glycerol (Lot no. 13/04, Srichand United Dispensary Co.,LTD, Thailand)
- Anaerobic Indicator BR0055B (Lot no. 1379200, RG24 8PW, England)

1.3 Other chemicals

- Gallic acid (Lot no. 1126284 32206107, % purity = 99.9, % LOD = 6.6, Sigma-Aldrich Chemie GmbH, Spain)
- Methanol HPLC (Lot no. 02726805, Fisher Scientific UK Limited, Leicestershire, UK)
- Trifluoroacetic acid (Lot no.53130 54705C05, Sigma-Aldrich Chemie GmbH, Steinheim, Germany)
- Sodium carbonate anhydrous (Lot no. 0569246, Fisher Scientific UK Limited, Leicestershire, UK)
- Folin-Ciocalteu's phenol reagent (Lot No. 1237128 13306001, Sigma-Aldrich Chemie GmbH, Buchs SG, Switzerland)
- Lithium chloride (Lot No. AF 601067, Ajax Finechem, Taren Point 2229, Australia)
- Magnesium nitrate (Lot No. AF 704325, Ajax Finechem, Taren Point 2229, Australia)
- Magnesium Chloride (Lot No. 0711208, Ajax Finechem, Taren Point 2229, Australia)
- Potassium nitrate (Lot No. 0710120, Ajax Finechem, Taren Point 2229, Australia)
- Sodium chloride (Lot No. 500408 and Lot No. 0709156, Ajax Finechem, Taren Point 2229, Australia)

2. Equipments

- High performance liquid chromatography system as follows:
    - Shimadzu liquid chromatography (Pump) (Model LC-10AD vp, Shimadzu, Kyoto, Japan)

  - Shimadzu degasser (Model DGU-14A, Shimadzu, Kyoto, Japan)
  - Shimadzu autosampler (Model SIL-10AD vp, Shimadzu, Kyoto, Japan)
  - Shimadzu diode array detector (Model SPD-M10A vp, Shimadzu, Kyoto, Japan)
  - Shimadzu UV-Vis detector (Model SPD-10A vp, Shimadzu, Kyoto, Japan)
  - Shimadzu system controller (Model SCL-10A vp, Shimadzu, Kyoto, Japan)
  - Shimadzu software (Model Class VP, Shimadzu, Kyoto, Japan)
  - Column oven (Model CTD-10 AS, Shimadzu, Kyoto, Japan)
- Spectrophotometer (Model V-530, Jasco, Kyoto, Japan)
- Alltima C18 column, 4.6 x 150 mm, 5µ (Lot no.060201066, AllTech, Illinois, USA, distributed by Ligand scientific Co.,LTD., Thailand)
- Analytical balance (Model A200S, Sartorius, Goettingin, Germany)
- Balance (No. MT-045, Mettler Toledo, Greifensee 8606, Switzerland)
- Moisture analyzer (Model HR83, Mettler Toledo, Greifensee 8606, Switzerland)
- Sieve shaker with 100 mm diameter of sieves (Model FT-150M, Filtra, Barcelona, Spain)
- pH meter (Model 201, ORION, Minnesota, USA)
- Ultrasonic cleaner (Type TP680DH, Elma, Singen, Germany)
- V-shape mixer (Fuji Electric CO., LTD., Tokyo, Japan)
- Oscillating granulator (ERWEKA AR 400, Hensenstamm, Germany)
- Hot air Oven (Model Tv40uL 998001, Memmert, Germany)
- Autoclave (Model HA-3D, Hirayama manufacturing corporation, Tokyo, Japan)
- Incubator (Edelstahl Rost frei, Memmert, Germany)
- Scanning electron microscope (Model S-2500, Hitachi, Tokyo, Japan)
- Petri dish with 9- cm diameter,

- Glass funnel with 1.5-cm orifice
- Desiccator with outlet,150-mm diameter and 300-mm diameter (Duran®, Mainz, Germany)
- Syringe filter, Nylon (Lot no. 49007, Vertical ™, Thailand, distributed by Ligand scientific Co., LTD., Thailand)
- 2 ml vial chamber (Lot no. 00064774 , Sun-sri, USA, distributed by Ligand scientific Co., LTD., Thailand)
- Filter paper, 110-mm diameter (Lot no. G1823824B ,Whatman®, ME16 0LS, England)
- Differential scanning calorimetry machine(Mettler Toledo, Zurich, Switzerland)
- Tablets hardness tester (Thermonik Tablet Tester, model DHT-250,Labquip(Thailand) Limited, Bangkok, Thailand)
- Roche friabilator (Erweka. Apparatebau,G.M.B.H, Western Germany)
- Single punch tabletting machine (โรงงานเหยี่ยวเฮง, Bangkok, Thailand)
- Dissolution tester (Vankel, Model VK7000, Ohio, USA, distributed by Meditop Co., LTD., Thailand

3. Methods

3.1 Preparation of *Phyllanthus emblica* Linn. fruit extract

Phyllanthus emblica Linn. spray dried fruit extract was prepared by the Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical sciences, Chulalongkorn University. *Phyllanthus emblica* Linn. fresh fruits were selected. They should not become rotten and should be cleaned with water. Next, 1.5 kilogram of *Phyllanthus emblica* Linn. fruits were dispersed in 1 liter of the distilled water and crushed by grinder for 10 minutes. The liquid part was separated by polyester cloth and kept in a container. Then, 0.5 liter of the distilled water was added to the remaining. The solid part was crushed again for 5 minutes. The liquid part was separated again. Then, both of the liquid parts were combined and filtered by

polyester cloth again. Finally, the liquid parts were dried by spray drying technique to obtain fine yellow powder

Some *Phyllanthus emblica* Linn. spray dried fruit extract batch no. 28122006 was coated with ethyl cellulose and Eudragit® RS PO. The ratios of extract powder to solid polymer were 99:1, 98:2 and 97:3, respectively. Absolute alcohol was used as solvent to prepare polymer solution. The coating pan was used to coat the powder with feed rate 6 RPM and atomizing air pressure 0.2 MPa. The coated extract was incubated at 45° C for 1 hour and screened through a 30-mesh sieve befor use.

3.2 Characterization of *Phyllanthus emblica* Linn. spray dried fruit extract and excipients

3.2.1 Morphology study

The sample of *Phyllanthus emblica* Linn. spray dried fruit extract was prepared by gold sputtering technique and viewed using a scanning electron microscope (SEM). Shape and surface topography were determined with magnification of 500x, 15 kv and 1,000x, 15 kv. For coated *Phyllanthus emblica* Linn. spray dried fruit extract coated granule was determined with magnification of 750 x, 15 kv.

3.2.2 Bulk density, Tapped density and Percent compressibility

Twenty grams of *Phyllanthus emblica* Linn. spray dried fruit extract were filled into a 100 ml graduate cylinder. The bulk volume was recorded and the bulk density was calculated as the following equation:

$$\text{Bulk density } (g/cm^3) = \frac{\text{Weight of the powder } (g)}{\text{Bulk volume } (cm^3)}$$

The tapped density was determined by dropping the filled graduated cylinder on a hard surface from 5 cm height, until the volume was constant. The tapped density was calculated as the following equation:

$$\text{Tapped density } (g/cm^3) = \frac{\text{Weight of the powder (g)}}{\text{Tapped volume } (cm^3)}$$

The value of bulk and tapped density was average from three replicates.

The percent compressibility was calculated from the bulk density and the tapped density by the following equation:

$$\text{Percent compressibility} = \frac{(T - B)}{T} \times 100$$

T = tapped density

B = bulk density

For the compressibility index, the generally accepted scale of flowability is given in table 1

Table 1 Scale of Flowability (The USP 31, 2008)

Compressibility Index (%)	**Flow Character**
<10	Excellent
11-15	Good
16-20	Fair
21-25	Passable
26-31	Poor
32-37	Very Poor
>38	Very, very poor

3.2.3 Flow rate

Twenty grams of *Phyllanthus emblica* Linn. spray dried fruit extract were filled in a glass funnel with 1.2 cm orifice. The glass funnel was fixed on the clamp at 5 cm height above smooth surface. The time taken for the powder started to flow until finished was record. The flow rate, in term of g/sec, was average from three replicates.

3.2.4 Loss on drying

Three grams of *Phyllanthus emblica* Linn. spray dried fruit extract or excipients was heated at 105°C by a moisture analyzer until the weight was constant. The moisture content, in term of percentage of loss on drying, was average from three replicates.

3.2.5 pH

The *Phyllanthus emblica* Linn. spray dried fruit extract was dispersed in deionized water to prepare 1% w/v of extract at ambient temperature. The pH of liquid was measured. The value of pH was average from three replicates.

3.2.6 Particle size distribution

The sieve analysis method was used to examine particle size distribution. The sieve shaker with sieve mesh size number 20 (0.850 mm), 40 (0.425 mm), 60 (0.250 mm), 80 (0.180 mm), 100 (0.150 mm), 200 (0.075 mm) and 325 (0.045 mm) was used. Five grams of *Phyllanthus emblica* Linn. spray dried fruit extract were accurately weighed and put on the top of sieve. Then, the set of sieves was shaken for 15 minutes at power = 9 and cycle = 9. Powders remained on each sieve size were weighed and calculated in percentage of weight distribution.

3.2.7 Total tannins analysis

Total tannins were determined by Folin-Ciocalteau method (Slinkard and Singleton, 1977). The method was described as the followings:

3.2.7.1 Standard preparation

Twenty-five milligrams of gallic acid were weighed into a 250 ml volumetric flask, dissolved in water and diluted to volume (concentration of the solution = 100

µg/ml). Then, this solution was diluted with water to six concentrations, 10, 20, 40, 50, 60 and 80µg/ml, which were used as standard solutions.

3.2.7.2 Sample preparation

Twenty-five milligrams of *Phyllanthus emblica* Linn. spray dried fruit extract were weighed into a 250 ml of volumetric flask, added water approximately 125 ml and sonicated for 15 minutes. Then, the volumetric flask was filled up to volume with water and well mixed (concentration of the solution = 100 µg/ml). The solution was filtered through a Whatman® no. 1 filter. Total tannins were determined in three replicates.

3.2.7.3 Diluted solution of Folin-Ciocalteau reagent

The Folin-Ciocalteau reagent was diluted with water to 1:10. The reagent was freshly prepared before use.

3.2.7.4 Colorimetric reaction

One milliliter of the filtrate was transferred into a 15 ml test tube. Five milliliters of diluted Folin-Ciocalteau reagent were added and mixed with the filtrate. After 3-8 minutes, 4 ml of 7.5% sodium carbonate anhydrous solution were added and the mixture was mixed by a vortex mixer. The test tubes were allowed to stay at ambient temperature in the dark cabinet for 2 hours. Then, the solution was assayed spectrophotometrically at 731.50 nm using gallic acid as the reference standard. The value of the total tannins content was average from three determinations and expressed as gallic acid equivalent (GAE).

3.2.7.5 Calculation

The standard curve was established by graphically plotting the absorbance and concentration of gallic acid solutions; and the coefficient of determination (R^2) was

calculated. The value of total tannins content was average from three determinations and expressed as gallic acid equivalent (GAE)

$$\% \text{ Total tannins} = \frac{\text{GAE concentration (µg/ml) of samples x 100}}{\text{Concentration of extract (µg/ml)}}$$

GAE = Total tannins in terms of gallic acid equivalence

3.2.8 Gallic acid analysis

The percentage of gallic acid in *Phyllanthus emblica* Linn. spray dried fruit extract was determined by high performance liquid chromatographic method (HPLC). The HPLC conditions were modified from Leewongpan and Laoruangsinchai (2004) and validated as the following:

HPLC chromatographic conditions:

HPLC colunn	: Alltima® C18 column (4.6 x 150 mm), 5 µm (AllTech) equipped with guard column packed with C18
Mobile phase	: Methanol : 0.3% v/v Trifluoroacetic acid (8:92, v/v)
Flow rate	: 1 ml/min
Detection	: UV detector at 270 nm
Injection volumn	: 20 µl
Temperature	: Ambient
Run Time	: 15 min

3.2.8.1 Validation of HPLC method

The analytical parameters for validation are linearity (R^2), accuracy (recovery), precision (%RSD) and system suitability.

3.2.8.1.1 Linearity

Gallic acid standard solutions with various concentrations ranging from 0.25, 1, 3, 5 and 7.5µg/ml were prepared and analyzed. The linearity of the curve obtained by plotting the peak area versus the concentrations of each standard solution was calculated using the least square method. The slope should be close to 1.0 (The USP 31, 2008).

3.2.8.1.2 Accuracy

Five concentrations of gallic acid solution (0.5, 1.0, 2.5, 4.0 and 5.0 µg/ml) and a certain of *Phyllanthus emblica* Linn. spray dried fruit extract solution (7.5 µg/ml) were prepared and analyzed on different days. Accuracy of the HPLC method was calculated as the percentage of recovery as the following equation:

$$\% \text{ recovery} = \frac{\text{Measured concentration (µg/ml) x 100}}{\text{Theoretical concentration (µg/ml)}}$$

Before % recovery was calculated, peak area of each standard solutions was substracted with the peak area of *Phyllanthus emblica* Linn. spray dried fruit extract. Peak area of the extract was obtained from Y-intercept value of the linear graph plotted between the peak areas of the mixture solutions versus the variation of gallic acid concentrations.

3.2.8.1.3 Precision

A. Within Run Precision

The solution containing gallic acid of 1.0 µg/ml and *Phyllanthus emblica* Linn. spray dried fruit extract of 7.5 µg/ml was prepared as described in section 3.2.8.1.2. The solution was injected in six replicates on the same day. Percentage of relative standard deviation (%RSD) was calculated for determination of the precision. The %RSD values must not be more than 2% (The USP 31, 2008).

B. Between Run Precision

The sample solutions were prepared as described in 3.2.8.1.2 and analyzed in 6 replicates on difference days for 3 days. Percentages of relative standard deviation (%RSD) were calculated for determination of the precision. The %RSD values must not be more than 2% (The USP 31, 2008).

3.2.8.1.4 Specificity

The specificity of the method was investigated by injection of gallic acid solution. Then, the chromatogram of gallic acid solution was compared with chromatogram of placebo solution. The chromatogram of gallic acid should not be interfered with those of excipients in the formulation.

3.2.8.1.5 System suitability

Gallic acid solution was prepared to obtain the final concentration of 1µg/ml as described in section 2.8.2.1. The results of analysis were obtained from six replicates of injections. The %RSD values should not more than 2% and tailing factors should not more than 2.0.

3.2.8.2 Assay for *Phyllanthus emblica* Linn. spray dried fruit extract

3.2.8.2.1 Standard preparation

Twenty-five milligrams of gallic acid were weighed into a 250 ml volumetric flask, then dissolved with methanol and the solution was diluted to volume (concentration of the solution = 100 µg/ml). Next, 3 ml of the solution were filled into a 100 ml volumetric flask and diluted to volume with 0.3% trifluoroacetic acid (concentration of the solution = 3 µg/ml). The final solution was filtered through 0.45 µm filter paper and injected into the HPLC column. The peak area was average from three replicates.

3.2.8.2.2 Sample solution preparation

Fifty milligrams of *Phyllanthus emblica* Linn. spray dried fruit extract was weighed into a 50 ml volumetric flask. Methanol of approximately 25 ml was added and the dispersion was sonicated for 15 minutes. Then, it was diluted to volume of concentration 1000 µg/ml. Next, 10 ml of the solution was filled into a 25 ml volumetric flask and diluted to a concentration of 400 µg/ml with 0.3% trifluoroacetic acid. The final solution was filtered through 0.45 µm filter paper and injected into HPLC column. Each sample was determined in three replicates.

3.2.8.2.3 Calculation

The percentage of gallic acid was calculated from peak areas according to the following equation:

$$\% \text{ Gallic acid} = \frac{PA_{sam}}{PA_{std}} \times \frac{Wt_{std}}{250} \times \frac{3}{100} \times \frac{50}{Wt_{sam}} \times \frac{25}{10} \times \% \text{ purity} \times \frac{(100 - \% \text{ LOD})}{100}$$

PA_{sam} = Peak area of *Phyllanthus emblica* Linn. spray dried fruit extract solution

PA_{std} = Peak area of gallic acid standard solution

Wt_{sam} = Weight of *Phyllanthus emblica* Linn. spray dried fruit extract

Wt_{std} = Weight of gallic acid standard

% purity = The gallic acid content in standard

% LOD = The moisture content in gallic acid standard

3.2.9 Microbial Limit Test

Phyllanthus emblica Linn. spray dried fruit extract was detected for the amount of microbial contamination according to the method described in The United States Pharmacopoeia 31 and Thai Herbal Pharmacopoeia 2000. The limit of microbial contamination in preparation of crude drugs and mixture of crude drugs for internal use, which contain whole or ground crude drugs according to Thai Herbal Pharmacopoeia 2000 is shown in table 2. The test for specified positive results on

selective agar and biochemical test of microorganisms, *Staphylococcus aureus, Pseudomonas aeruginosa,* Enterobacteriaceae, *Salmonella* spp*., Escherichia coli*, and *Clostridium* spp., is shown as Table 3 – Table 8.

Table 2 Limit of microbial contamination (Thai Herbal Pharmacopoeia 2000).

Type	Requirements
Total aerobic bacteria	Not more than 5.0×10^5 in sample 1 g
Yeasts and moulds	Not more than 5.0×10^3 in sample 1 g or 1ml
Escherichia coli	Not more than 5.0 in sample 1 g or 1 ml
Enterobacteriaceae	Not more than 5.0×10^3 in sample 1 g or 1ml
Staphylococcus aureus	Absent in sample 1 g or 1 ml
Clostridium spp.	Absent in sample 10 g or 10 ml
Salmonella spp.	Absent in sample 10 g or 10 ml

Table 3 Morphologic characteristics of *Staphylococcus aureus* on Mannitol-Salt Agar Medium and diagnostic test (The USP 31).

Colonial morphology	Yellow colonies surrounded by yellow zone
Gram strain	Positive cocci (in cluster)
Catalase test	Positive

Table 4 Morphologic characteristics and biochemical test of *Pseudomonas aeruginosa* on selective and diagnostic agar media (The USP 31).

Medium / Test	Cetrimide Agar	Pseudomonas Agar for detection of	
		Fluorescin	Pyocyanin
Colonial morphology	Generally greenish	Generally colorless or yellowish	Generally greenish
Gram stain	Negative rods	Negative rods	Negative rods
Oxidase test	Positive	Positive	Positive

Table 5 Morphologic characteristics of Enterobacteriaceae on Crystal Violet Neutral Red-Bile-Dextrose-Agar (The USP 31).

Type	Description of colony
Lactose fermenters	Purple-red colonies, with or without a zone of precipitate around the colonies
Lactose non-fermenter	Colorless to transparent colonies
Gram positive Cocci	Colorless, pinpoint colonies

Table 6 Morphologic characteristics of *Salmonella* spp. on selective agar media (The USP 31).

Medium	Description of colony
Brilliant Green Agar Medium	Small, transparent, colorless or pink to white opaque (frequently surrounded by pink or red zones)
Xylose-Lysine-Deoxycholate Agar Medium	Red with or without black centres
Bismuth Sulfite Agar Medium	Black or green

Table 7 Morphologic characteristics of *Escherichia coli* on selective and diagnostic agar media (The USP 31).

Medium	Description of colony	Gram strain
MacConkey Agar	Brick-red, may have surrounding zone of precipitated bile	Negative rods (coccobacilli)
Eosin-Methylene Blue Agar	Metallic sheen under reflected light and blue-black appearance under transmitted light	

Table 8 Morphologic characteristics of *Clostridium* species on 5% defibrinated blood agar medium (The USP 31).

Selective species	*C. botulinum*	*C. perfringens*	*C. tetani*
Colonies	Irregular, translucent with a granular surface and indefinite fimbriated spreading edge	Large, circular, convex Semi-translucent, Smooth with an entire edge	Transparent with long feathery spreading projections
Hemolysis	Positive	Double zone	Positive
Spores	Oval, central, subterminal distend bacilli	Absent	Spherical and terminal (drum stick)

3.2.9.1 Sampling preparation for microbial contamination test

Ten grams of *Phyllanthus emblica* Linn. spray dried fruit extract was used for each of the tests.

3.2.9.2 Test for total aerobic bacterial count

Suspend 10 g of *Phyllanthus emblica* Linn. spray dried fruit extract in Fluid Soybean-Casein Digest Medium to make 100 ml. Dilute the sample to be $1:10^2$, $1:10^3$ and $1:10^4$ and pipette 1 ml of each dilution onto each of three sterile petri dishes. Promptly add to each dish 15 to 20 ml of Soybean-Casein Digest Agar Medium that previously has been melted and cooled to approximately 45°C. Cover the petri dishes, mix the sample with the agar by tilting or rotating the dishes, and allow the contents to solidify at room temperature. Invert the petri dishes, and incubate for 2days at 35°C. Following incubation, examine the plates for growth, count the number of colonies, and express the average for the three plates in terms of the number of microorganisms per g of sample.

3.2.9.3 Test for total fungal count

Suspend 10 g of *Phyllanthus emblica* Linn. spray dried fruit extract in Fluid Soybean-Casein Digest Medium to make 100 ml. Dilute the sample to be 1:2.5, 1:5, 1:10 and pipette 1 ml of each dilution onto each of three sterile petri dishes. Promptly add to each dish 15 to 20 ml of Sabouraud Dextrose Agar Medium that previously has been melted and cooled to approximately 45°C. Cover the petri dishes, mix the sample with the agar by tilting or rotating the dishes, and allow the contents to solidify at room temperature. Invert the petri dishes, and incubate for 5 days at 25°C. Following incubation, examine the plates for growth, count the number of colonies, and express the average for the three plates in terms of the number of microorganisms per g of sample.

3.2.9.4 Test for specified microorganisms

A. Test for *Staphylococcus aureus* and *Pseudomonas aeruginosa*

To the sample add Fluid Soybean Casein Digest Medium to make 100 ml, mix and incubate for 24 hours at 35°C. Examine the medium for growth, use an inoculating loop to streak a portion of the medium on the surface of Mannitol Salt Agar Medium and of Cetrimide Agar Medium, each plated on petri dishes. Cover and invert the dishes, and incubate for 24 hours at 35°C. Following incubation, examine the plates for growth, none of the plates contains colonies having the characteristic listed in table 3 and table 4 for the media used, the test sample meets the requirements for freedom from *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

B. Test for Enterobacteriaceae

Suspend 10 g of *Phyllanthus emblica* Linn. fruit extract in Fluid Lactose Medium to make 100 ml. Incubation for 2 hours at 35°C, dilute the sample with Fluid Enterobacteria Enrichment Medium until a suspension to be $1:10^2$ and $1:10^3$ dilution, incubate for 24 hours at 35°C, pipette 0.1 ml of each dilution on the surface of Crystal

Violet Neutral Red-Bile-Dextrose-Agar Medium, each plated on petri dishes, spread the sample thoroughly the surface of medium. Invert the petri dishes, and incubate for 24 hours at 35°C. Following incubation, examine the plates for growth, none of the plates contains colonies having the characteristic listed in table 5 for the media used, the test sample meets the requirements for freedom from Enterobacteriaceae.

C. Test for *Salmonella* species and *Escherichia coli*

Suspend 10 g of *Phyllanthus emblica* Linn. fruit extract in Fluid Lactose Medium to make 100 ml, mix and incubate for 5 hours at 35°C. Examination the medium for growth, mix by gently shaking. Pipette 1 ml portions into vessels containing, 10 ml of Fluid Selenite-Cysteine Medium and Fluid Tetrathionate Medium, respectively, mix and incubate for 24 hours at 35°C. (Retain the remaining of the Fluid Lactose Medium)

Test for *Salmonella* species

Use an inoculating loop to streak portions from both the Fluid Selenite-Cysteine Medium and Fluid Tetrathionate Medium on the surface of Brilliant Green Agar Medium, Xylose-Lysine-Desoxycholate Agar Medium and Bismuth Sulfite Agar Medium contained in petri dishes. Cover and invert the dishes, and incubate for 24 hours at 35°C. Following incubation, examine the plates for growth, if none of the colonies conforms to the description given in Table 6, the requirements of the test for freedom of the genus *Salmonella*.

If colonies of Gram-negative rods matching the description in Table 5 are found, proceed with further identification by transferring representative suspect colonies individually, by means of an inoculating wire, to a butt-slant tube of Triple Sugar-Iron-Agar Medium by first streaking the surface of the slant and then stabbing the wire well beneath the surface. Incubate for 24 hours at 35°C, if examination discloses no evidence of tubes having alkaline (red) slants and acid (yellow) butts (with or without concomitant blackening of the butt from hydrogen sulfide

production), the sample meets the requirements of the test for absence of the genus *Salmonella*.

Test for *Escherichia coli*

Use an inoculating loop to streak a portion from the remaining of the Fluid Lactose Medium on the surface of MacConkey Agar Medium. Cover and invert the dishes, and incubate for 24 hours at 35°C. Following incubation, examine the plates for growth, if none of the colonies conforms to the description given in Table 7, the requirements of the test for freedom of *Escherichia coli*.

If colonies matching the description are found, proceed with further identification by transferring representative suspect colonies individually, by means of an inoculating loop, to the surface of Levine Eosin-Methylene Blue Agar Medium. If none of the colonies exhibits the characteristic given in Table 7, the sample meets the requirements of the test for freedom of *Escherichia coli*.

D. Test for *Clostridium* species

Two flasks of Cooked Meat Medium were boiled for 2 minutes at 100°C and allow the contents to cool at 37°C. Suspend 10 g of *Phyllanthus emblica* Linn. fruit extract in flask 1 to make 100 ml, mix and incubate for 24 hours at 35°C, examination every day for 4 days. The another flask (flask 2) was suspend with 10 g of sample, mix and warm for 30 minutes at 65°C, allow the contents to cool at room temperature, added with sterile paraffin, incubate for 24 hours at 35°C, examination every day for 4 days. Examine the medium for growth, pipette 0.1 ml of the medium from flask 2 on the surface of 5% Defibrinated Sheep Blood Agar Medium to make 2 set. Cover and invert the dishes, set 1 was incubate for 48 hours at 35°C. For set 2 was placed in Anaerobic Jar, added Gas Pack and anaerobic indicator, close the lid and waiting until anaerobic indicator appears white color, incubate for 48 hours at 35°C. Following incubation, examine the plates for growth. If none of the plates contains colonies have the characteristic listed in table 8 for the media used, the test sample meets the requirements for freedom from *Clostridium* species.

3.2.10 Moisture sorption study

Five saturated salt solutions, lithium chloride, magnesium chloride, magesium nitrate, sodium chloride and potassium nitrate were chosen to various control of relative humidity. Since the relative humidity of these solutions somewhat varies with temperature, the actual relative humidity levels for these solutions obtained from the reference (กาญจน์พิมล ฤทธิเดช และคณะ, 2546) are 11, 33, 52, 75 and 93% RH at 25° C, respectively. A thin layer of approximately 200 mg of the coated extract granule was spread in each amber glass drying bottle of 1.90 cm in diameter and 3.80 cm in height. The bottles were placed in the desiccators which contained a saturated salt solution that generated a desired relative humidity. Then, the desiccators were stored in a constant temperature incubator at 25° C. At predetermined time intervals, each sample bottle was removed and covered with aluminum cap to prevent moisture exchange with the ambient during the transportation. After weighing, the bottle was immediately placed back into the desiccators for the next time interval use. The net weight of the coated extract granule in each bottle was calculated and the coated extract granule, which protected the highest moisture sorption, was selected to prepare the chewable tablets containing *Phyllanthus emblica* Linn. spray dried fruit extract coated (กาญจน์พิมล ฤทธิเดช และคณะ, 2546).

3.3 Formulation and preparation of chewable tablets containing *Phyllanthus emblica* Linn. spray dried fruit extract

3.3.1 Formulation of chewable tablets

The quantities of *Phyllanthus emblica* Linn. spray dried fruit extract, mannitol and xylitol to be used to make the tablets, were studied. The *Phyllanthus emblica* Linn. spray dried fruit extract is very hygroscopic and its content in the formula might affect to properties of the product such as its flowability of the mixtured powder. Thus, the extract contents in range of 30, 40 and 50% were designed to study the flowability of the formulations. The result found that 30% range of the extract had good flowability of mixture powder. The designated formulations are presented in

Table 9. Mannitol and xylitol were usually used as excipients for the chewable tablets. They have sweetness and few calories. The quantity of mannitol and xylitol were varied due to the consideration of good mouth-feel for on 1:1 ratio. Microcrystalline cellulose PH 102, 10% and copovidone, 10% were used as binders. Colloidal silicon dioxide, 0.3% was used as a glidant. Talcum, 10% and magnesium stearate, 1% were used as lubricants. Aspartame, 1% and sucralose, 1% were used as sweetening agents. Finally, Citric acid and sodium chloride were used to improve the taste of formulation.

The formulations were paired to study respectively the effects of the binder (microcrytalline cellulose PH 102 and copovidone), the flavoring agents (citric acid and sodium chloride), alkalinity of magnesium stearate and the sweetening agents (aspartame and sucralose) for the properties and stability of the chewable tablets. Pairs of formulations A1/A5, A2/A6, A3/A7 and A4/A8 were investigated the effect of binders. In formulations A1, A2, A3 and A4 had microcrystalline cellulose PH 102 but in formulations A5, A6, A7 and A8 had copovidone as binder. Pairs of formulations A1/A3, A2/A4, A5/A7, A6/A8 and B1/B3 were investigated on the effect of alkalinity of magnesium stearate. Formulations A1, A2, A5, A6 and B1 had 1% magnesium stearate and 10% talcum but formulations A3, A4, A7, A8 and B3 had only 30% talcum as lubricant. Pairs of formulations A1/A2, A3/A4, A5/A6, A7/A8 and B1/B2 were investigated on the effect of citric acid and sodium chloride. Formulations A1, A3, A5, A7 and B1 had citric acid and sodium chloride but formulations A2, A4, A6, A8 and B2 had no citric acid and sodium chloride. Finally, a pair of formulation B1/B4 was investigated on the effect of sweetening agent. Formulation B1 had 1% aspartame but formulation B4 had 1% sucralose as sweetening agent.

Table 9 Formulations of chewable tablets containing *Phyllanthus emblica* Linn. spray dried fruit extract (750 mg/tablet) (Batch size = 1500 g)

Ingredients	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**B1**	**B2**	**B3**	**B4**
P. emblica L. spray dried fruit extract (%)	30	30	30	30	30	30	30	30	-	-	-	-
P. emblica L. spray dried fruit extract coated granule (%)	-	-	-	-	-	-	-	-	30	30	30	30
Mannitol (%)	20.35	23.85	10.85	14.35	20.35	23.85	10.85	14.35	20.35	23.85	10.85	20.35
Xylitol (%)	20.35	23.85	10.85	14.35	20.35	23.85	10.85	14.35	20.35	23.85	10.85	20.35
MCC PH 102 (%)	10	10	10	10	-	-	-	-	10	10	10	10
Copovidone (%)	-	-	-	-	10	10	10	10	-	-	-	-
Silicon dioxide (%)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Magnesium Stearate (%)	1	1	-	-	1	1	-	-	1	1	-	1
Talc (%)	10	10	30	30	10	10	30	30	10	10	30	10
Aspartame (%)	1	1	1	1	1	1	1	1	1	1	1	-
Sodium Chloride (%)	2	-	2	-	2	-	2	-	2	2	2	2
Sucralose (%)	-	-		-	-	-	-	-	-	-	-	1
Citric acid (%)	5	-	5	-	5	-	5	-	5	-	5	5

3.3.2 Preparation of chewable tablets containing *Phyllanthus emblica* Linn. spray dried fruit extract.

A batch size of 1500 g of chewable tablets containing 30% or 225 mg of *Phyllanthus emblica* Linn. spray dried fruit extract equivalent to 56.41-57.62 mg total tannins or 1.37-1.71 mg gallic acid were prepared using direct compression process. The total tannins and gallic acid contents varied batch to batch from variation of the extract. First, *Phyllanthus emblica* Linn. spray dried fruit extract was mixed with half amount of talc in the formulation using V-shape mixer for 10 minutes. Next, xylitol, mannitol, copovidone and microcrystalline cellulose PH 102 were added geometrically and the mixing was carried out for 20 minutes. Then, aspartame or sucralose, citric acid, sodium chloride, silicon dioxide, magnesium stearate and/or the remaining amount of talc were added and the mixing continued for 10 minutes. Finally, the mixture was compressed into 750 mg tablets using a single punch tableting machine assembled with a round plate of stainless chromium steel N695 punch 10 mm in diameter. The compression force was kept constant. The process was carried out in a controlled humidity and temperature room at 38-40% relative humidity and 25 °C. The tablets were filled in an amber glass bottle and aluminum cap before evaluation.

3.4 Evaluation of chewable tablets containing *Phyllanthus emblica* Linn. spray dried fruit extract

The product was characterized for physical appearance (section 3.4.1), average weight and weight variation (section 3.4.2), hardness (section 3.4.3), friability (section 3.4.4), loss on drying (section 3.4.5), pH (section 3.4.6), dissolution study (3.4.7), amount of total tannins (section 3.4.8) and amount of gallic acid (section 3.4.9).

3.4.1 Physical appearance

The appearance of the product was visually observed.

3.4.2 Average weight and weight variation

Twenty chewable tablets were randomly sampled and weighed on an analytical balance. The average weight and standard deviation were calculated.

3.4.3 Hardness

Ten chewable tablets were randomly sampled and their hardness was determined individually by a hardness tester. The value of hardness of chewable tablets was average on the ten tablets.

3.4.4 Friability

Twenty chewable tablets were randomly sampled and weighed using an analytical balance and their total weight, W_0, was recorded. Then, the tablets were placed in a Roche Friabilator which was allowed to operate at 25 rpm for 4 minutes. After removal of fines, the remaining tablets were weighed again and their weight, W, was recorded. The percent friability was calculated by the following equation.

$$\% \text{ friability} = \frac{(W_0 - W)}{W_0} \times 100$$

3.4.5 Loss on drying

Ten tablets were ground. Three grams of ground powder were weighed and heated at 105°C by a moisture analyzer until the weight remained constant. The moisture content, in term of percentage of loss on drying, was average from three replicates.

3.4.6 pH

The pH of chewable tablets were determined using the method as described in section 3.2.5

3.4.7 Dissolution study

Dissolution of the crushed tablets was studied according to the USP paddle method at the paddle speed of 50 rpm, using 0.1 N hydrochloric acid as the dissolution medium. The crushed tablet was prepared by mechanically breaking tablets, using tablet cutter, into eight pieces with similar fragment size, simulating chewed tablet (Hiroyuki et al., 2003)**.**

All fragments obtained from one crushed tablet were put into a vessel containing in 900 ml of the dissolution medium pre-warmed at 37 °C. Ten milliliter of the tested medium was taken after 5, 10, 15, 20, 30, 45, 60 and 70 minutes and filtered through a membrane filter of 10 µm pore size. Immediately after each sampling, 10 ml of fresh medium was replaced. The amount of total tannins released was determined by spectrophotometer (Model V-530, Jasco, Japan) at 731.50 nm. The study was carried out in three replicates.

3.4.8 Amount of total tannins content

4.8.1 Sample preparation of chewable tablets

Total tannins content was analyzed by Folin-Ciocalteu method. Twenty chewable tablets were randomly sampled and ground, the ground product, equivalent to 225 mg of *Phyllanthus emblica* Linn. spray dried fruit extract, was accurately weighed and filled into a 100 ml volumetric flask. Approximately 50 ml of water was added and then the dispersion was sonicated for 15 minutes. After that, the volumetric flask was filled up to volume with water. The dispersion was well mixed and filtered through a Whatman® no. 1 filter. One milliliter of the filtrate was transferred into a 15 ml test tube. Five milliliters of 10 times diluted Folin-Ciocalteau reagent, were added and mixed with the filtrate. After 3-8 minutes, 4 ml of 7.5% sodium carbonate anhydrous solution were added and the mixture was mixed by a vortex mixer. The test tubes were allowed to stay at ambient temperature in a dark cabinet for 2 hours. Then, the solution was assayed spectrophotometrically at 731.50 nm using gallic acid as the reference standard. The value of total tannins content was average from three

determinations and expressed as gallic acid equivalent (GAE). Each sample was determined in three replicates.

3.4.8.2 Calculation of product

The amount of total tannins in product was calculated as the following equation.

$$\% \text{ Total tannins} = \frac{\text{GAE concentration (µg/ml) of samples x 100}}{\text{Concentration of extract in product (µg/ml)}}$$

GAE = Total tannins in terms of gallic acid equivalence

3.4.9 Amount of Gallic acid contents

HPLC chromatographic conditions and standard preparation as described in section 2.8

3.4.9.1 Sample preparation of chewable tablets

Gallic acid content was determined by HPLC in a Shimadzu HPLC-10AT system. Twenty chewable tablets were randomly sampled and ground. The ground product, equivalent to 225 mg of *Phyllanthus emblica* Linn. spray dried fruit extract, was accurately weighed and filled into a 50 ml volumetric flask, added with 25 ml of methanol. The dispersion was sonicated for 15 minutes and filled up to volume with methanol and mixed. Next, 2 ml of the dispersion was filled into a 25 ml volumetric flask and diluted to volume with 0.3% trifluoroacetic acid. The final dispersion was filtered through 0.45 µm filter paper. The filtrate of 20 µl was injected into HPLC column (Alltima® C18 column (4.6 x 150 mm), 5 µm (AllTech) equipped with guard column packed with C18). The mobile phase was methanol and 0.3% v/v trifluoroacetic acid (8:92, v/v) with flow rate 1 ml/min for 15 minutes at 270 nm. The value of gallic acid content, expressed as % gallic acid, was average from three determinations.

3.4.9.2 Calculation of product

The amount of gallic acid in product was calculated as the following equation.

$$\% \text{ Gallic acid} = \frac{PA_{sam}}{PA_{std}} \times \frac{Wt_{std}}{250} \times \frac{3}{100} \times \frac{50}{Wt_{sam}} \times \frac{25}{2} \times \% \text{ purity} \times \frac{(100 - \% \text{ LOD})}{100}$$

PA_{sam} = Peak area of *Phyllanthus emblica* Linn. spray dried fruit extract solution in chewable tablets

PA_{std} = Peak area of gallic acid standard solution

Wt_{sam} = Weight of *Phyllanthus emblica* Linn. spray dried fruit extract in chewable tablets

Wt_{std} = Weight of gallic acid standard

% purity = The gallic acid content in standard

% LOD = The moisture content in gallic acid standard

3.4.10 Microbial contamination test

The chewable tablets were ground and ten grams of ground powder were weighed. The microbial contamination test were then carried out as methods described in section 3.2.9

3.5 Stability of chewable tablets containing *Phyllanthus emblica* Linn. spray dried fruit extract.

The chewable tablets stored in amber glass bottles closed with aluminum caps were placed in four stability conditions including refrigeration, ambient condition, ambient temperature and 75%RH, and 40°C and 75%RH for 3 months. One glass bottled filled with chewable tablets was randomly selected at time intervals of 1, 2 and 3 months and determined for physical appearance, weight variation, hardness,

friability, loss on drying, pH, amounts of total tannins and amount of gallic acid. After 3 months the dissolution of the tablets were investigated.

3.6 Consumer acceptance simulation testing

Thirty volunteers were included in the study. Chewable tablets were given to volunteers. The evaluation of taste, mouth-feel and appearance, were subjective. One tablet of each formulation was chewed by volunteer who then gargled with 250 ml of water before testing the subsequent formulation.

The simple “like” rating scale was selected in this investigation. Statistical analysis was possible for multiple factors in order to assure the highest probability so that the final formulation could demonstrate the most appealing possibilities to the consumers.

3.7 Statistical analysis

For the comparison, statistical analyses were performed using student *t*-test, ANOVA with Scheffe test for post-hoc comparisons. The results of the statistical analyses with at $P < 0.05$ concluded that there was significant difference.

CHAPTER IV

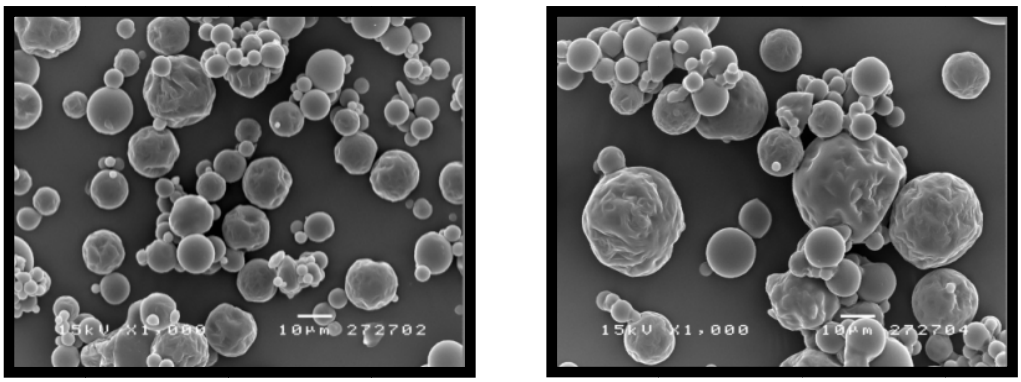
RESULTS AND DISSCUSSION

1. Characterization of *Phyllanthus emblica* Linn. spray dried fruit extract and excipients

Phyllanthus emblica Linn. spray dried fruit extract and the extract coated with 1% ethyl cellulose, which absorbed the least of moisture content from the result of moisture sorption study, were characterized as follows:

1.1 Morphology study

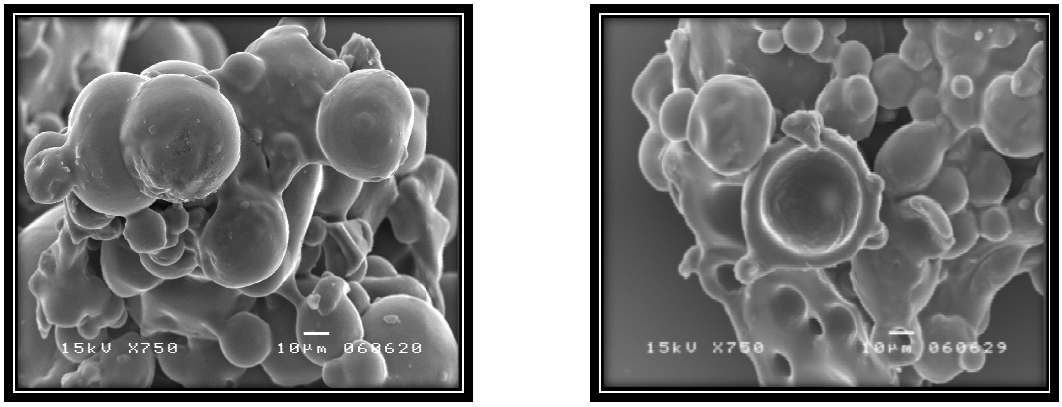
The *Phyllanthus emblica* Linn. spray dried fruit extract was investigated with a scanning electron microscope. The shape and surface topography of the extract batch no. 08122006 and batch no. 28122006 appeared mostly as spherical particles with smooth surface as shown in Figure 4.



A. Batch No. 081229008; 1,000x B. Batch No. 28122006; 1,000x

Figure 4 SEM photomicrograph of *Phyllanthus emblica* Linn. spray dried fruit extract A. Batch no. 081229008, 1,000x; B. Batch no. 28122006, 1,000x

The 1% ethyl cellulose coated extract were mostly spherical particles with smooth surface as shown in Figure 5.



A.Batch no. 28122006 coated with 1% ethyl cellulose, 750x

B.Cross section of batch no.28122006 coated with 1% ethyl cellulose, 750x

Figure 5 SEM photomicrograph of *Phyllanthus emblica* Linn. spray dried fruit extract coated granule. Batch no. 28122006 coated with 1% ethyl cellulose, 750x (A); and Cross section of batch no. 28122006 coated with 1% ethyl cellulose, 750x (B).

1.2 Bulk density, Tapped density and Percent compressibility

Bulk density, tapped density and % compressibility of *Phyllanthus emblica* Linn. spray dried fruit extract of batch no. 08122006 were 0.56 g/ml, 0.86 g/ml and 34.86%, respectively. Bulk density, tapped density and % compressibility of the spray dried fruit extract of batch no. 28122006 were 0.62 g/ml, 0.87 g/ml and 29.23%, respectively as shown in Table 10. As for the compressibility index in Table 1, the *Phyllanthus emblica* Linn. spray dried fruit extract of both batch no. 08122006 and batch no. 28122006 were classified as having a very poor flowing characteristic. Bulk density, tapped density and % compressibility of coated *Phyllanthus emblica* Linn. spray dried fruit extract of batch no. 28122006 were 0.65 g/ml, 0.74 g/ml and 9.00 %, respectively as shown in Table 10. As for the compressibility index in Table 1, the coated extract was classified as excellent flowing characteristic.

Table 10 Physicochemical properties of *Phyllanthus emblica* Linn. spray dried fruit extract and *Phyllanthus emblica* Linn. spray dried fruit extract coated with 1% ethyl cellulose (n=3)

***Phyllanthus emblica* Linn. spray dried fruit extract**	**Batch no. 08122006 Mean (SD)**	**Batch no. 28122006 Mean (SD)**	**Batch no. 28122006 coated with 1% ethyl cellulose Mean (SD)**
Bulk density (g/ml)	0.56 (0.00)	0.62 (0.04)	0.65 (0.02)
Tapped density (g/ml)	0.86 (0.07)	0.87 (0.03)	0.74 (0.05)
% Compressibility	34.86 (4.93)	29.23 (2.95)	9.00 (0.63)
Flow rate (g/sec)	NA	NA	0.28 (0.15)
Angle of repose **(θ)**	NA	NA	23.78 (0.62)
% Loss on drying	3.69 (0.06)	2.97 (0.14)	4.59 (0.19)
pH	4.26 (0.05)	4.61 (0.09)	4.07 (0.05)
% Total tannins	25.64 (0.45)	25.07 (0.36)	25.41 (0.25)
% Gallic acid	0.61 (0.02)	0.76 (0.06)	0.72 (0.23)

As shown in Table 11 the compressibility values of xylitol and mannitol were classified as good flowing characteristic. In contrast, MCC PH 102 and copovidone were classified as having a passable flowing characteristic. Talc was classified as very poor flowing characteristic.

1.3 Flow rate

The flow rate of *Phyllanthus emblica* Linn. spray dried fruit extract could not be measured because the sample could not flow through a glass funnel. After coating with 1% ethyl cellulose, the coated extract of batch no. 28122006 showed improved flowability.

1.4 Loss on drying

The loss on drying of the coated extract was higher than the loss on drying of the uncoated *Phyllanthus emblica* Linn. spray dried fruit extracts as shown in Table 10 because coating process was made under ambient condition. Although, absolute

alcohol was used as solvent to dissolve polymer the extract could absorb moisture from environment.

The loss on drying of xylitol, mannitol, MCC PH 102 and copovidone were 0.04%, 0.35%, 4.70% and 5.56%, respectively as shown in Table 11. From the results MCC PH 102 and copovidone possessed relatively high moisture content.

1.5 pH

The pH of the solutions of *Phyllanthus emblica* Linn. spray dried fruit extracts of batch no. 08122006, batch no. 28122006 and that of coated extract were 4.26, 4.61 and 4.07, respectively, as shown in Table 10. These results indicated that *Phyllanthus emblica* Linn. spray dried fruit extracts could be classified as acidic compound.

1.6 Particle size distribution

The particle size distribution of the *Phyllanthus emblica* Linn. spray dried fruit extract were giveen in Figure 6. Most of the batch no. 08122006 were in the size range of 0.250-0.425 mm, while most of the batch no. 28122006 were in the size range of 0.180 mm – 0.250 mm. The batch no. 28122006 coated with 1% ethyl cellulose got slightly bigger size but the majority was still in the size range of 0.250 mm – 0.425 mm.

Similarly, the analyses of excipients gave the following results: most of mannitol, microcrystalline cellulose PH 102, copovidone and talcum are in the size range of 0.075 mm – 0.150 mm. For sodium chloride, xylitol, citric acid, aspartame, sucralose and silicon dioxide, their majority were in the size range of 0.180 mm – 0.425 mm, 0.250 mm – 0.425 mm, 0.425 mm – 0.850 mm, 0.425 mm – 0.850 mm, 0.425 mm – 0.850 mm and 0.425 mm – 0.850 mm, respectively as shown in Figure 7 and Figure 8.

Table 11 Physicochemical properties of excipients (Mean (SD), n=3)

Ingredients	Mean (SD)					
	Bulk Density (g/ml)	**Tapped Density (g/ml)**	**Compressibility Index**	**Flow rate (g/sec)**	**Angle of repose (θ)**	**% Loss on drying**
Xylitol	0.67 (0.00)	0.75 (0.01)	10.61 (1.14)	0.23 (0.08)	24.19 (0.78)	0.04 (0.02)
Mannitol	0.53 (0.00)	0.61 (0.00)	12.80 (0.58)	0.20 (0.07)	27.57 (2.99)	0.35 (0.03)
MCC PH 102	0.35 (0.01)	0.47 (0.00)	25.59 (1.06)	0.10 (0.29)	28.24 (3.09)	4.70 (0.05)
Copovidone	0.25 (0.00)	0.34 (0.00)	24.49 (0.49)	NA	NA	5.56 (0.04)
Talcum	0.61 (0.01)	1.03 (0.03)	40.31 (0.81)	NA	NA	0.11 (0.02)

NA = Not available

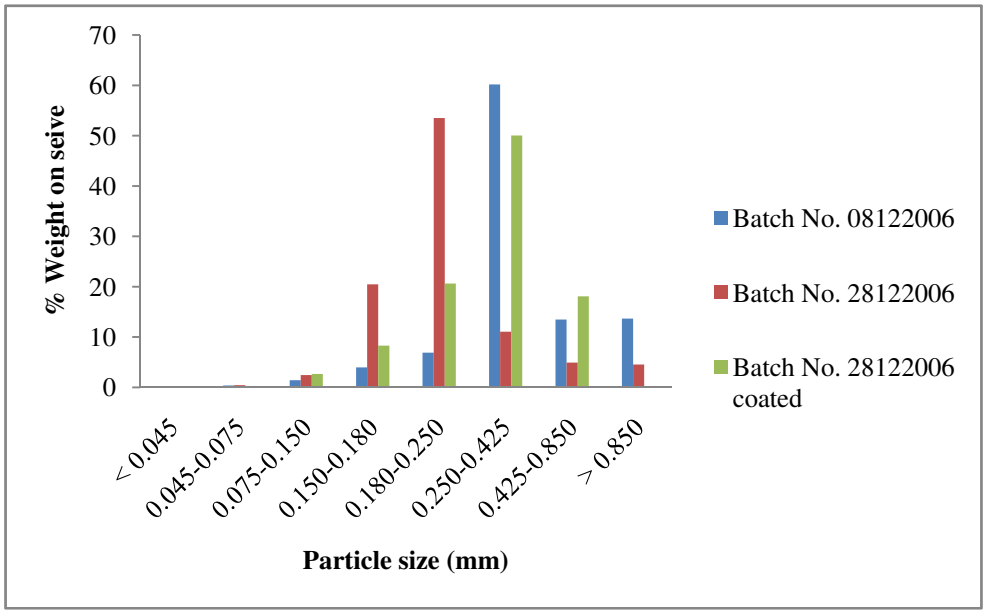


Figure 6 Size distribution of *Phyllanthus emblica* Linn. spray dried fruit extract.

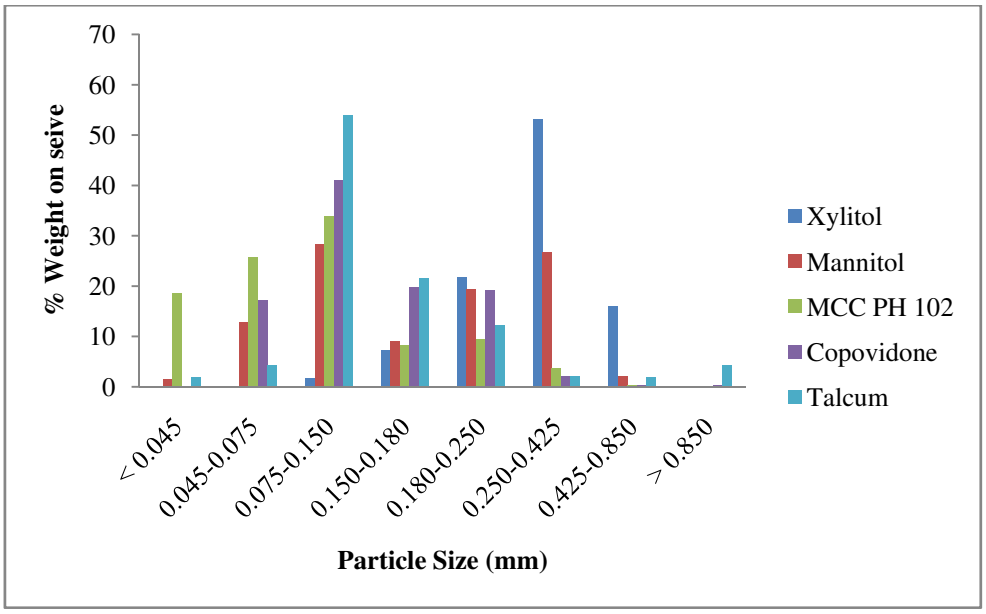


Figure 7 Size distribution of xylitol, mannitol, MCC PH 102, copovidone and talcum.

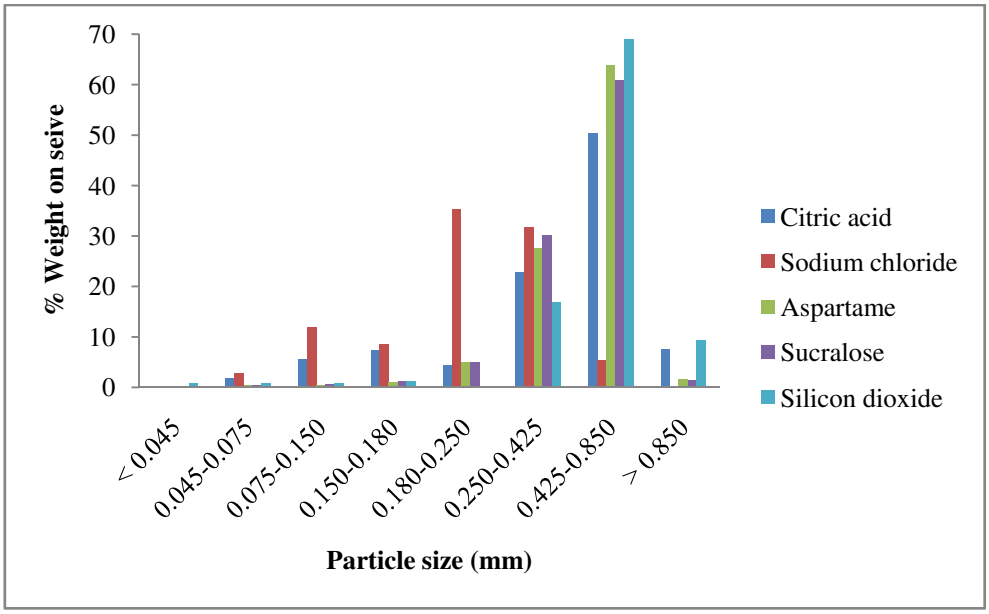


Figure 8 Size distribution of citric acid, sodium chloride, aspartame, sucralose and silicon dioxide.

1.7 Total tannins analysis

Total tannins of *Phyllanthus emblica* Linn. spray dried fruit extract batch no. 08122006, batch no. 28122006 and the extract coated with 1% ethyl cellulose analysed by Folin-Ciocalteau method were 25.64%, 25.07% and 25.41%, respectively as shown in Table 10. These were complied with the specification of *Phyllanthus emblica* Linn. fruit extract of Thai Herbal Pharmacopoeia (2000).

1.8 Gallic acid analysis

1.8.1 Validation of HPLC method

Analytical method validation is a process to evaluate that the method is suitable and consistent for application. The analytical parameters considered in this validation study were linearity, accuracy, precision and specificity.

1.8.1.1 Linearity

A linearity study was carried out to determine whether this method could measure accurately different concentrations of gallic acid. The linearity curve of the peak area versus the concentrations of standard gallic acid is shown in Figure 2A and Table 2A (Appendix A). The standard concentration that gave linear standard curve was in range from 0.25 to7.5 µg/ml. The regression coefficient (R^2) for standard curve was 0.9998. The results showed a good linearity of peak area and standard concentration.

1.8.1.2 Accuracy

The accuracy of the method defined as the percentage of recovery is calculated as deviation agreement between the measured value and the theoretical value. The range of recovery of *Phyllanthus emblica* Linn. spray dried fruit extracts on three days were 90.80-93.92%, 89.96-93.87% and 89.42-94.26%. The percentages of relative standard deviation (%RSD) of percentage of recovery on three days were 0.39-1.75%, 0.12-1.29% and 0.18-1.17% as shown in Tables 3A - 5A (Appendix A), respectively. The results of %RSD were less than 2%, so it indicated the good accuracy of this method.

1.8.1.3 Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of a homogeneous sample. Precision of this method is expressed as the percentage of relative standard deviation (%RSD) and the data are shown in Table 6A (Appendix A). The % RSD of mixture solution on three days was in the range of 0.78-1.62. The results, %RSD were less than 2%, so it indicated the good accuracy of this method.

1.8.1.4 Specificity

The chromatograms were presented in Figure 3A to Figure 8A (Appendix A). The excipients in formulation did not interfere with the peak of gallic acid.

1.8.1.5 System suitability

The RSD percentage of peak area of gallic acid solution was 1.31 and its tailing factor was 1.10. Both of RSD percentages were less than 2%, thus indicating a good system suitability of this method as shown in Table 7A (Appendix A).

1.8.2 Assay for *Phyllanthus emblica* Linn. spray dried fruit extracts

The amount of gallic acid content in *Phyllanthus emblica* L. spray dried fruit extract batch no. 08122006, batch no. 28122006 and the extract coated with 1% ethyl cellulose analysed by HPLC method were 0.61%, 0.76% and 0.72%, respectively as shown in Table 10.

1.9 Microbial contamination test of *Phyllanthus emblica* Linn. spray dried fruit extract

Total aerobic microbial count of *Phyllanthus emblica* Linn. spray dried fruit extract batch no. 08122006 and batch no. 28122008 were 8.8×10^4 CFU/g and 1.97×10^3 CFU/g, respectively. Total combined molds and yeasts counts of the extract batch no. 08122006 and batch no. 28122008 were 52 CFU/g and 148 CFU/g, respectively. The results should that specified microorganisms, *Staphylococcus aureus, Pseudomonas aeruginosa,* Enterobacteriaceae, *Salmonella* spp*., Escherichia coli*, and *Clostridium* spp., were not found in the extracts as shown in Table 46B, Table 47B and Table 48B (Appendix B).

These results were in the limitation of microbial contamination test from Thai Herbal Pharmacopoeia.

1.10 Moisture sorption study of *Phyllanthus emblica* Linn. spray dried fruit extract coated with polymers

Phyllanthus emblica Linn. spray dried fruit extract was coated with ethyl cellulose and Eudragit® RS PO for moisture sorption study. The weight ratios of extract powder to ethyl cellulose or to Eudragit® RS PO were 99:1, 98:2 and 97:3**.** At 25°C and 93%RH the extract coated with 1% ethyl cellulose had the least moisture sorption at about 0.67%, as shown in Figure 9.

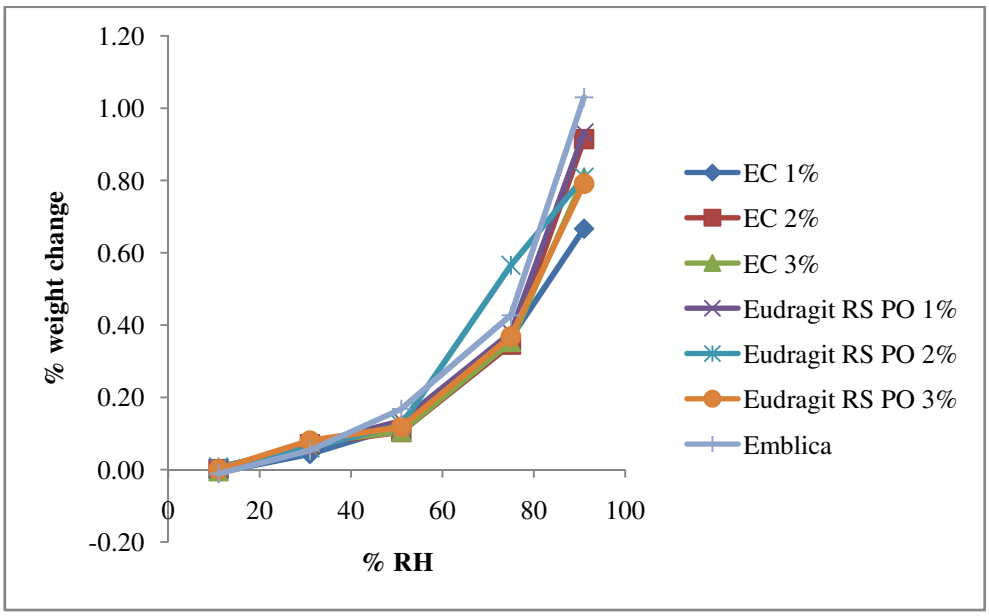


Figure 9 The moisture sorption isotherm of *Phyllanthus emblica* L. spray dried fruit extract coated granule at 25°C.

Consequently, the *Phyllanthus emblica* Linn. spray dried fruit extract coated with 1% ethyl cellulose was selected to be used as active ingredient in some formulations because it showed protective property against moisture.

2. Chewable tablets containing *Phyllanthus emblica* Linn. spray dried fruit extract

2.1 Formulation of chewable tablets

Dry powders of the extracts did not inherenly exhibit the appropriate flowability and compressibility required for direct compression. Numerous reports have addressed techniques to solve this kind of problems, such as wet granulation with non-aqueous solvents, direct compression of spray dried extracts**,** and selection of suitable excipients (Plazier-Vercamen and Bruwier, 1986; Dı´az et al., 1996; Renoux et al., 1996; Palma et al., 2002). However, few studies have aimed at eliciting dry plant extracts of both good flowability and compactibility.

Phyllanthus emblica Linn. fruit extract that was hygroscopic powders and that could show severe problems of tablet picking, if insufficient lubricants were in the formulation. In preliminary study, the attempts were made to add 50%, 40% and 30% into the formulation. The results showed that only could the formulation contained 30% of the extract flow. Excipients commonly used in tablet formulation are also applicable to formulation of chewable tablets due to their ability to provide sweetness and chewability. The polyols such as xylitol and mannitol providing, respectively 100 % and 60% sweetness relative to sucrose, and contributing cooling effect were thus selected as the fillers in the formulation (Saulo, 2005). Xylitol may have additional benefits as it has been shown to have a protective effect and reduce tooth decay by reducing the levels of *Streptococcus mutans* in plaque and saliva and the level of lactic acid produced by these bacteria (Amaechi et al., 1998; Hietala, and Larmas, 1995). In preliminary study the amount of xylitol per mannitol was varied to 1:0, 0:1, 1:1, 2:1 and 1:2, the result was found that the 1:1 provide sweetness and chewability. The binding property of MCC PH 102 and copovidone in the formulation were studied. The results were found that when there was no binder the tablets were capping. The amount of binder, 10% MCC PH 102 or 10% copovidone, was appropriate. The lubricants were added to avoid the tablet sticking. It was found that incorporation of up to 30% talc, or mix of 1% magnesium stearate and 10% talc into the formulation could overcome the problems. However, magnesium stearate, alkaline

lubricant, may be incompatible with acidic compounds in the extract. Thus, its level was kept at minimum. For protection against the interaction between iron and tannins to form complex (Andjelkovic et al., 2006), a round plate stainless chromium steel N695, containing 17% chromium, was used to compress these tablets. Finally, the amount of sweetening agent, aspartame or sucralose, and flavoring agents, sodium chloride and citric acid, were studied. The results were found that 1% aspartame or 1% sucralose, 2% sodium chloride and 5% citric acid had satisfying taste.

2.2 Properties of chewable tablets containing *Phyllanthus emblica* Linn. spray dried fruit extract.

2.2.1 Physical appearance

The appearance of chewable tablets containing *Phyllanthus emblica* Linn. spray dried fruit extract (formulations A1, A2, A3, A4, A5, A6, A7 and A8) and chewable tablets containing *Phyllanthus emblica* Linn. spray dried fruit coated extract granule (formulations B1, B2, B3 and B4) were observed. *Phyllanthus emblica* Linn. fruit spray dried extract was fine yellowish powder. It was considerably hygroscopic. The color of chewable tablets formulations A1, A2, A3, A4, A5, A6, A7 and A8 were yellowish color because the extract was fine, yellow powder, but the excipients in formulation were white color as shown in Figure 10. In contrast, the color of chewable tablets formulations contained the coated extract were darker than formulations contained the uncoated extract. This because the tablets contained the coated extract having larger particle size, in relative to particle size of the uncoated extract as shown in Figure 10. Moreover, both formulations had cooling effect because the formulation contained mannitol and xylitol, which have negative heat characteristics.

(A) (B)

Figure 10 The appearance of chewable tablets containing uncoated (A) and coated (B) *Phyllanthus emblica* Linn. extract.

2.2.2 Average weight and weight variation

The average weight and weight variation of the tablets after preparation were complied with USP 31 as shown in Table 1B (Appendix B) and Table 2B (Appendix B).

2.2.3 Hardness

After preparation, the average hardness of formulations contained spray dried fruit extract were in range from 8.46 to 14.61 KP as shown in Table 13 and Table 11B (Appendix B). The average hardness of formulations contained the extract coated granule were in range from 6.30 to 8.75 as shown in Table 12 and Table 12B (Appendix B). The results are shown that the hardness of tablets is in the range of acceptance of chewable tablets, which is in range from 4 to 7 KP (Robert, 1989).

2.2.4 Friability

After preparation, the friability of formulations contained spray dried fruit extract were in range from 0.11% to 1.14% as shown in Table 13 and Table 21 (Appendix B). The friability of formulations contained the extract coated granule were in range from 0.56% to 1.16% as shown in Table 12 and Table 22B (Appendix B).

2.2.5 Loss on drying

The loss on drying of chewable tablets was determined by a moisture analyzer. As shown in Table 31B (Appendix B) % loss on drying lied between 1.79-2.31%. The coated extract showed the relatively high loss on drying, about 4.59% because it migh attract moisture from environment during coating process under ambient condition.

2.2.6 pH

The formulations containing citric acid, formulations A1, A3, A5, A7, B1 and B4, had pH value of solution lower than formulations A2, A4, A6, A8 and B2 as shown in Table 36B (Appendix B).

2.2.7 Dissolution of the tablets

The types of binder also significantly affected on *in vitro* dissolution of chewable tablets. The release of total tannins was markedly faster when MCC PH 102 was used as the binder in formulations A1, A2, A3, A4, B1, B2, B3 and B4. This resulted from that these tablets further disintegrated in the dissolution medium, while the chewable tablets with copovidone swelled and did not disintegrate; thus the release of total tannins was retarded. In addition, the total tannins was slowly released from chewable tablets of formulations A7 and A8 containing copovidone and high level of talc due to hydrophobic nature of these materials as shown in Figure 11.

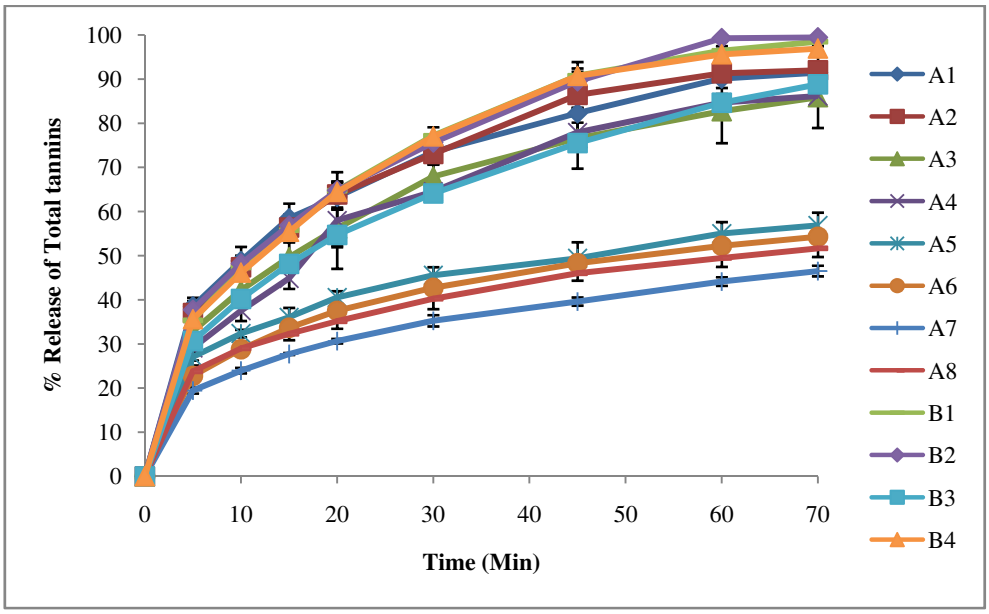


Figure 11 Total tannins release profiles of chewable tablets containing uncoated or coated *Phyllanthus emblica* Linn. fruit extract after preparation.

2.2.8 Amount of total tannins

Total tannins contents were measured by the Folin-Ciocalteau method. Chewable tablets formulations A1, A2, A3, A4, A5, A6, A7 and A8 had total tannins content 22.77%, 22.22%, 22.63%, 22.96%, 21.06%, 21.65%, 20.18% and 20.67%. Moreover, total tannins contents of chewable tablets formulations B1, B2, B3 and B4 were 23.06 %, 23.36 %, 22.67 % and 23.50 %, respectively as presented in Table 41B (Appendix B).

Total tannins contents of chewable tablets were 45.32 mg/tablet to 52.88 mg/tablet, depending on the batch of the extract incorporated into the formulatioins and thus % label amount of total tannins contents were 80.51% to 93.72 % as presented in Table 12.

2.2.9 Amount of gallic acid

The amount of gallic acid was determined by high performance liquid chromatographic method. Chewable tablets formulations A1, A2, A3, A4, A5, A6, A7

and A8 had gallic acid 0.55%, 0.53%, 0.54%, 0.52%,0.50%, 0.49%, 0.64% and 0.64%. Moreover, gallic acid contents in the chewable tablets formulations B1, B2, B3 and B4 were 0.79 %, 0.90 %, 0.76 % and 0.76 %, respectively as presented in Table 41B (Appendix B).

Gallic acid contents in the chewable tablets were 1.12 mg/tablet to 2.02 mg/tablet, depending on the batch of the extract incorporated into the formulatioins and hence % label amount of gallic acid contents were 81.03% to 118.78 % as presented in Table 12.

3. Stability of chewable tablets containing *Phyllanthus emblica* Linn. fruit extract

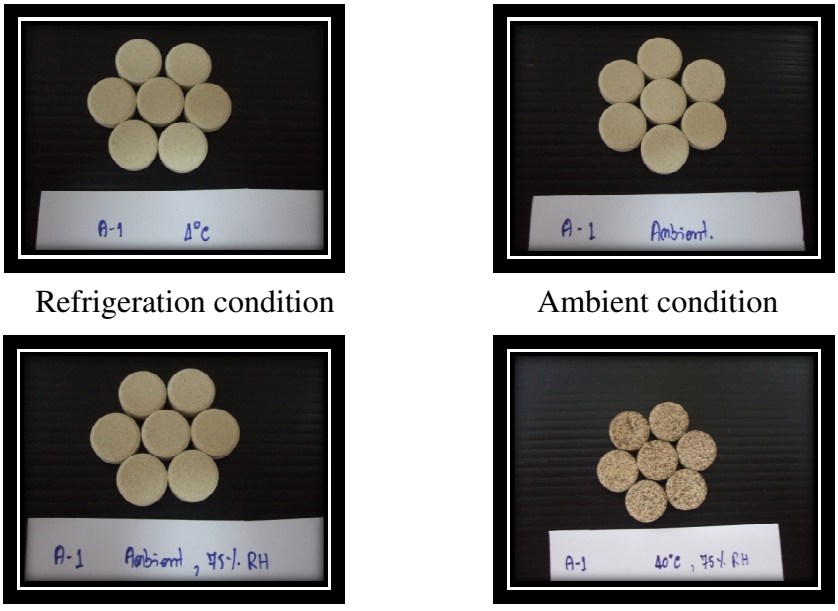
3.1 Physical appearance

After storage for 3 months, the color of chewable tablets under relatively low temperatures did not change significantly, whereas that of chewable tablets under 40°C and 75%RH started to change after the first month, and it appeared to be brownish after 3 months as shown in Figure 12.1 and Figure 12.2. The browning of food results from both enzymatic browning and non-enzymatic browning of phenolic compounds. The enzymatic browning is discoloration resulted when monophonic compounds of plants, in the presence of atmosphere oxygen and polyphenol oxidase (PPO), are hydroxylated to o-diphenols; and the latter are oxidized to o-quinones resulting in pigments and flavor (Li et al., 2008). The PPO is relatively heat labile and the pH influences to it. Heat inactivation of PPO is feasible by applying temperature of over 60°C and the optimum pH for PPO activity is between pH 5-7 (Icier et al., 2008). However the browning of the *Phyllanthus emblica* Linn. fruit extract might be non enzymatic oxidation because the heat was applied in the spray dry process about 180°C and the pH of *Phyllanthus emblica* Linn. fruit extract in batch no. 08122006 is 4.26 and in batch no. 28122006 is 4.61; so the PPO was inactivated in this study.

For the non-enzymatic browning include Maillard reaction (Buedo et al., 2001), ascorbic acid degradation (Kennedy et al., 1992), and the oxidation and condensation of tannins (Rassis and Saguy, 1995). The major reaction responsible for the non-enzymatic browning of *Phyllanthus emblica* Linn. fruit extract might be

oxidation and condensation of tannins because the polyphenolic compounds containing a great number of hydroxyl functional group that is easily oxidized to o-quinone by oxidation (Edwin, 1998). The cause of the mechanism could be oxygen and moisture, describing for chewable tablets under 40°C and 75%RH in which the rate of interaction was faster than those stored at other conditions; and the pigment of the tablets was darkest.

In order to control the browning process, sulfiting agent (Iyengar and McEvilly, 1992), ascorbic acid (Son et al., 2001), PPO inhibitors (Marecik and Czapski, 2007), complexing agent (e.g. EDTA, sodium acid pyrophosphate, citric acid) (Marecik and Czapski, 2007), organic halides (Marecik and Czapski, 2007), exclusion of oxygen (Rassis and Saguy, 1995), have been used. In this study, although certain amounts of citric acid and sodium chloride were added into some formulations, they could not inhibit the browning effect. In addition, ascorbic acid, major compound in *Phyllanthus emblica* Linn. fruit (Khopde et al., 2001), also reported as anti-oxidation could have been destroyed during spray drying of the extract at the high temperature (Nisha et al., 2004).



Refrigeration condition | Ambient condition

Ambient temperature and 75% RH | 40 °C and 75% RH.

Figure 12.1 Appearance of chewable tablets formulation A1 stored under refrigeration, ambient conditions, ambient temperature and 75% RH, and 40 °C and 75% RH.

Refrigeration condition

Ambient condition

Ambient temperature and 75% RH

40 ˚C and 75% RH.

Figure 12.2 Appearance of chewable tablets formulation B1 stored under refrigeration, ambient conditions, ambient temperature and 75% RH, and 40 ˚C and 75% RH.

3.2 weight variation

After storage for 3 months, the average weight and weight variation of all formulations were not changed under refrigeration, ambient condition, ambient temperature and 75%RH and 40˚C and 75%RH as shown in Table 3 to Table 10 (Appendix B).

Table 12 Hardness, friability, %total tannins and %gallic acid of chewable tablets containing uncoated or coated *Phyllanthus emblica* Linn. fruit extract after preparation.

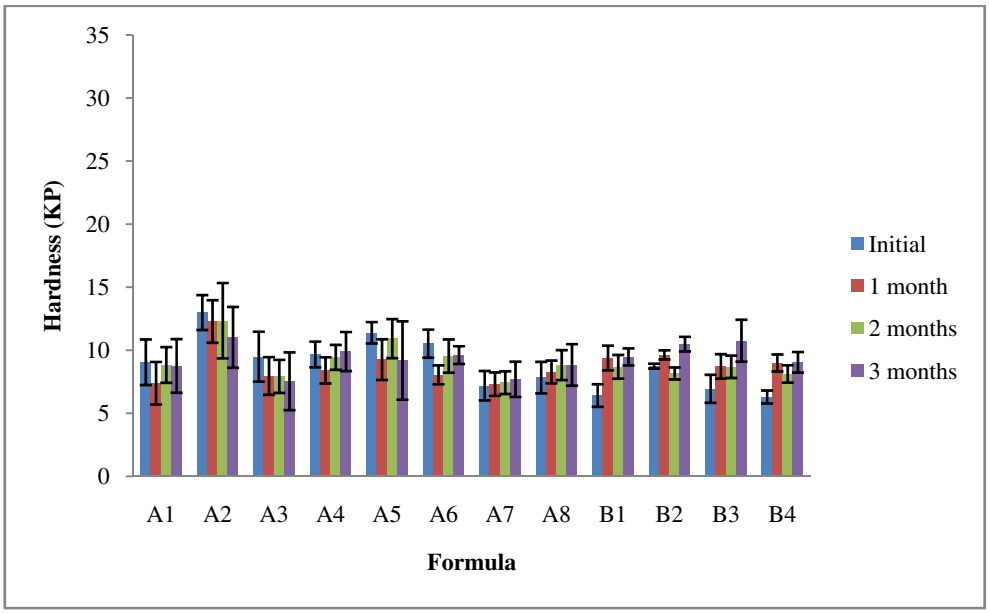
Formulation	Hardness (KP) (mean (SD), n =10)	Friability (%) (mean, n =1)	Total tannins (mg/tablet) (mean (SD), n =3)	Total tannins (%LA[a]) (mean (SD), n =3)	Gallic acid (mg/tablet) (mean (SD), n =3)	Gallic acid (%LA[a]) (mean (SD), n =3)
A1	10.04 (1.81)	0.96	51.71 (0.68)	88.80(2.64)	1.24 (0.01)	89.44 (1.35)
A2	14.61 (1.38)	0.11	50.19 (0.11)	86.66 (0.42)	1.19 (0.02)	86.37 (2.72)
A3	10.24 (1.98)	0.65	50.94 (0.21)	88.25 (0.83)	1.21 (0.01)	88.23 (1.27)
A4	10.43 (1.02)	0.48	51.41 (0.04)	89.57 (0.15)	1.18 (0.02)	86.05 (3.36)
A5	13.03 (0.85)	0.44	47.03 (0.86)	82.12 (3.34)	1.12 (0.02)	82.54 (3.68)
A6	11.72 (1.11)	0.69	49.01 (1.05)	84.42 (4.11)	1.12 (0.01)	81.03 (1.93)
A7	8.46 (1.17)	1.14	45.32 (0.48)	80.51 (1.90)	1.43 (0.01)	83.70 (1.82)
A8	8.51 (1.25)	0.84	46.36 (0.71)	82.43 (2.85)	1.45 (0.01)	84.77 (1.12)
B1	6.41 (0.89)	1.08	49.63 (0.52)	92.00 (2.06)	1.78 (0.02)	103.60 (2.46)
B2	8.75 (0.19)	0.56	52.56 (0.24)	93.18 (0.96)	2.02 (0.06)	118.78 (8.21)
B3	6.95 (1.11)	0.68	51.01 (0.52)	90.44 (2.09)	1.71 (0.00)	100.53 (0.49)
B4	6.30 (0.51)	1.16	52.88 (0.71)	93.72 (2.82)	1.71 (0.00)	99.78 (0.30)

[a] %LA was calculated based on the loading amounts of total tannins and gallic acid in the chewable tablet.

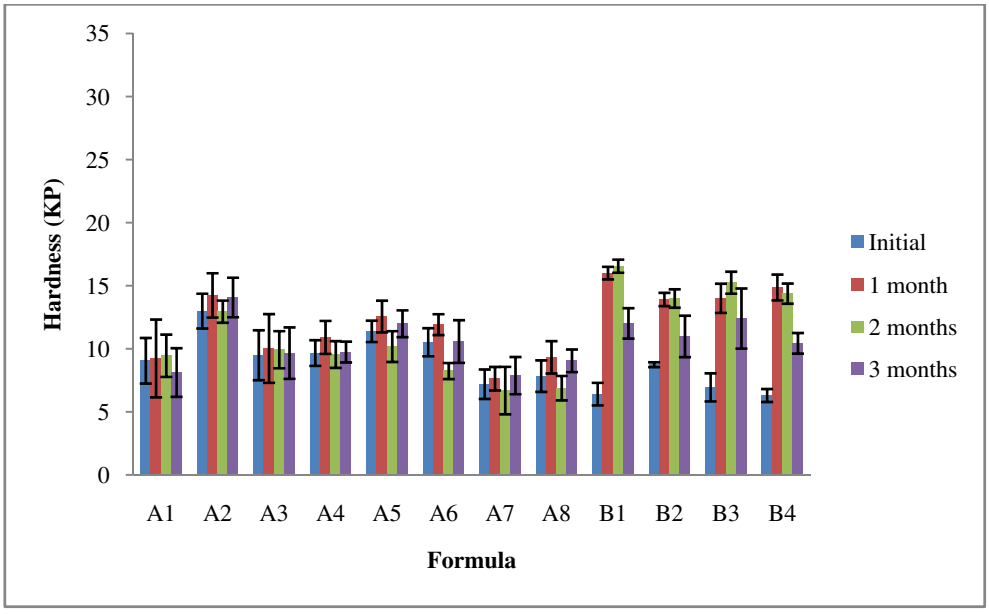
3.3 Hardness

After storage for 3 months, the hardness of these chewable tablets containing *Phyllanthus emblica* Linn. spray dried fruit extract was considerably increased, in particular, those prepared with MCC PH102, where their hardness was increased up to 14.61KN. However, the results of hardness were confounded with incorporated lubricant and the addition of sodium chloride and citric acid. Less hardness of formulations A7 and A8 in which talc was incorporated up to 30% was observed. In addition, increased hardness was clearly dependent on storage temperature. When chewable tablets were stored at relatively low temperatures, refrigeration and ambient temperatures, the formulation A6 containing 10% copovidone and 10% talc, the hardness of the tablets decreased significantly ($p<0.05$), whereas at the high temperature, 40°C and 75%RH, the hardness of tablets in all of the formulations increased significantly ($p<0.05$) after 1 months and remained relatively constant after 2 and 3 months as shown in Figure 13, Figure 14 and Table 13B to Table 20B (Appendix B).

However, the hardness of these chewable tablets containing the coated extract granule, formulations B1, B2, B3 and B4, was increased significantly ($p<0.05$) under all storage conditions; except for that at the high temperature, 40°C and 75%RH, the hardness of the formulations B1 and B4 were increased significantly ($p<0.05$) after 1 month but after 2 months and 3 months the hardness decreased significantly ($p<0.05$) as shown in Figure 14(B) and Table 14.

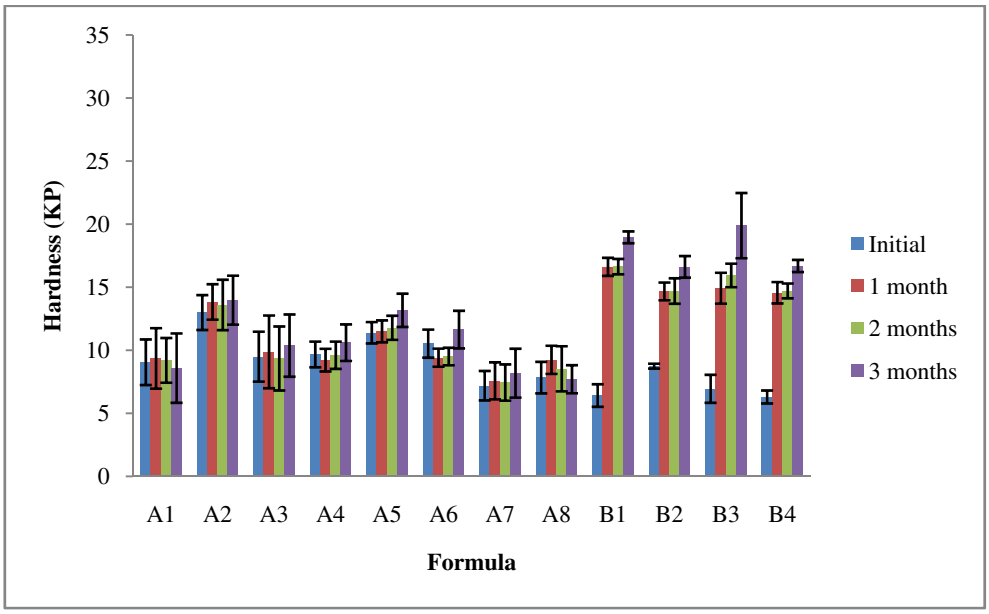


(A)

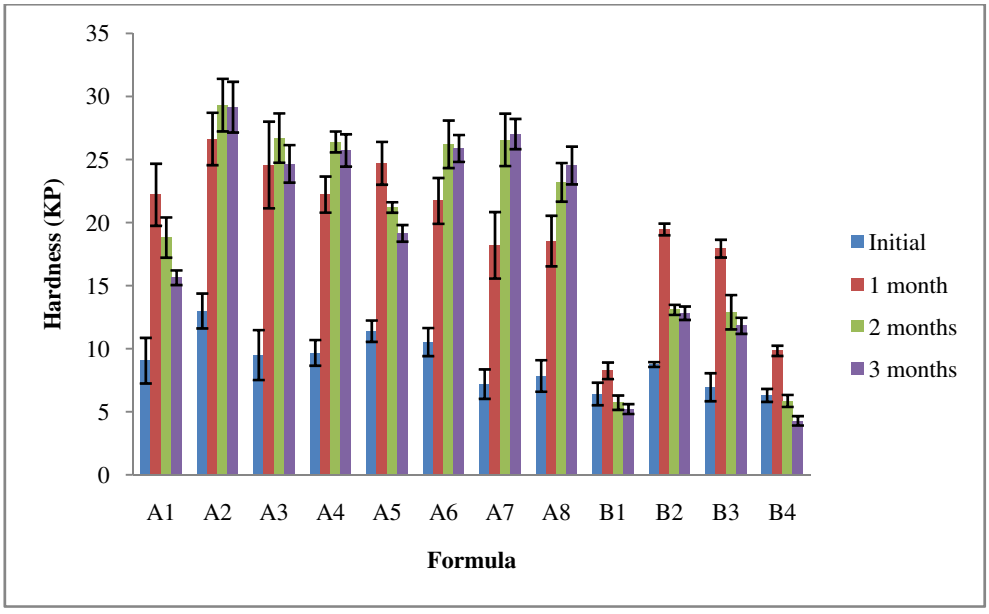


(B)

Figure 13 Hardness of chewable tablets containing *Phyllanthus emblica* Linn. fruit extract stored under refrigeration for 3 months (A); and under ambient condition for 3 months (B).



(A)



(B)

Figure 14 Hardness of chewable tablets containing *Phyllanthus emblica* Linn. fruit extract stored under ambient temperature and 75%RH for 3 months (A); and under 40°C and 75%RH for 3 months (B).

Table 13 The statistical analysis of hardness of chewable tablets after stability study stored under refrigeration, ambient conditions, ambient temperature and 75% RH, and 40 ˚C and 75% RH.

Property	Condition	Post Hoc Test	A1	A2	A3	A4	A5	A6	A7	A8	B1	B2	B3	B4
Hardness	Refrigeration	Initial-1month	D	-	-	D	D	D	-	-	I	I	I	I
		Initial-2months	-	-	-	-		D	-	-	I	I	I	I
		Initial-3months	-	D	D	-	D	D	-	-	I	I	I	I
	Ambient	Initial-1month	-	-	-	-	-	-	-	-	I	I	I	I
		Initial-2months	-	-	-	-	D	D		D	I	I	I	I
		Initial-3months	-	-	-	-	-	-	-	-	I	I	I	I
	Ambient and 75% RH	Initial-1month	-	-	-	-	D	D	-	-	I	I	I	I
		Initial-2months	-	-	-	-	-	D	-	-	I	I	I	I
		Initial-3months	-	-	-	-	-	-	-	-	I	I	I	I
	40 ˚C and 75% RH	Initial-1month	I	I	I	I	I	I	I	I	I	I	I	I
		Initial-2months	I	I	I	I	I	I	I	I	D	I	I	D
		Initial-3months	I	I	I	I	I	I	I	I	D	I	I	D

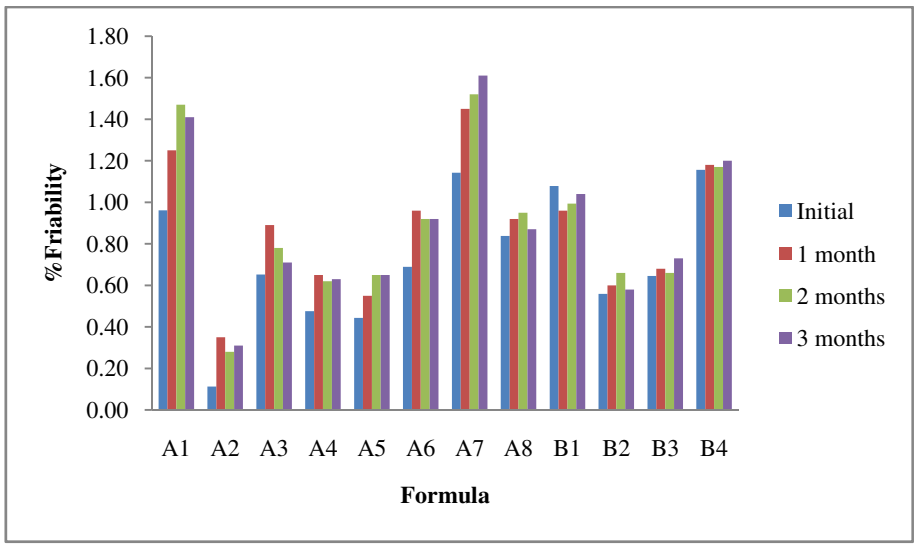
D = The hardness of chewable tablets decreased significant difference at a significant level ($p < 0.05$)

I = The hardness of chewable tablets increased significant difference at a significant level ($p < 0.05$)

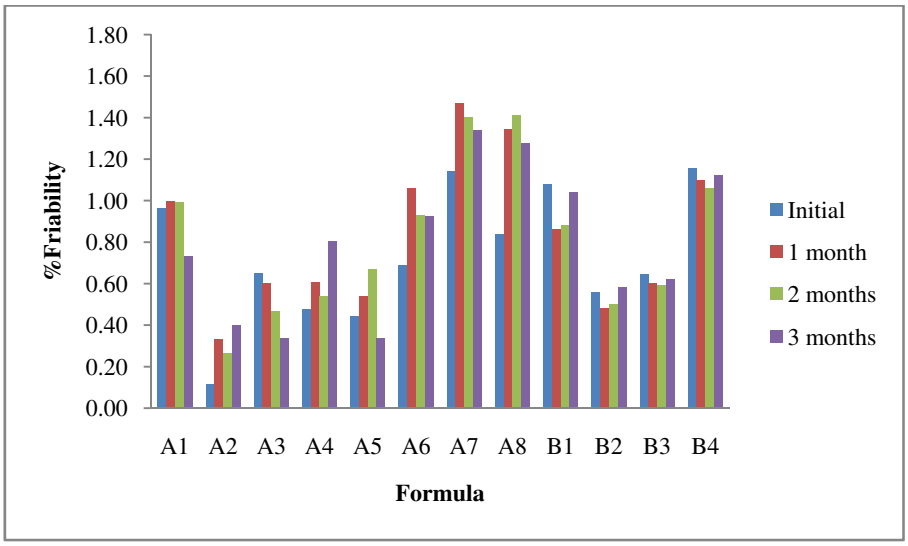
- = The hardness of chewable tablets showed no significant difference at a significant level ($p < 0.05$)

3.4 Friability

After storage for 3 months, the friability was rather low, reflecting in hardness of the tablets as shown in Figure 15, Figure 16 and Table 23B to Table 30B (Appendix B).

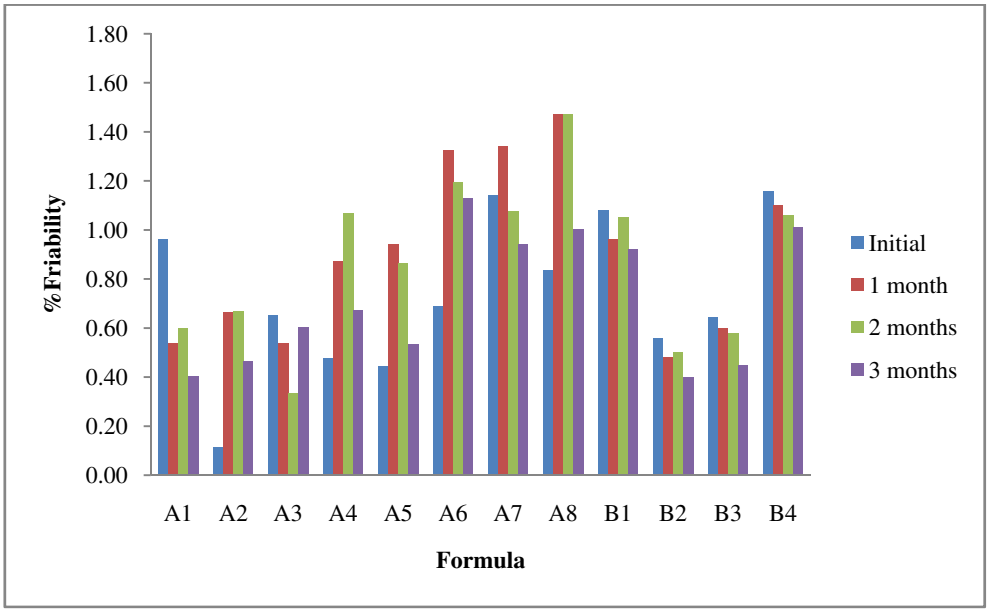


(A)

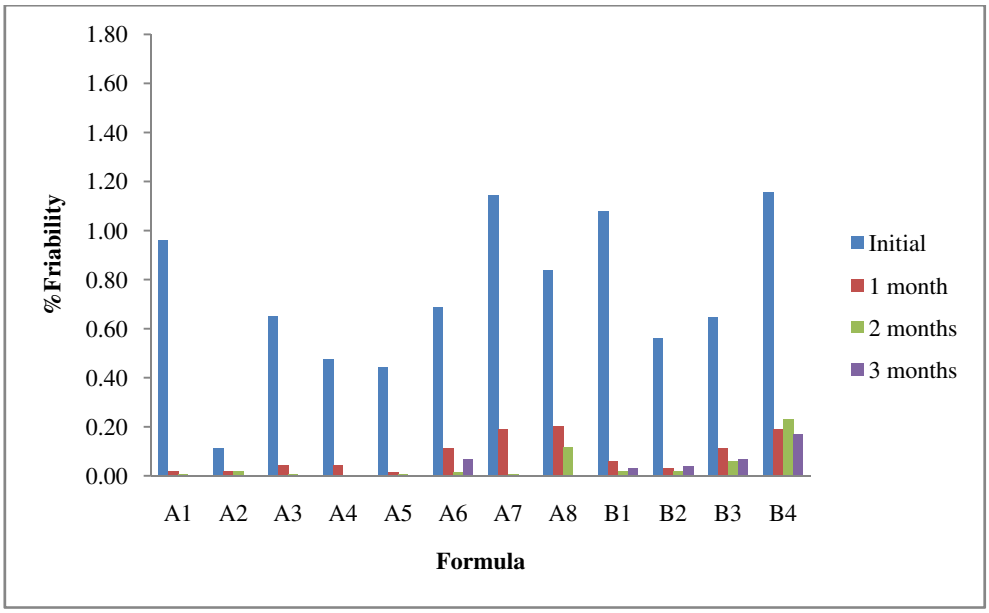


(B)

Figure 15 Friability of chewable tablets containing *Phyllanthus emblica* Linn. fruit extract stored under refrigeration for 3 months (A); and under ambient condition for 3 months (B).



(A)

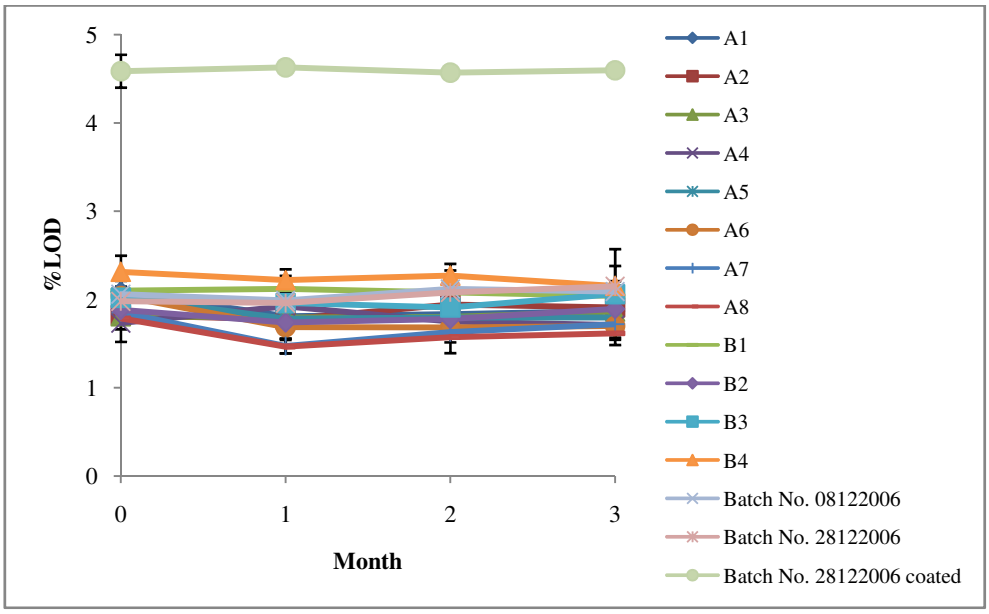


(B)

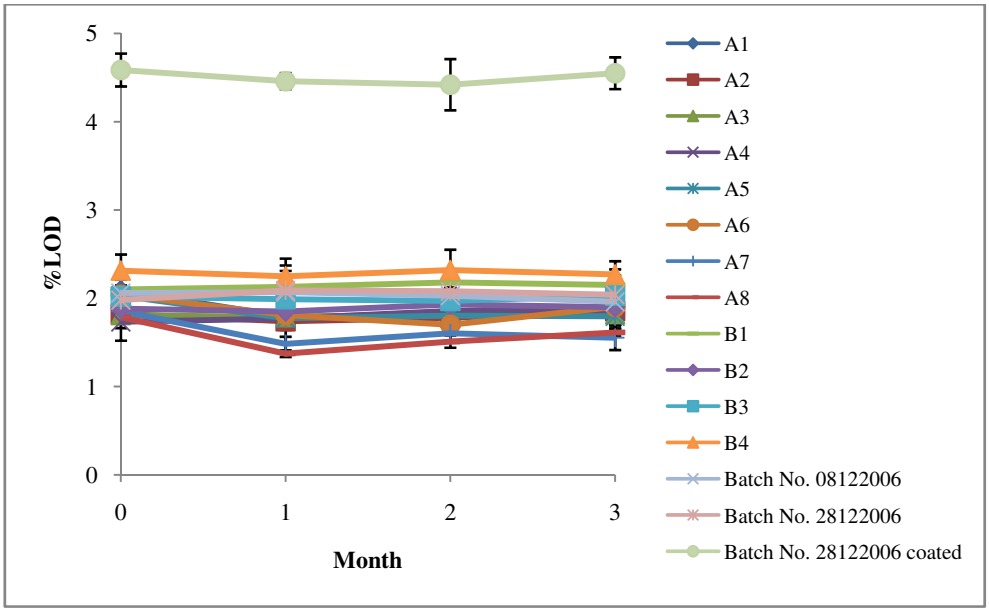
Figure 16 Friability of chewable tablets containing *Phyllanthus emblica* Linn. fruit extract stored under ambient temperature and 75%RH for 3 months (A); and under 40°C and 75%RH for 3 months (B).

3.5 Loss on drying

After storage for 3 months, all of the storage condition, the loss on drying was not change from after preparation as shown in Figure 17, Figure 18 and Table 32B to Table 35B (Appendix B).

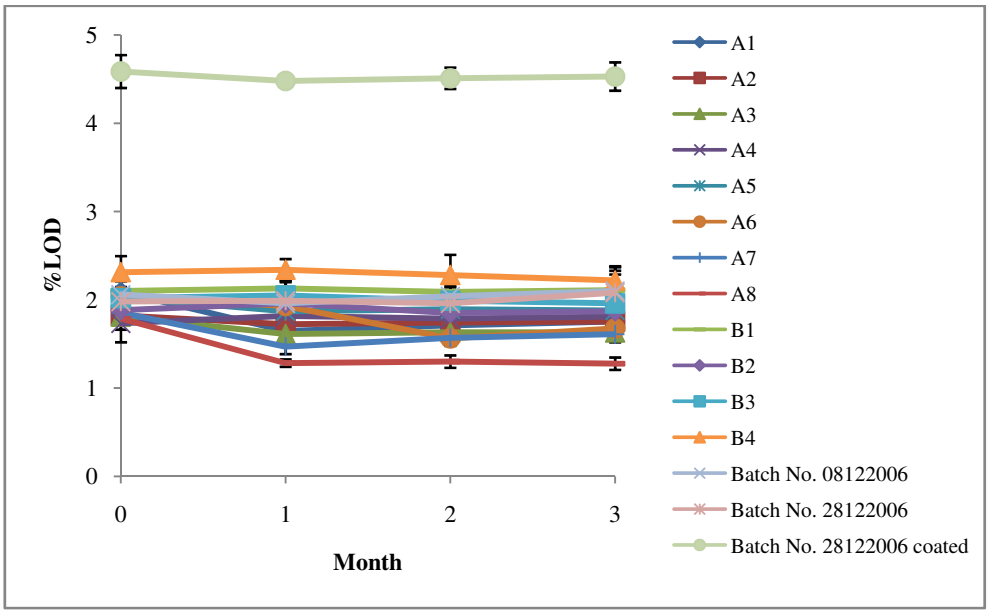


(A)

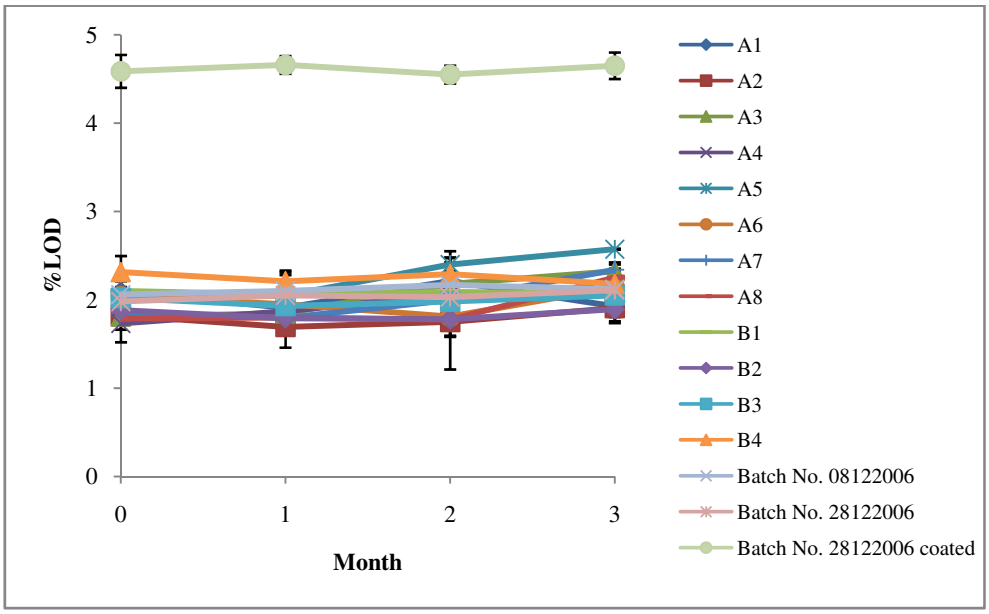


(B)

Figure 17 Loss on drying of *Phyllanthus emblica* Linn. fruit extract and chewable tablets containing *Phyllanthus emblica* Linn. fruit extract stored under refrigeration for 3 months (A); and under ambient condition for 3 months (B).



(A)

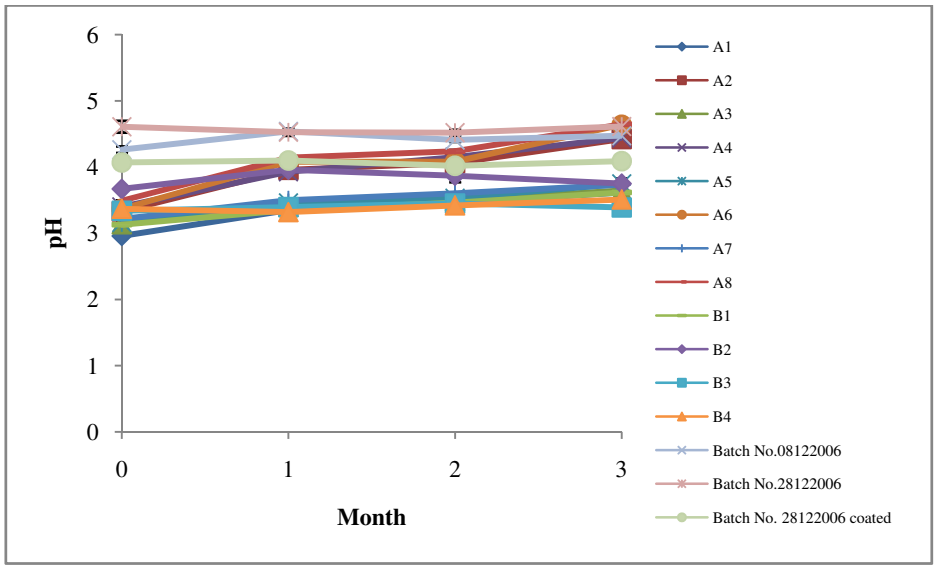


(B)

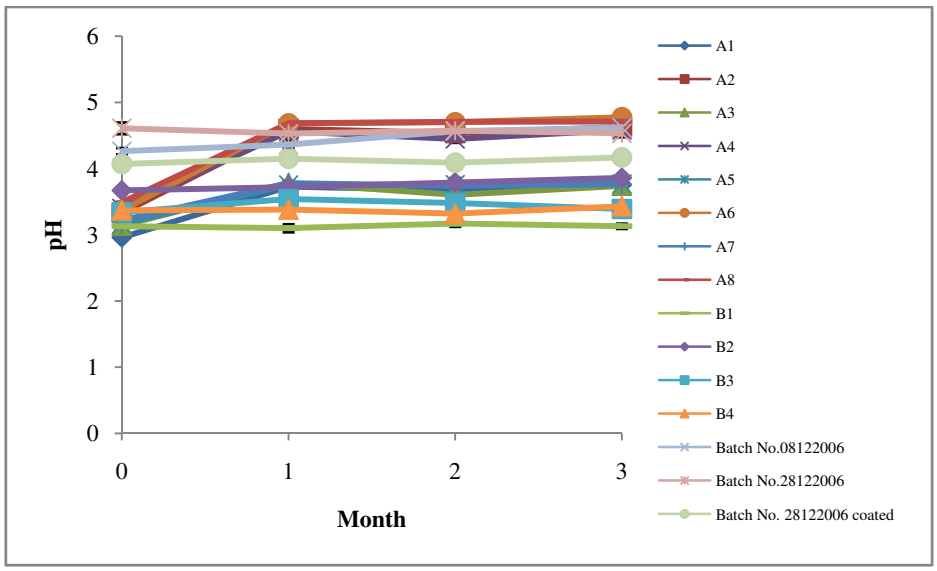
Figure 18 Loss on drying of *Phyllanthus emblica* Linn. fruit extract and chewable tablets containing *Phyllanthus emblica* Linn. fruit extract stored under ambient temperature and 75%RH for 3 months (A); and under 40°C and 75%RH for 3 months (B).

3.6 pH

After storage for 3 months, in all storage conditions, pH of the solutions of chewable tablets was not changed as shown in Figure 19, Figure 20 and Table 37B to Table 40B (Appendix B).

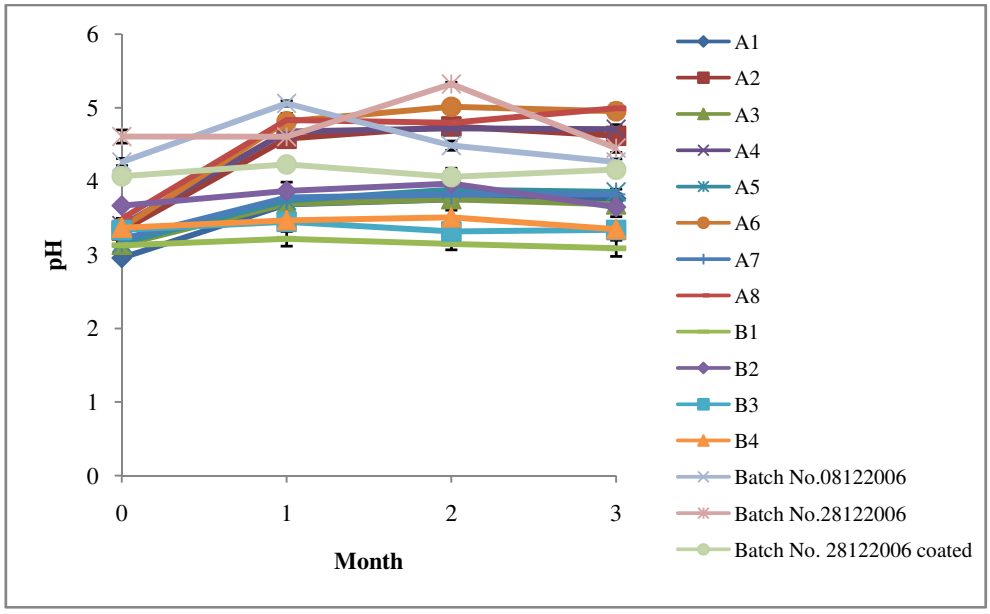


(A)

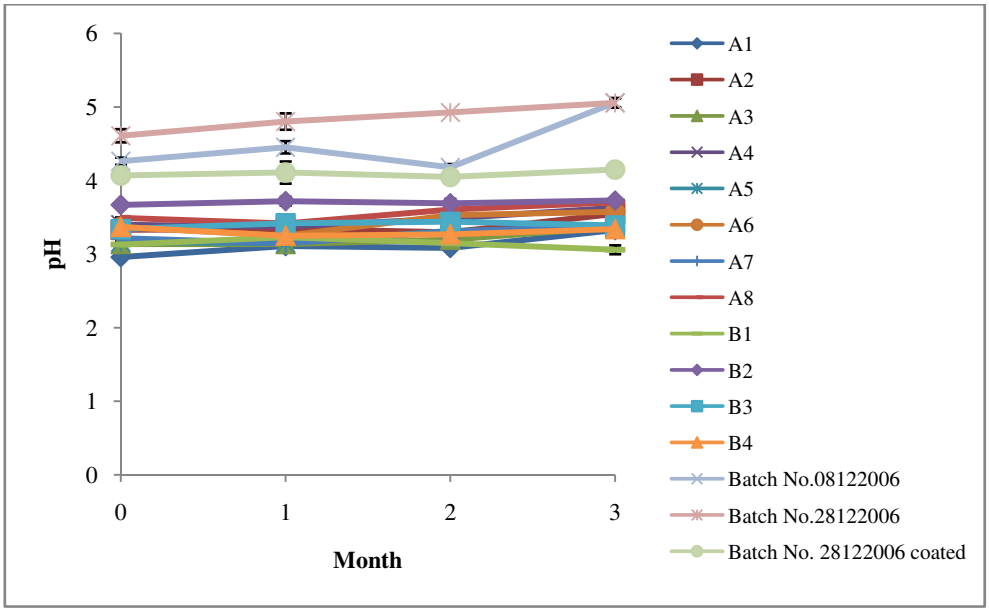


(B)

Figure 19 pH of solutions of *Phyllanthus emblica* Linn. fruit extract and chewable tablets containing *Phyllanthus emblica* Linn. fruit extract stored under refrigeration for 3 months (A); and under ambient condition for 3 months (B).



(A)

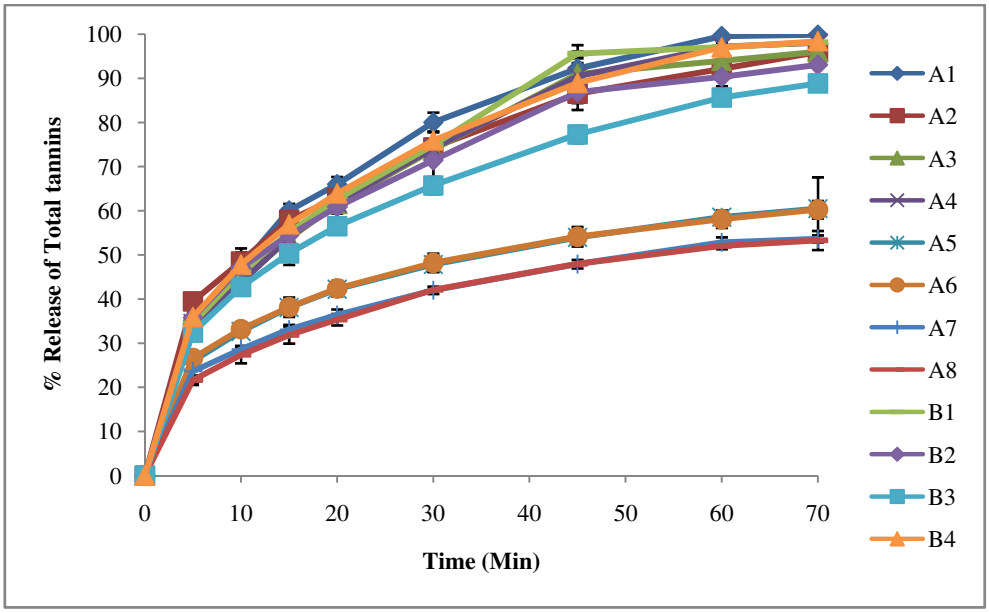


(B)

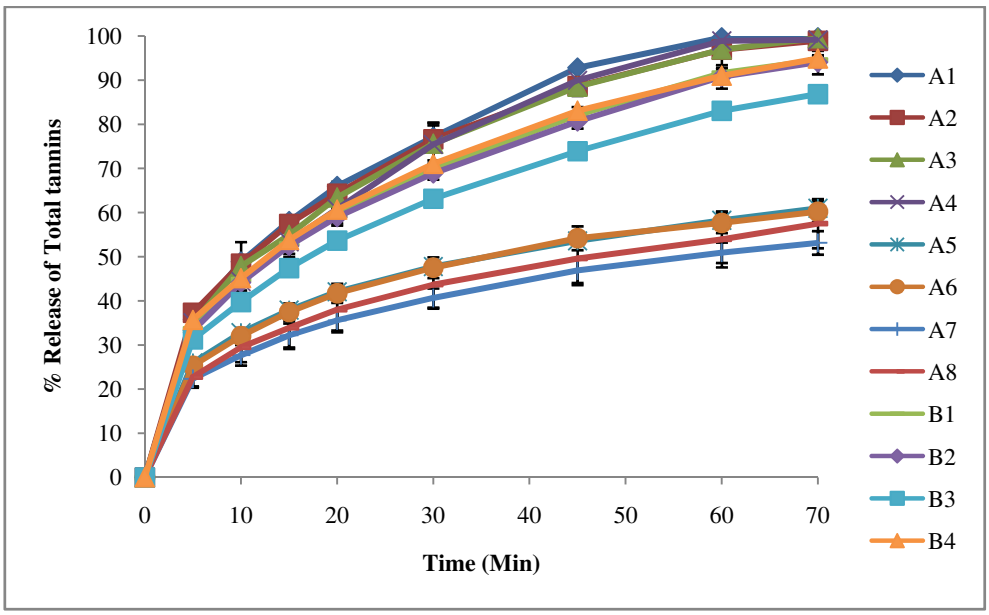
Figure 20 pH of solutions of *Phyllanthus emblica* Linn. fruit extract and chewable tablets containing *Phyllanthus emblica* Linn. fruit extract stored under ambient temperature and 75%RH for 3 months (A); and under 40°C and 75%RH for 3 months (B).

3.7 Dissolution study

After storage for 3 months, the types of binder were still markedly affected on *in vitro* dissolution of chewable tablets same as after preparation as shown in Figure 21 and Figure 22.

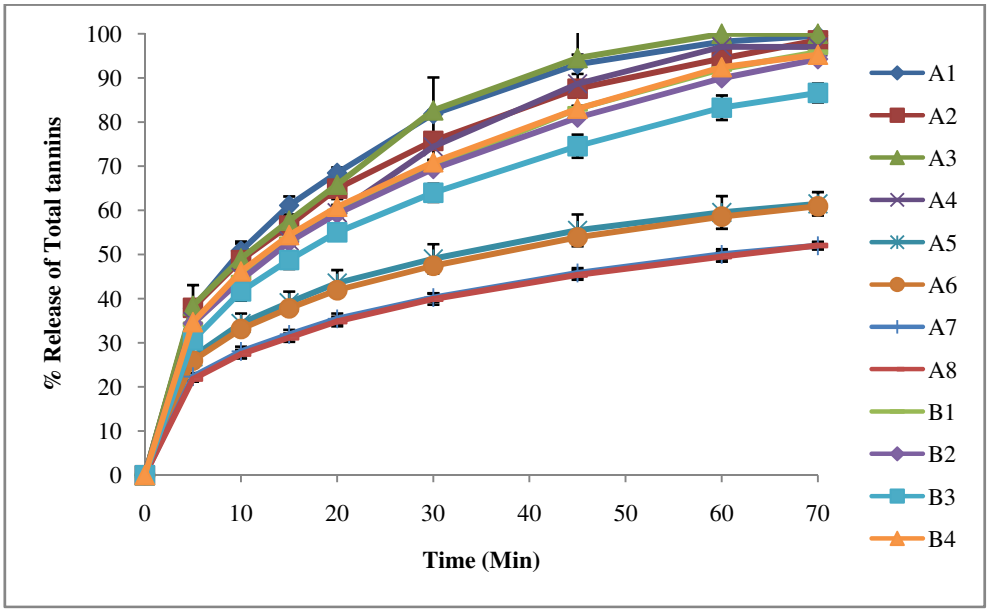


(A)

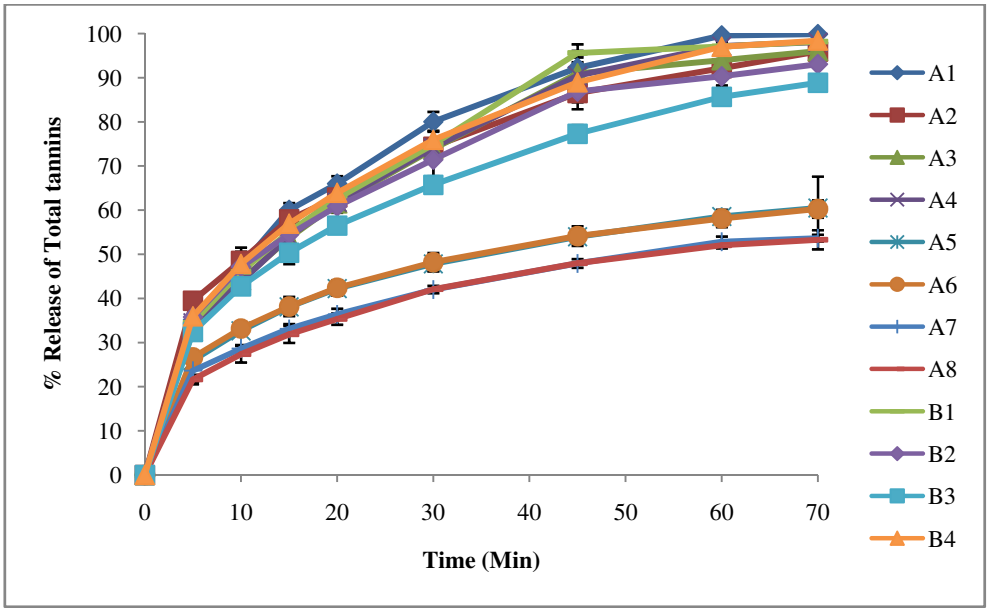


(B)

Figure 21 Total tannins release profiles of chewable tablets containing *Phyllanthus emblica* Linn. fruit extract stored under refrigeration for 3 months (A); and under ambient condition for 3 months (B).



(A)



(B)

Figure 22 Total tannins release profiles of chewable tablets containing *Phyllanthus emblica* Linn. fruit extract stored under ambient temperature and 75%RH for 3 months (A); and under 40°C and 75%RH for 3 months (B).

3.8 Amount of total tannins contents

After storage for 3 months, the amount of total tannins contents of chewable tablets containing *Phyllanthus emblica* Linn. spray dried fruit extract, the main compounds in the extract, were monitored through the period of 3 months. Initially, the total tannins in the chewable tablets were within the range of 45.32 – 52.88 mg/tablet, as presented in Table 41B (Appendix B), and with relative standard deviation was less than 2.0%. Variation in the amount of total tannins in the chewable tablets was dependent on storage temperature. However, only at ambient temperatures, the total tannins content in the chewable tablets formulations A2, A7 and A8 were increased significantly (p<0.05), but in others formulations the content was insignificantly changed as shown in Figure 22(B), Figure 23(A), Table 43B and Table 44B (Appendix B). In addition, those in the chewable tablets formulations A3, A4 and A8 stored under refrigeration and in all formulations stored under 40°C and 75%RH were decreased significantly (p<0.05) as shown in Figure 22(A), Figure 23(B), Table 42B and Table 45B (Appendix B). The amount of total tannins contents of chewable tablets containing coated *Phyllanthus emblica* Linn. spray dried fruit extract decreased significantly (p<0.05) when stored in any condition as shown in Table 14.

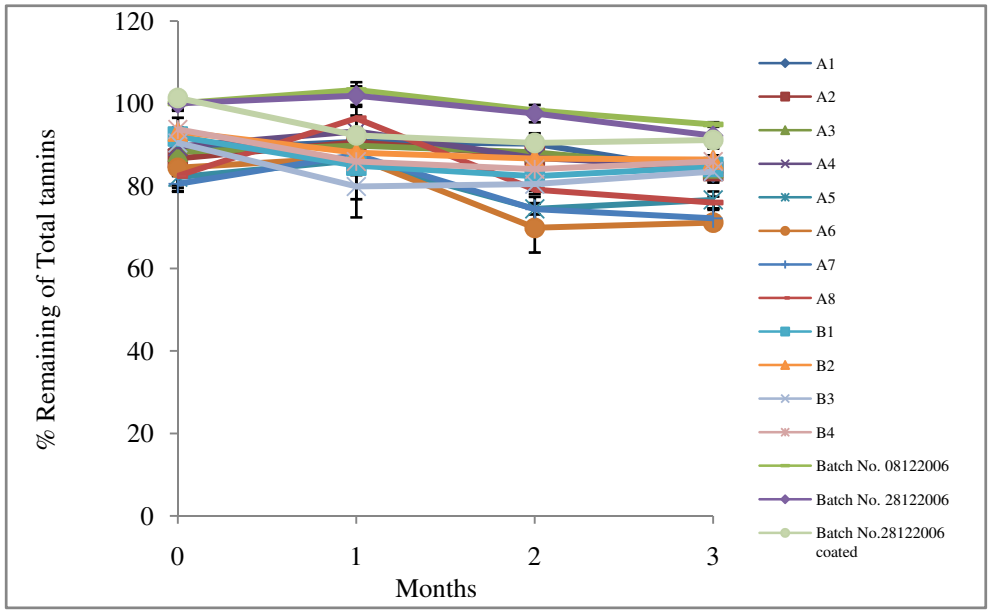
In the previous study, with inactivated PPO, Thitima Kuljarachanan et al. (2009). investigated that the amounts of vitamin C and phenolic compound decreased when the temperature was increased during drying lime residues and Andrea Bunea et al. (2008) found that the amount of total phenolic compounds decreased after storage of spinach (*Spinacia oleracea* Linn.) at low temperature (4°C or -18°C). However, decrease in total tannins contents stored under refrigeration in this study could not be directly related to the stable appearance and hardness as above mentioned.

It was found that the binder types may contribute to some effects on the remaining amount of total tannins. With copovidone, the remaining amount of total tannins in formulations A5, A6, A7 and A8 were significantly lower than that in formulations A1, A2, A3 and A4 containing MCC PH 102 as the binder (p<0.05), when the tablets were stored under refrigeration for 3 months. This is confirmed by the remaining amount of total tannins in formulation A5 which was significantly

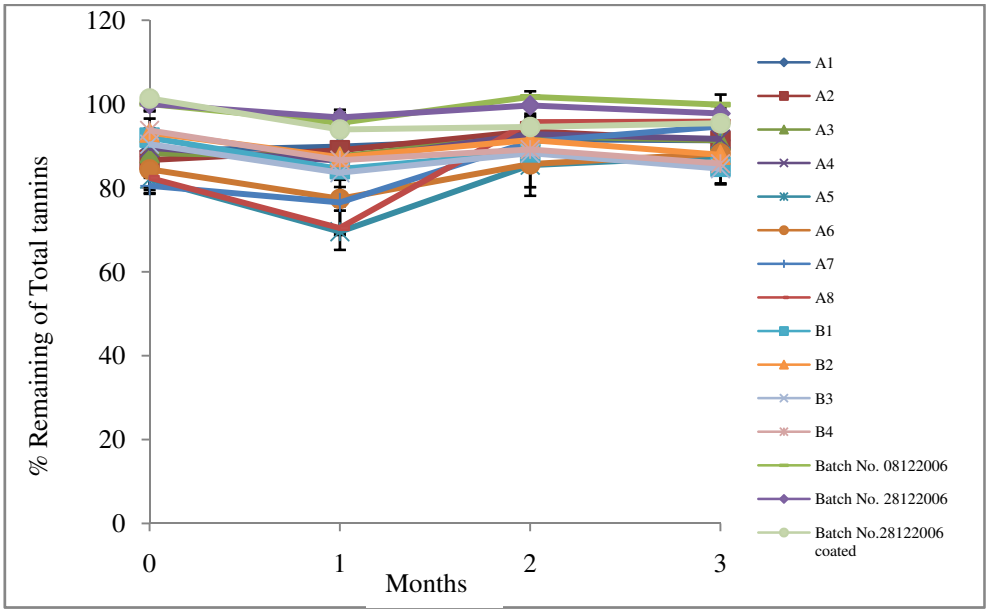
lower than that in formulation A1 when stored under 40°C and 75%RH (p<0.05). In a previous study, Gustavson (1956) established that both soluble and insoluble polyvinyl pyrrolidone form stable insoluble complexes with tannins. The polymer is a strong H-acceptor, which has ketone functional group in structure, can bind with hydroxyl functional groups of tannins by strong H-bonding characteristics which make polyvinyl pyrrolidone effective in adsorbing phenols. Moreover, the chain of polyvinyl pyrrolidone can entrap tannins in the structure (Loomis and Battaile, 1966). In contrast to microcrystalline cellulose, the planar structure containing hydroxyl functional groups, can bind with hydroxyl functional groups of tannins by weak H-bonding characteristics to form complex, so the tannins of tannins-cellulose complex can release easier than the tannins of tannins- polyvinyl pyrrolidone complex (Loomis and Battaile, 1966). In addition, copovidone (Moroni, 2001 and Heng et al., 2004), citric acid and sodium chloride are hygroscopic materials that can absorb moisture which then may induce hydrolysis of tannins that contained many hydroxyl functional group and ester bond in structure. The incorporation of the high amount of hydrophobic material i.e. talc can reduce absorption of moisture to tablets (Uhumwangho et al., 2007and Goss et al., 2003). Thus, hydrolysis degradation of total tannins occurred less in these formulations (A7 and A8).

However, for all of the storage conditions in this study, the amount of total tannins contents of chewable tablets containing coated *Phyllanthus emblica* Linn. spray dried fruit extract, decreased significantly (p<0.05) as shown in Table 15 because interaction between tannins and cellulose as the above mentioned.

The stability study of *Phyllanthus emblica* Linn. spray dried fruit extract was found under ambient temperature the total tannins contents were insignificantly changed but under refrigeration and 40°C the total tannins contents were significantly decreased (p<0.05). In contrast, for the coated extract was stored under any storage conditions in this study the total tannins content was significantly decreased (p<0.05) as shown in Figure 23, Figure 24, Table 42B, Table 43B, Table 44B and Table 45B (Appendix B). These results were same as the tablets that at high and low temperature total tannins contents were decreased.

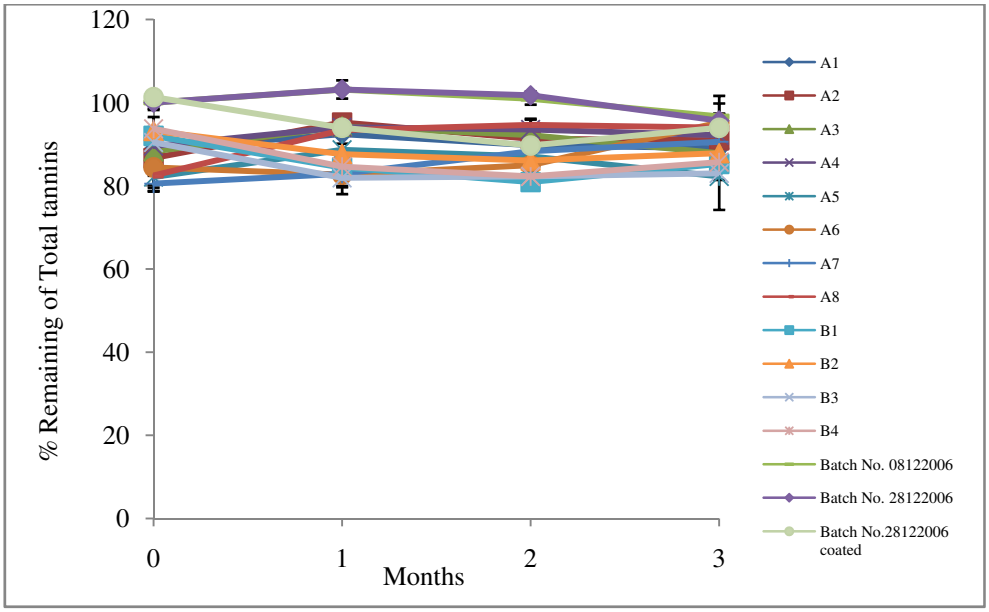


(A)

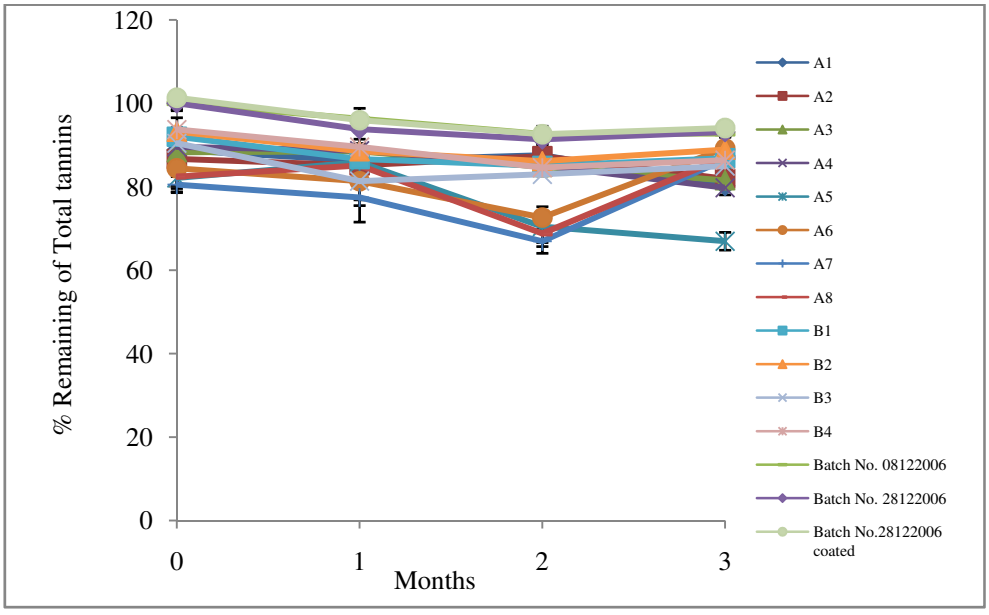


(B)

Figure 23 % Remaining of total tannins in *Phyllanthus emblica* Linn. fruit extract and chewable tablets containing *Phyllanthus emblica* Linn. fruit extract stored under refrigeration for 3 months (A); and under ambient condition for 3 months (B).



(A)



(B)

Figure 24 % Remaining of total tannins in *Phyllanthus emblica* Linn. fruit extract and chewable tablets containing *Phyllanthus emblica* Linn. fruit extract stored under ambient temperature and 75%RH for 3 months (A); and under 40°C and 75%RH for 3 months (B).

Table 14 The statistical analysis of total tannins content of chewable tablets after stability study stored under refrigeration, ambient conditions, ambient temperature and 75% RH, and 40 ˚C and 75% RH.

Property	Condition	Post Hoc Test	A1	A2	A3	A4	A5	A6	A7	A8	B1	B2	B3	B4	Batch No 08122006	Batch No 28122006	coated Batch No 28122006
Total tannins content	Refrigeration	Initial-1month	-	I	-	-	-	-	-	I	D	D	-	D	-	-	D
		Initial-2months	-	-	-	-	-	-	-		D	D	-	D	-	-	D
		Initial-3months	-	-	D	D	-	-	-	D	D	D	-	D	D	D	D
	Ambient	Initial-1month	-	-	-	-	-	-	-	D	D	D	D	D	-	-	D
		Initial-2months	-	I	I	-	-	-	-	I	-	-	-	-	-	-	D
		Initial-3months	-	-	-	-	-	-	I	I	D	D	D	D	-	-	D
	Ambient and 75% RH	Initial-1month	-	I	I	-	-	-	-	I	D	D	D	D	-	-	D
		Initial-2months	-	I	-	-	-	-	I	I	D	D	D	D	-	-	D
		Initial-3months	-	I	-	-	-	-	I	I	D	D	D	D	-	-	D
	40 ˚C and 75% RH	Initial-1month	-	-	-	-	-	-	-	-	D	D	D	-	D	D	D
		Initial-2months	-	-	D	D	D	D	D	D	D	D	-	D	D	D	D
		Initial-3months	D	D	D	D	D	-	-	-	D	D	-	-	D	D	D

D = The total tannins content of chewable tablets decreased significant difference at a significant level ($p < 0.05$)

I = The total tannins content of chewable tablets increased significant difference at a significant level ($p < 0.05$)

- = The total tannins content of chewable tablets showed no significant difference at a significant level ($p < 0.05$)

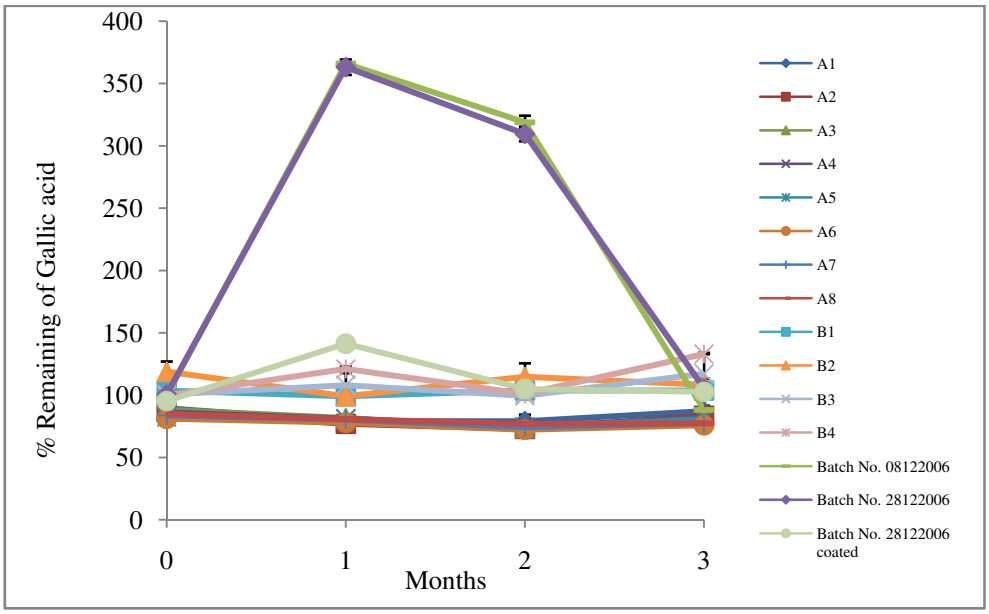
3.9 Amount of gallic acid contents

Variation in the amounts of gallic acid in the chewable tablets containing *Phyllanthus emblica* Linn. spray dried fruit extract was also dependent on storage temperature. In chewable tablets stored at low temperature, i.e. under refrigeration and ambient temperature, the gallic acid content decreased significantly ($p<0.05$) except in formulation A4 but in formulation A5 it increased significantly ($p<0.05$) at 3 months as shown in Figure 25, Table 15 and Table 42B (Appendix B). Moreover, those in the chewable tablets stored at 40°C were rather increased ($p<0.05$). In contrast, the formulations A7 and A8 containing 30% talc, after storage at 40°C, the gallic acid contents were decreased significantly ($p<0.05$) as shown in Table 15.

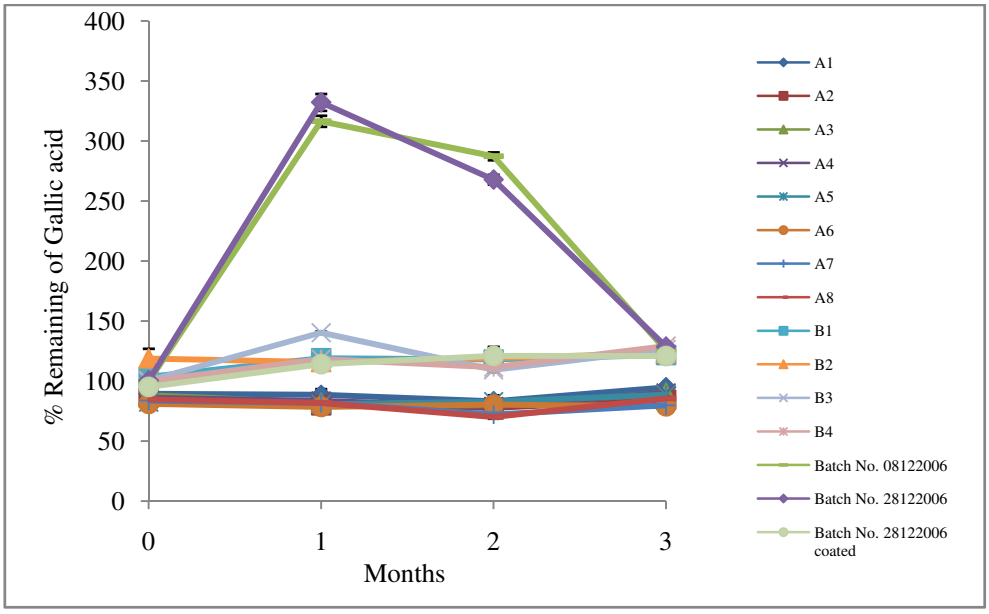
The gallic acid contents of chewable tablets containing the extract coated granule, under ambient condition and ambient temperature and 75%RH, formulation B3 and formulation B4, increased significantly ($p<0.05$) as shown in Figure 25(B), Figure 26(A), Table 16 and Table 44B (Appendix B). However, under refrigeration, only formulation A4, the gallic acid contents in tablets increased significantly ($p<0.05$). Moreover, the gallic acid contents of formulation B1, increased significantly ($p<0.05$) at ambient and formulation B2, increased significantly ($p<0.05$) under ambient temperature and 75%RH. In addition, under 40°C and 75%RH, all of the formulation, gallic acid contents increased significantly ($p<0.05$) as shown in Figure 26(B), Table 15 and Table 45B (Appendix B).

The gallic acid contents of the extract and the extract coated granule were increased significantly ($p<0.05$) when storage all of conditions as shown in Table 15.

The results correlated with the previous study by Ghosal et al. described that the degradation of low molecular hydrolysable tannin groups produces gallic acid and emblicanin A by hydrolysis process. According to their results, there was possibility that at the high temperature, 40°C, the formulation A5 containing copovidone, magnesium stearate, citric acid and sodium chloride had the highest amounts of gallic acid content because at high temperature tannins would degraded to gallic acid.

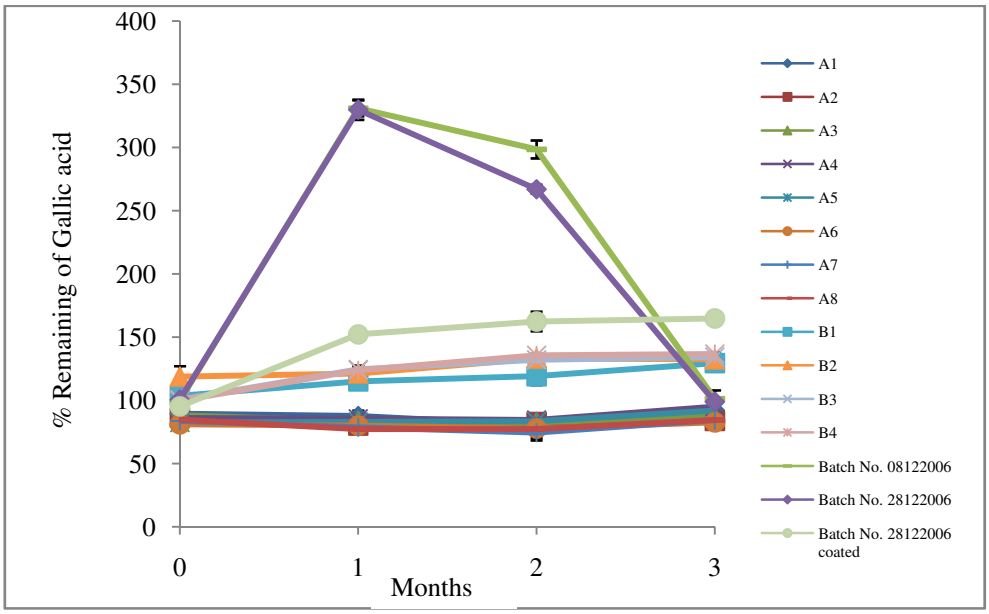


(A)

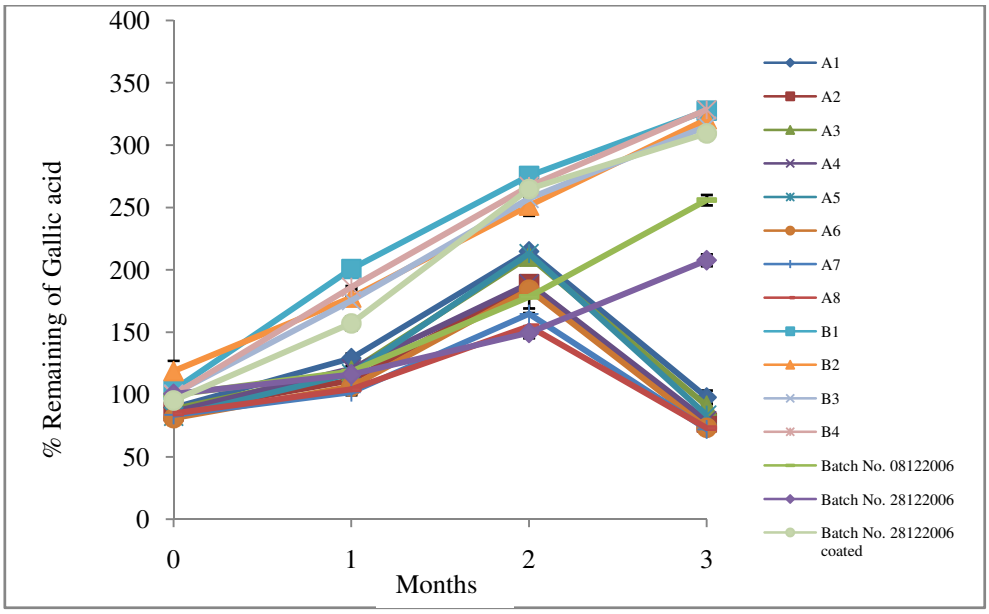


(B)

Figure 25 %Remaining gallic acid in *Phyllanthus emblica* Linn. fruit extract and chewable tablets containing *Phyllanthus emblica* Linn. fruit extract stored under refrigeration for 3 months (A); and under ambient condition for 3 months (B).



(A)



(B)

Figure 26 %Remaining gallic acid in *Phyllanthus emblica* Linn. fruit extract and chewable tablets containing *Phyllanthus emblica* Linn. fruit extract stored under ambient temperature and 75%RH for 3 months (A); and under 40°C and 75%RH for 3 months (B).

Table 15 The statistical analysis of gallic acid content of chewable tablets stored under refrigeration, ambient conditions, ambient temperature and 75% RH, and 40 ˚C and 75% RH.

Property	Condition	Post Hoc Test	A1	A2	A3	A4	A5	A6	A7	A8	B1	B2	B3	B4	Batch No 08122006	Batch No 28122006	coated Batch No 28122006
Gallic acid content	Refrigeration	Initial-1month	D	D	D	D	-	-	-	D	-	D	-	I	I	I	I
		Initial-2months	-	D	D	D	D	D	D	D	-	-	-	-	I	I	-
		Initial-3months	-	-	D	-	-	D	D	D	-	-	-	I	-	-	-
	Ambient	Initial-1month	-	-	D	-	-	-	-	D	I	-	I	I	I	I	I
		Initial-2months	-	-	D	D	-	-	D	D	I	-	I	I	I	I	I
		Initial-3months	-	-	-	-	-	-	-	-	I	-	I	I	-	-	I
	Ambient and 75% RH	Initial-1month	-	-	D	-	-	-	-	-	-	-	I	I	I	I	I
		Initial-2months	-	-	D	-	-	-	-	-	-	I	I	I	I	I	I
		Initial-3months	-	-	-	I	I	-	-	-	-	I	I	I	-	-	I
	40 ˚C and 75% RH	Initial-1month	I	I	I	I	I	I	I	I	I	I	I	I	-	-	I
		Initial-2months	I	I	I	I	I	I	I	I	I	I	I	I	I	-	I
		Initial-3months	-	-	-	-	-	-	D	D	I	I	I	I	I	I	I

D = The gallic acid content of chewable tablets decreased significant difference at a significant level ($p < 0.05$)

I = The gallic acid content of chewable tablets increased significant difference at a significant level ($p < 0.05$)

- = The gallic acid content of chewable tablets showed no significant difference at a significant level ($p < 0.05$)

4. Microbial limit of chewable tablets containing *Phyllathus emblica* Linn. spray dried fruit extract

The chewable tablets formulations A1, A2, B1 and B4, which were selected to evaluate consumer acceptance simulation testings, were determined microbial contamination for quality control of product to volunteers. The total aerobic microbial count of formulations A1, A2, B1 and B4 were $5.3x10^3$ CFU/g, $5.7x10^3$ CFU/g, $2.0x10^3$ CFU/g and $3.8x10^3$ CFU/g, respectively as shown in Table 46 (Appendix B). When comparative the aerobic microbial count of chewable tablets and the total aerobic microbial count of emblica extract, the results shown that formulations A1 and A2 were decreased while formulations B1 and B4 were increased. These results could describe that all of materials were heated at 50°C for 30 minutes to reduce moisture content thus amout of aerobic microbial contents were decreased. And, before the preparation of formulations B1 and B4, the emblica extract was coated by polymer, in which the process could increase aerobic microbial content. However, the aerobic microbial count of formulations A1, A2, B1 and B4 were complied to limit of microbial contamination of Thai Herbal Pharmacopoeia 2000

Total combined molds and yeasts counts of formulations A1, A2, B1 and B4 were 121 CFU/g, 79 CFU/g, 19 CFU/g and 145 CFU/g, respectively as shown in Table 47 (Appendix B). The comparative total combined molds and yeasts count of chewable tablets and total combined molds and yeasts count of emblica extract, the results shown formulations A1 and A2 were not increased, while the formulations B1 and B2 were decresed. However, the total combined molds and yeastes count of formulations A1, A2, B1 and B4 were complied to limit of microbial contamination of Thai Herbal Pharmacopoeia 2000

The specified microorganisms, *Staphylococcus aureus, Pseudomonas aeruginosa,* Enterobacteriaceae, *Salmonella* spp*., Escherichia coli*, and *Clostridium* spp. of formulations A1, A2, B1 and B4 were not found in any formulation same as the emblica extract as shown in Table 48 (Appendix B).

These results were complied to limit the microbial contamination test from Thai Herbal Pharmacopoeia 2000.

5. Consumer acceptance simulation testing

The formulations were paired to evaluate in consumer accepatance simulation test, formulations A1/A2 was investigated for the effect of flavoring agent. In formulation A1 had sodium chloride and citric acid but formulation A2 had no sodium chloride and citric acid. Formulations A1/B1 was investigated for the effect of coating material. In formulation A1 had the uncoted extract but formulation B1 had the extract coated with 1% ethyl cellulose. Finally, formulations B1/B4 investigated for the effect of sweetening agent. In formulation B1 had aspartame but formulation B4 had sucralose.

The volunteers who were recruited in consumer acceptance simulation test consisted of 16 men and 14 women. Among these, 12 people were below 35 years old and 18 people were above 35 years old. Chewable tablets formulations A1, A2, B1 and B4 were given to the volunteers had passed the evaluation of microbial contamination test. In general, the acceptance in appearance, flavor/taste (sweetness, sour and bitterness) and mouth-feel showed insignificant different ($p>0.05$) comparing between men and women groups and between groups of people under 35 years and above 35 years; except for that women's satisfaction in sour taste of formulations A1 and A2 were significantly differenct ($p<0.05$). Formulation A1 had satisfied score of sour taste of 42.86% higher than formulation A2, which got 28.57%, as presented in Table 16 and Figure 27 - Figure 40.

The results of acceptance test for appearance and flavor showed that the tablets were suitably satisfying. Formulation B4 generally obtained most satisfying acceptance score. However, formulation B4 was not suitable to develop further since the stability data showed more variation in total tannins and gallic acid content.

The results for mouth-feel showed that all formulations had more grittiness than stickness and tongue burn effects because the fillers were used in formulation had relatively large particle size, as shown in Figure 32 and Figure 39.

Both formulations A1 and A2 were stable, as the total tannins in the chewable tablets were rather consistant during 3 months of storage, in particular when they were stored under refrigeration and at ambient temperature. From the results of consumer acceptance simulation test, as shown in Figure 33 and Figure 40, formulation A1 were

more accepatable than formulation A2. However, the satisfied score for these formulations were insignificant different (p>0.05). In terms of stability data, there fore, formulation A1 might be a better candidate for future development with the need of improving consumer acceptance.

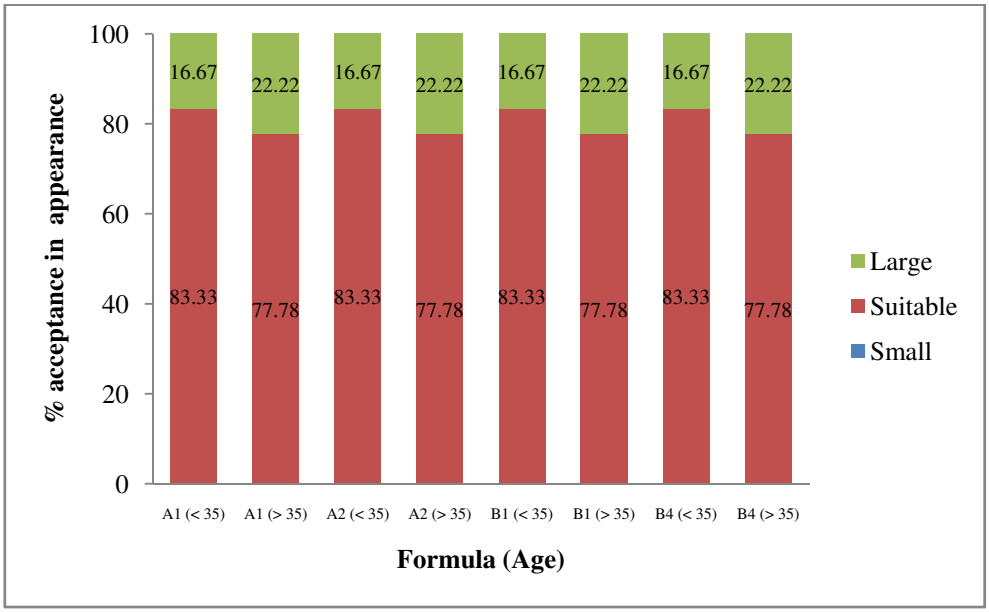


Figure 27 Consumer acceptance simulation testing result comparing percent acceptance in appearance between two groups of people whose ages were different (< 35, below 35 years old; > 35, above 35 years old).

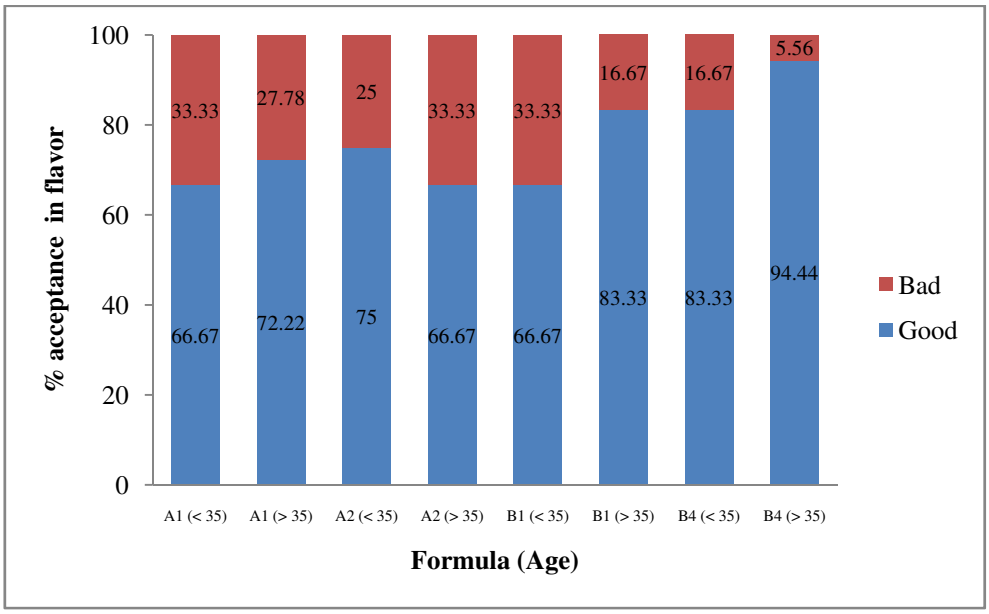


Figure 28 Consumer acceptance simulation testing result comparing percent acceptance in flavor between two groups of people whose ages were different (< 35, below 35 years old; > 35, above 35 years old).

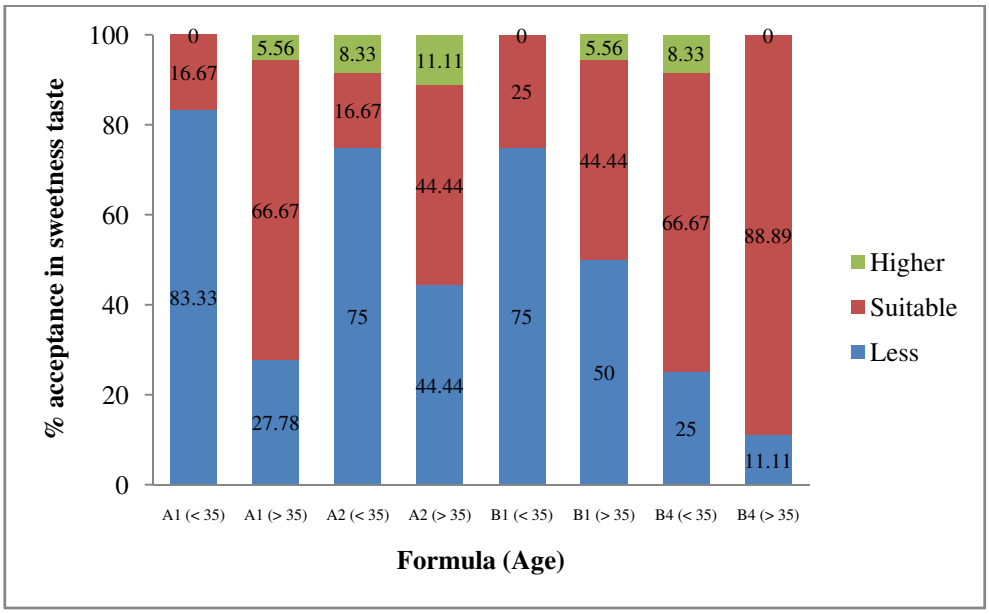


Figure 29 Consumer acceptance simulation testing result comparing percent acceptance in sweetness taste between two groups of people whose ages were different (< 35, below 35 years old; > 35, above 35 years old).

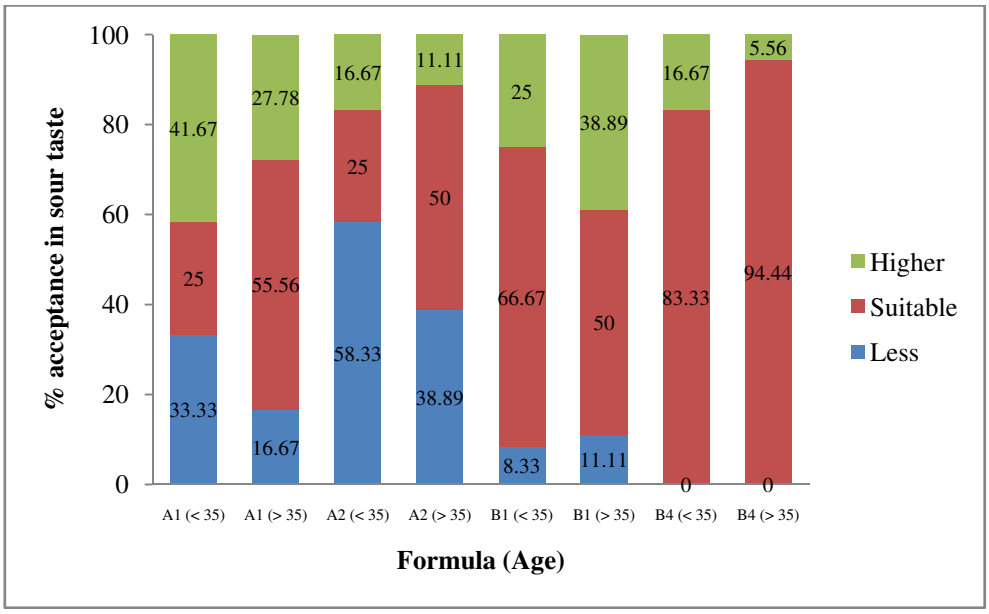


Figure 30 Consumer acceptance simulation testing result comparing percent acceptance in sour taste between two groups of people whose ages were different (< 35, below 35 years old; > 35, above 35 years old).

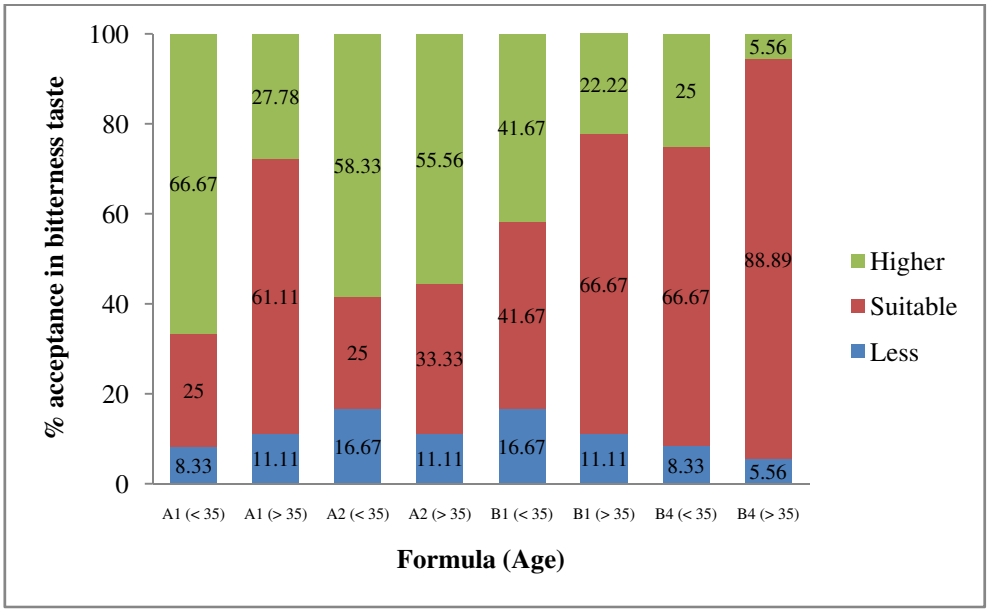


Figure 31 Consumer acceptance simulation testing result comparing percent acceptance in bitterness taste between two groups of people whose ages were different (< 35, below 35 years old; > 35, above 35 years old).

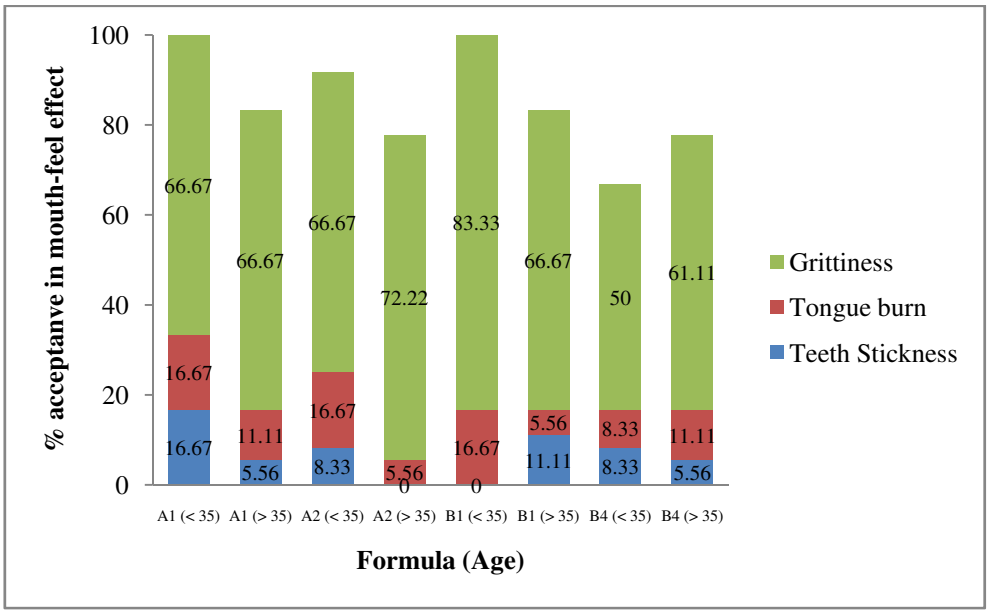


Figure 32 Consumer acceptance simulation testing result comparing percent acceptance in mouth-feel effect between two groups of people whose ages were different (< 35, below 35 years old; > 35, above 35 years old).

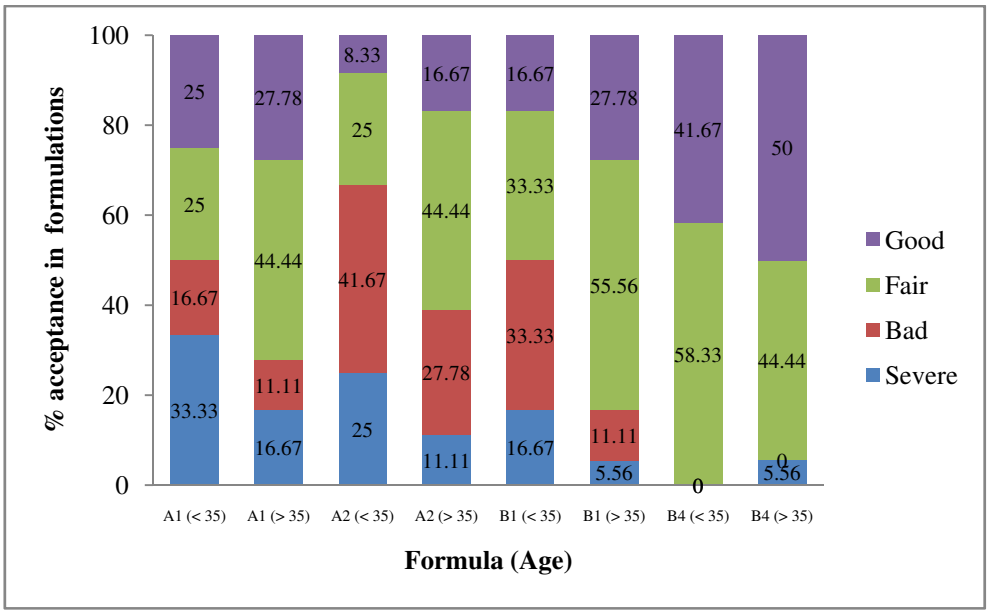


Figure 33 Consumer acceptance simulation testing result comparing percent acceptance in formulations between two groups of people whose ages were different (< 35, below 35 years old; > 35, above 35 years old).

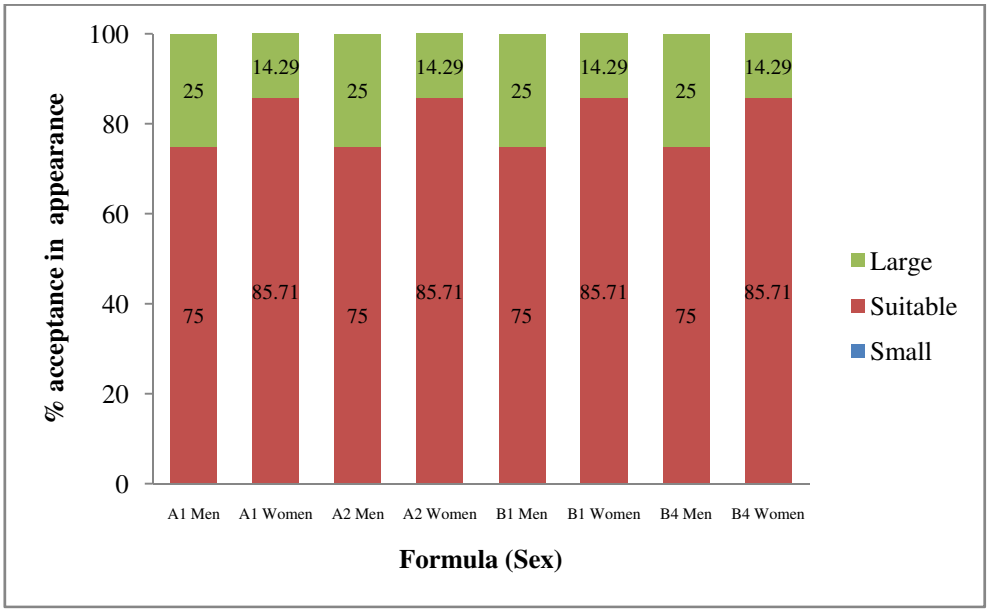


Figure 34 Consumer acceptance simulation testing result comparing percent acceptance in appearance between men and women.

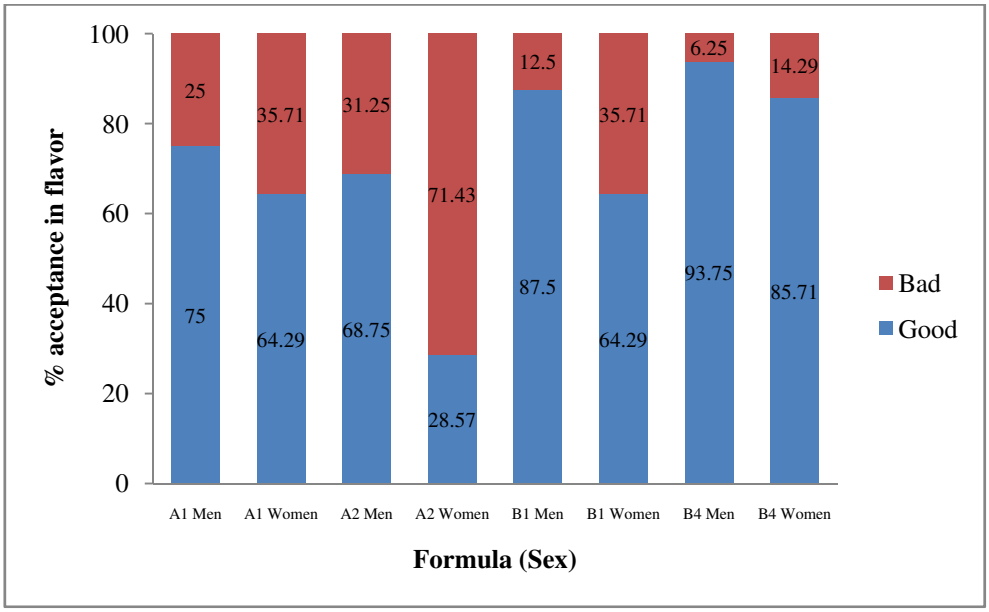


Figure 35 Consumer acceptance simulation testing result comparing percent acceptance in flavor between men and women.

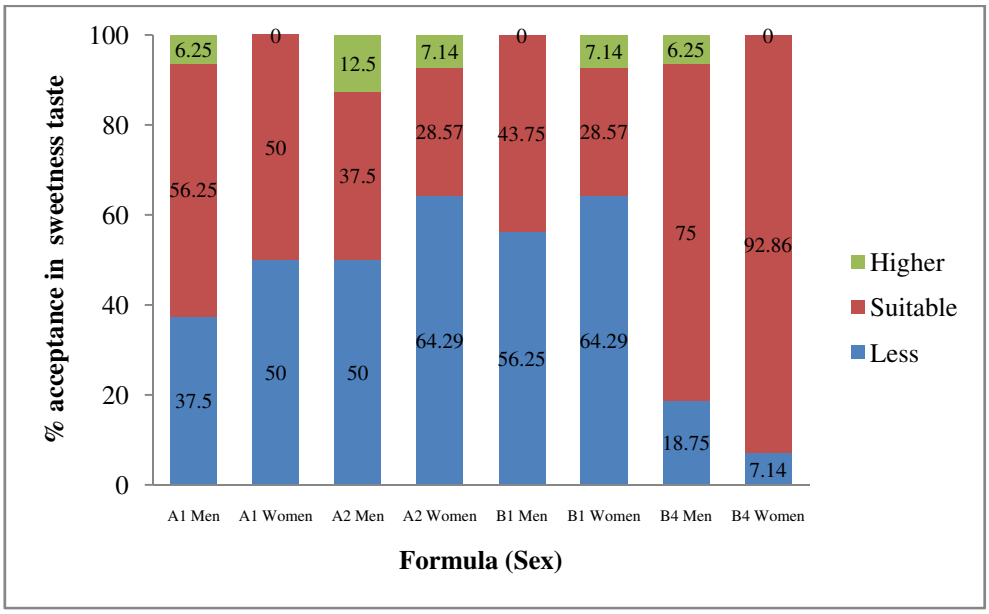


Figure 36 Consumer acceptance simulation testing result comparing percent acceptance in sweetness taste between men and women.

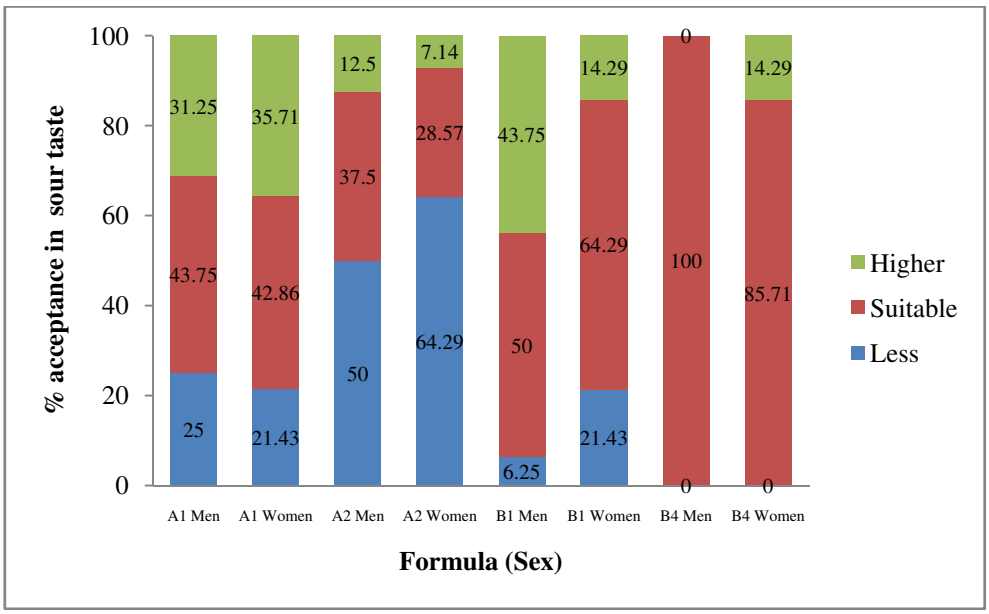


Figure 37 Consumer acceptance simulation testing result comparing percent acceptance in sour taste between men and women.

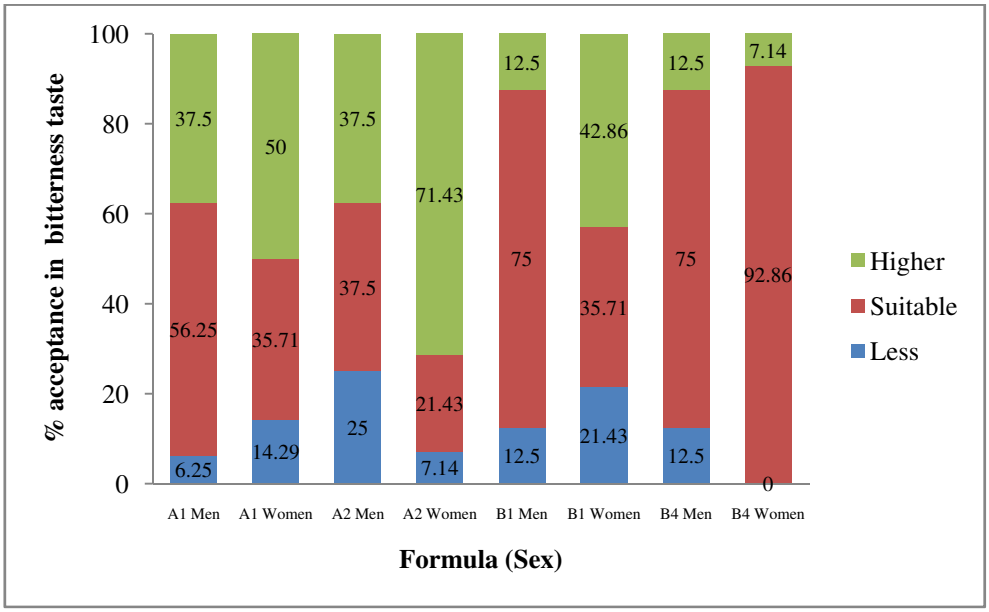


Figure 38 Consumer acceptance simulation testing result comparing percent acceptance in bitterness taste between men and women.

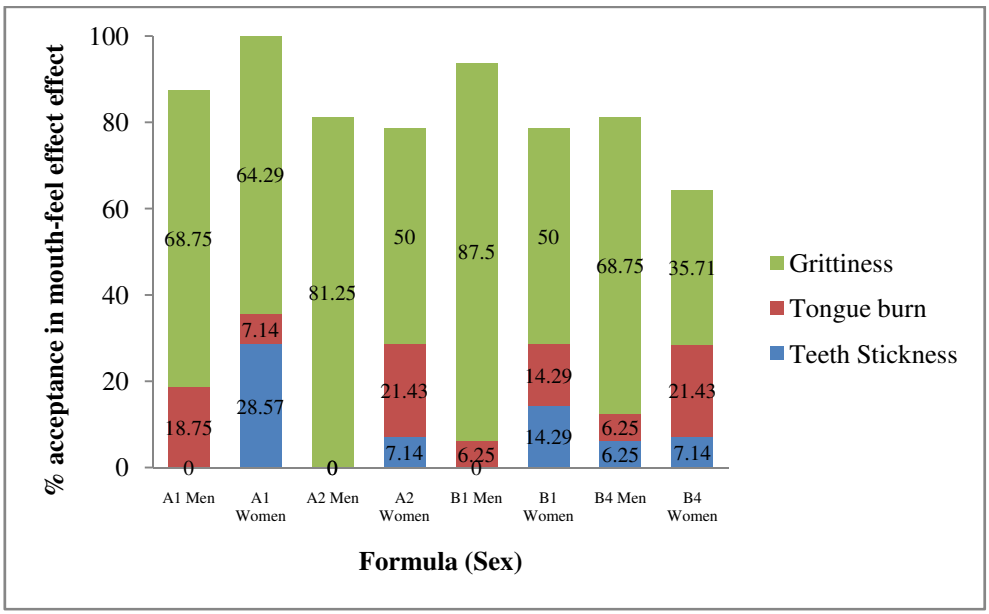


Figure 39 Consumer acceptance simulation testing result comparing percent acceptance in mouth-feel effects between men and women.

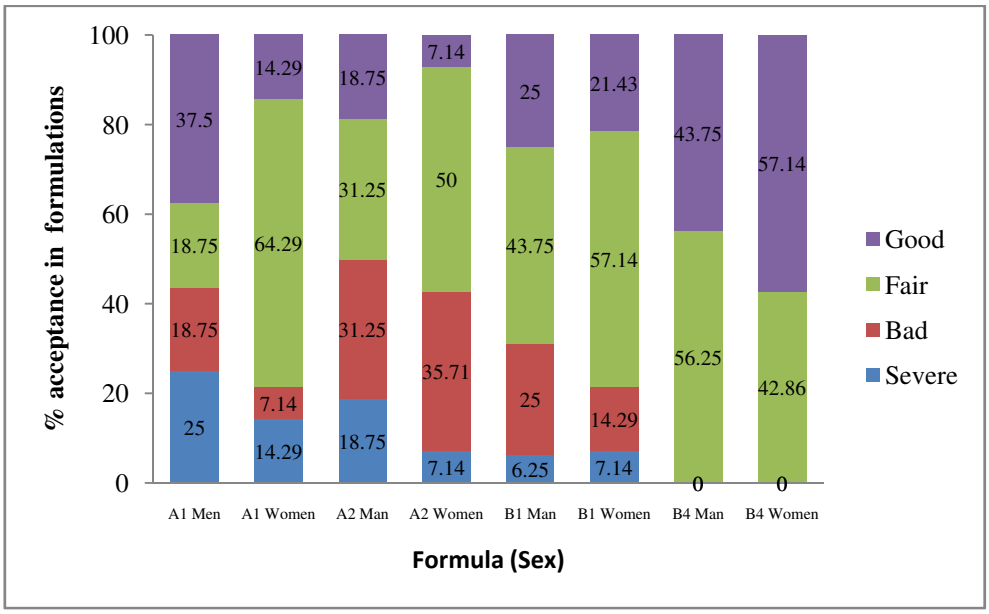


Figure 40 Consumer acceptance simulation testing result comparing percent acceptance in formulations between men and women.

Table 16 The statistical analysis of consumer acceptance simulation testing of chewable tablets containing *Phyllathus emblica* Linn. fruit extract.

Parameters	Compared Formulations	Sex		Age	
		Men	Women	Below 35 years	Above 35 years
Appearance	A1/A2	NS	NS	NS	NS
	A1/B1	NS	NS	NS	NS
	B1/B4	NS	NS	NS	NS
Smell	A1/A2	NS	NS	NS	NS
	A1/B1	NS	NS	NS	NS
	B1/B4	NS	NS	NS	NS
Sweetness	A1/A2	NS	NS	NS	NS
	A1/B1	NS	NS	NS	NS
	B1/B4	NS	NS	NS	NS
Sour	A1/A2	NS	S	NS	NS
	A1/B1	NS	NS	NS	NS
	B1/B4	NS	NS	NS	NS
Bitterness	A1/A2	NS	NS	NS	NS
	A1/B1	NS	NS	NS	NS
	B1/B4	NS	NS	NS	NS
Mouth-feel effect	A1/A2	NS	NS	NS	NS
	A1/B1	NS	NS	NS	NS
	B1/B4	NS	NS	NS	NS
Formula	A1/A2	NS	NS	NS	NS
	A1/B1	NS	NS	NS	NS
	B1/B4	NS	NS	NS	NS

CHAPTER V

CONCLUSIONS

In this study, chewable tablets of *Phyllanthus emblica* Linn. were prepared by direct compression process. The conclusions can be drawn as the followings:

The extract was hygroscopic. It possessed very poor flowability and could cause severe problems of tablet picking during the process. Sufficient lubrication was required.

The formulation which was relatively stable and accepted by volunteers contained 30% *Phyllanthus emblica* Linn. spray dried fruit extract, 20.35% mannitol, 20.35% xylitol, 10% microcrystalline cellulose PH 102, 10% talc, 5% citric acid, 1% aspartame, 2% sodium chloride, 1% magnesium stearate and 0.3% silicon dioxide.

The ambient temperature was found to be a suitable condition to store the chewable tablets containing *Phyllanthus emblica* Linn. spray dried fruit extract because total tannins and gallic acid contents of chewable tablets were stable and hardness of the chewable tablets was insignificantly changed.

The release of total tannins from the tablets cut into pieces was dependent on the type of binder used. With copovidone, the release was retarded

Coating the extract with ethyl cellulose before incorporating into the formulations did not increase stability of the chewable tablets.

The spray dried extract and the chewable tablets obtained from direct compression process in the present study were proved to contain acceptable limit of microbial, complying with the microbial contamination limits of Thai Herbal Pharmacopoeia 2000.

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APPENDICES

APPENDIX A

Table 1A Calibration data of total tannins at 731.50 nm.

Concentration (µg/ml)	Absorbance at 731.50 nm
9.37	0.1154
18.74	0.2316
37.47	0.4641
46.84	0.5781
56.21	0.6887
74.94	0.9183

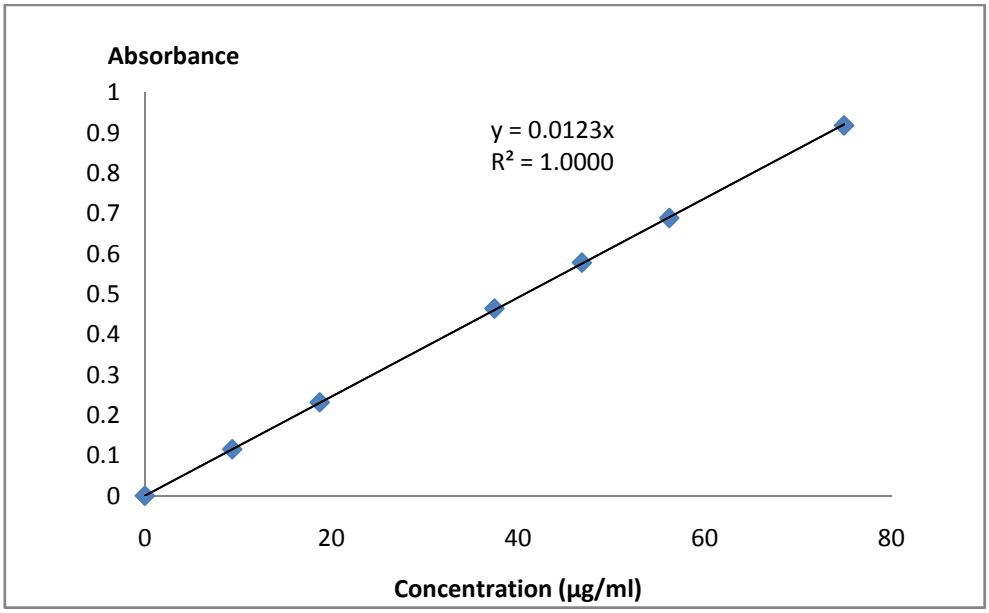

Figure 1A Calibration curve of total tannins at 731.50 nm.

Table 2A Calibration data of gallic acid at 270 nm.

Concentration (µg/ml)	Peak area at 270 nm
0.233	14815.33
0.933	59433.00
2.799	183553.67
4.665	304749.33
6.998	466430.67

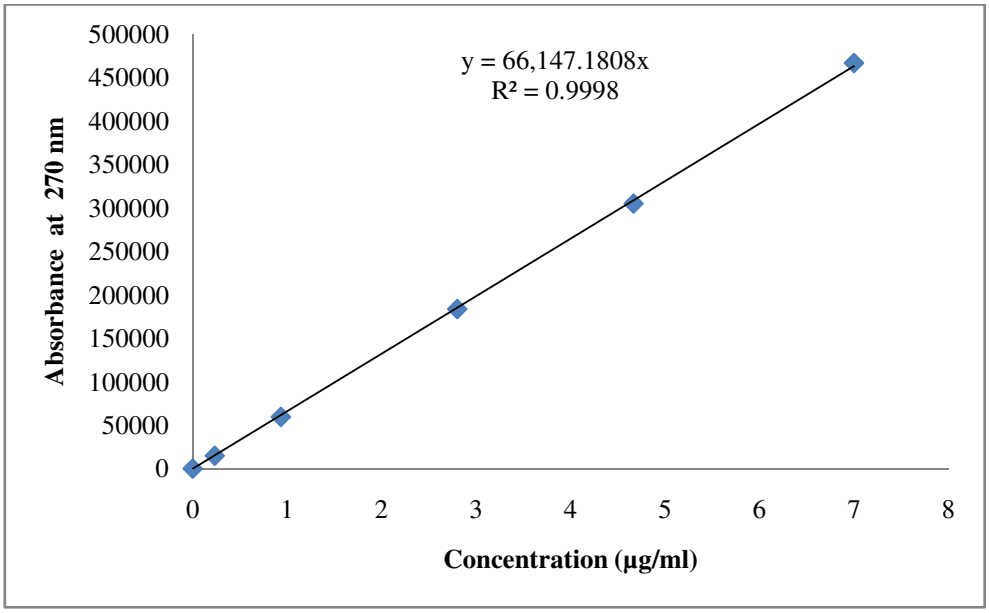

Figure 2A Calibration curve of gallic acid at 270 nm.

Table 3A The percentage of recovery of gallic acid on 1st day.

Theoretical Concentration (µg/ml)	Measured Concentration (µg/ml)			Recovery (%)					
	n1	n2	n3	n1	n2	n3	Mean	SD	% RSD
0.504	0.4668	0.4555	0.4605	92.61	90.38	91.37	91.45	1.12	1.22
1.008	0.9123	0.9209	0.9160	90.51	91.36	90.87	90.91	0.43	0.47
2.519	2.2800	2.2843	2.2972	90.51	90.68	91.19	90.80	0.35	0.39
4.031	3.6021	3.6942	3.7296	89.36	91.65	92.52	91.18	1.63	1.79
5.039	4.7840	4.6769	4.7369	94.94	92.81	94.00	93.92	1.07	1.13

Table 4A The percentage of recovery of gallic acid on 2nd day.

Theoretical Concentration (µg/ml)	Measured Concentration (µg/ml)			Recovery (%)					
	n1	n2	n3	n1	n2	n3	Mean	SD	% RSD
0.491	0.4518	0.4587	0.4602	92.12	93.51	93.83	93.15	0.91	0.98
1.020	0.9154	0.9209	0.9165	89.74	90.29	89.85	89.96	0.29	0.32
2.506	2.2951	2.2934	2.2987	91.58	91.52	91.73	91.61	0.11	0.12
4.026	3.7533	3.7094	3.7447	93.23	92.14	93.01	92.79	0.58	0.62
5.063	4.8142	4.6920	4.7520	95.09	92.67	93.86	93.87	1.21	1.29

Table 5A The percentage of recovery of gallic acid on 3rd day.

Theoretical Concentration (µg/ml)	Measured Concentration (µg/ml)			Recovery (%)					
	n1	n2	n3	n1	n2	n3	Mean	SD	% RSD
0.508	0.4564	0.4647	0.4663	89.84	91.48	91.79	91.03	1.05	1.15
1.029	0.9199	0.9134	0.9270	89.40	88.76	90.09	89.42	0.67	0.74
2.515	2.2966	2.2949	2.3032	91.32	91.25	91.58	91.38	0.17	0.19
4.050	3.7684	3.7547	3.7598	93.05	92.71	92.83	92.86	0.17	0.18
5.010	4.7840	4.6769	4.7067	95.49	93.35	93.95	94.26	1.10	1.17

Table 6A Precision of HPLC method.

Number	**Peak area at 270 nm**		
	1st day	**2nd day**	**3rd day**
1	153760	161008	142270
2	154585	160420	146156
3	155747	164025	144497
4	154367	162194	143501
5	154932	161364	147614
6	152206	167505	145213
Mean	154266	162753	144875
%RSD	0.78	1.62	1.31

Table 7A System suitability of HPLC method.

	Mean	**%RSD**
Peak area	144875	1.31
Tailing factor	0.56	1.10

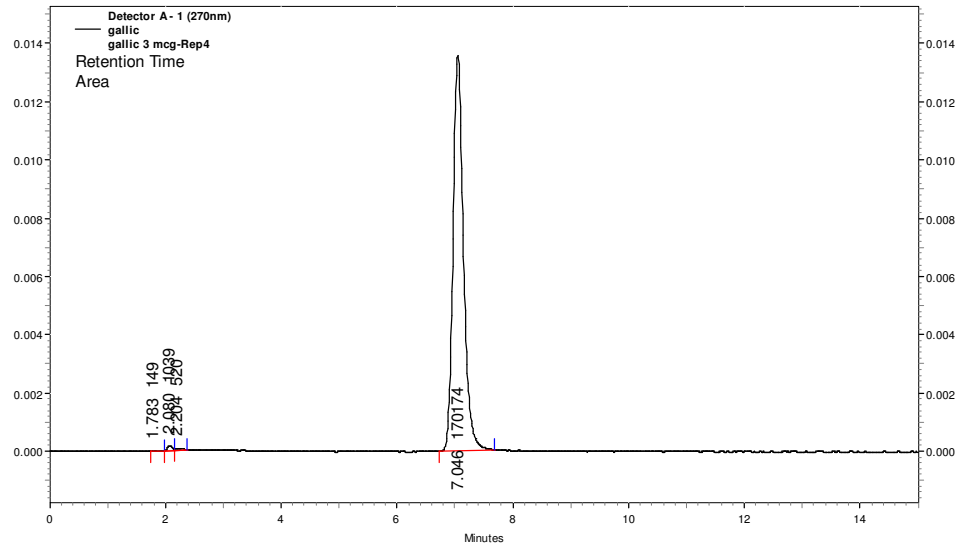

Figure 3A Chromatogram of gallic acid solution (1).

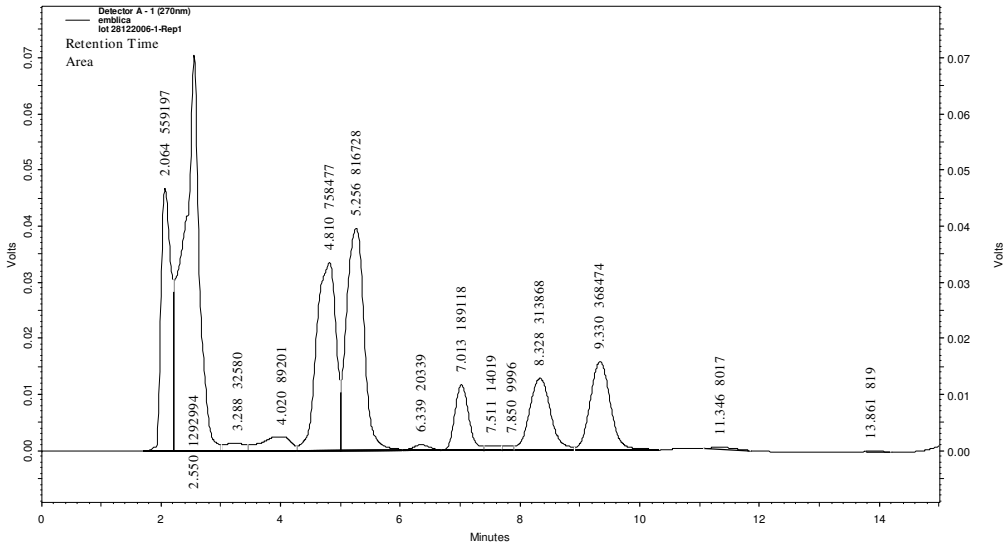

Figure 4A Chromatogram of *Phyllanthus emblica* Linn. fruit extract solution.

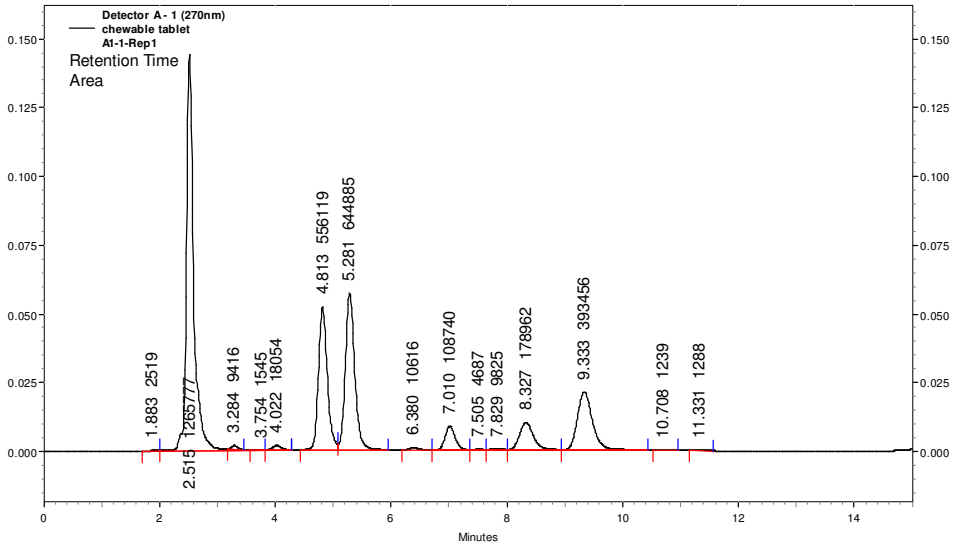

Figure 5A Chromatogram of the solution of chewable tablets containing *Phyllanthus emblica* Linn. fruit extract formulation A1.

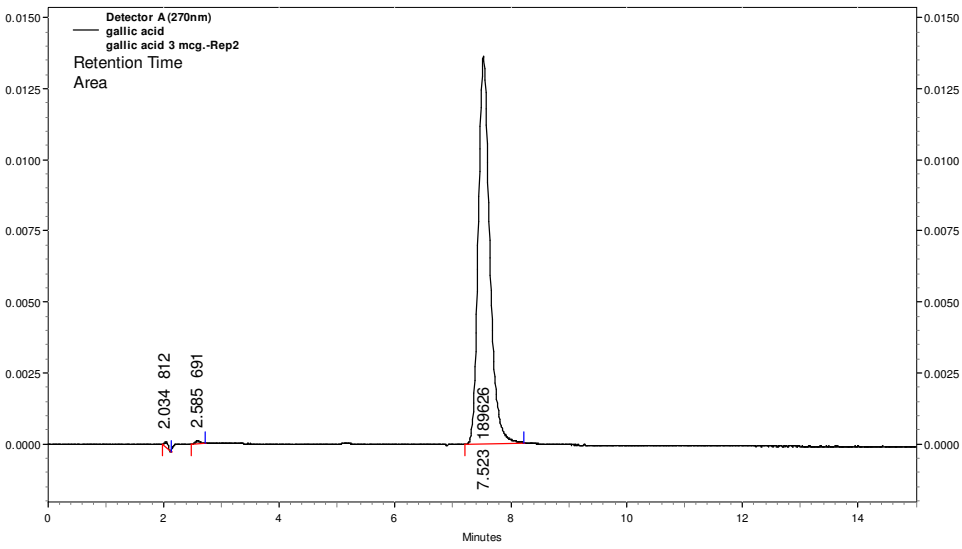

Figure 6A Chromatogram of gallic acid solution (2).

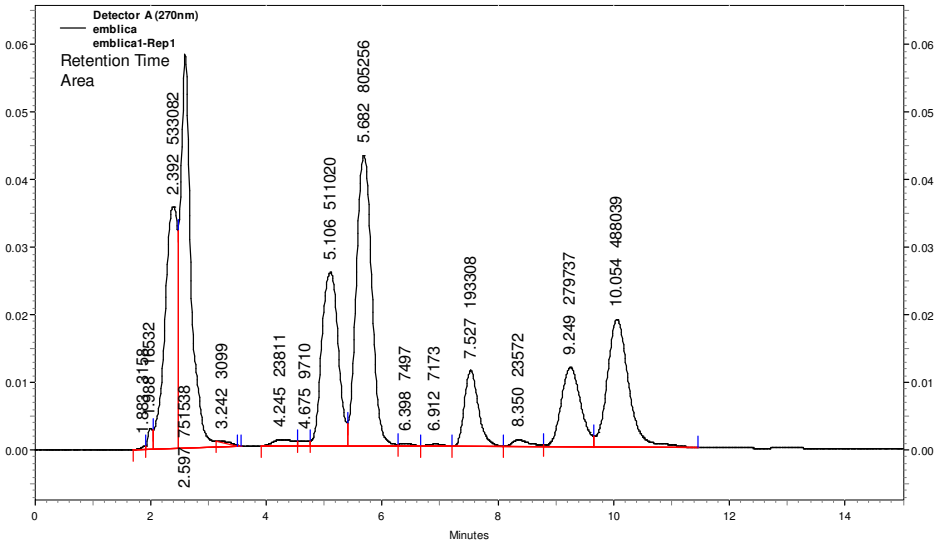

Figure 7A Chromatogram of the solution of coated *Phyllanthus emblica* Linn. fruit extract.

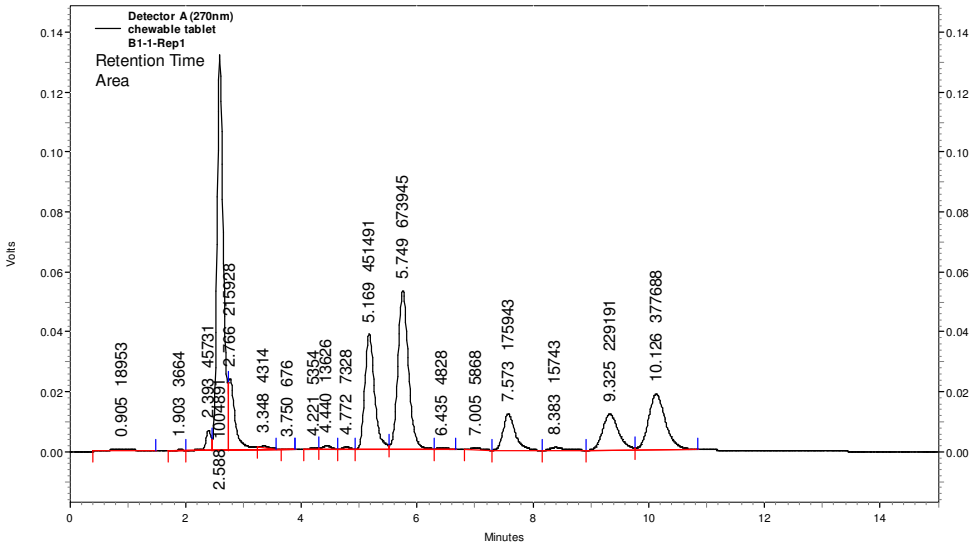

Figure 8A Chromatogram of the solution of chewable tablets containing *Phyllanthus emblica* Linn. fruit extract formulation B1.

APPENDIX B

Table 1B Weight variation of chewable tablets containing *Phyllanthus emblica* Linn. spray dried fruit extract after preparation.

No.	Weight (mg)							
	A1	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
1	740	740	739	745	738	745	740	738
2	742	742	740	745	740	747	741	738
3	743	746	742	740	740	747	741	741
4	743	749	743	741	740	749	742	741
5	747	749	744	742	741	753	743	742
6	748	750	745	743	741	753	743	743
7	748	750	747	743	742	753	744	743
8	749	750	748	744	742	753	746	744
9	749	751	750	744	742	754	746	748
10	749	751	751	746	743	755	748	748
11	750	751	752	747	744	756	749	748
12	750	752	752	747	745	757	751	751
13	751	752	753	748	745	757	752	752
14	753	753	754	748	746	758	752	752
15	753	753	755	748	748	758	753	753
16	756	754	757	748	749	759	754	754
17	756	756	758	750	749	759	754	754
18	758	758	759	754	751	759	754	758
19	758	759	760	761	752	760	758	758
20	759	760	761	761	753	764	760	760
Average	750.10	751.30	750.50	747.25	744.55	754.80	748.55	748.30
SD	5.53	5.00	6.79	5.72	4.44	4.93	6.00	6.81
%CV	0.74	0.66	0.91	0.77	0.60	0.65	0.80	0.91

Table 2B Weight variation of chewable tablets containing coated *Phyllanthus emblica* Linn. fruit extract after preparation.

No.	Weight (mg)			
	B1	**B2**	**B3**	**B4**
1	738	740	738	750
2	742	740	740	751
3	746	742	740	751
4	746	742	740	751
5	747	742	741	752
6	751	743	741	753
7	751	743	742	753
8	752	743	743	753
9	752	743	745	753
10	752	743	745	754
11	752	745	745	754
12	753	745	749	754
13	753	745	750	754
14	753	745	750	755
15	753	746	750	755
16	754	746	752	755
17	754	747	752	755
18	754	748	755	755
19	755	748	755	755
20	756	749	755	756
Average	750.70	744.25	746.40	753.45
SD	4.60	2.57	5.71	1.70
%CV	0.61	0.35	0.76	0.23

Table 3B Weight variation of chewable tablets containing *Phyllanthus emblica* Linn. spray dried fruit extract stored under refrigeration for 3months.

No.	Weight variation (mg)							
	A1	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
1	740	745	740	741	740	740	740	742
2	740	747	743	742	742	742	742	742
3	741	748	743	743	743	744	743	742
4	742	749	746	744	744	745	745	746
5	744	750	746	745	745	747	747	746
6	749	750	747	745	745	749	749	746
7	750	751	748	745	746	750	750	746
8	750	751	749	745	746	750	751	749
9	750	751	750	746	746	751	752	750
10	750	751	751	746	746	752	752	750
11	751	752	752	746	749	754	752	750
12	754	752	752	747	750	755	752	753
13	755	753	753	747	750	756	753	754
14	755	753	753	748	750	756	753	754
15	756	754	753	748	752	757	754	754
16	757	754	754	749	753	758	755	755
17	758	756	756	750	754	758	755	755
18	758	758	757	751	754	758	756	756
19	758	760	757	753	754	758	757	756
20	759	761	758	757	756	759	758	759
Average	750.85	752.30	750.40	746.90	748.25	751.95	750.80	750.25
SD	6.46	4.08	5.08	3.78	4.59	5.91	5.05	5.21
% CV	0.86	0.54	0.68	0.51	0.61	0.79	0.67	0.69

Table 4B Weight variation of chewable tablets containing coated *Phyllanthus emblica* Linn. fruit extract stored under refrigeration for 3 months.

No.	Weight (mg)			
	B1	**B2**	**B3**	**B4**
1	741	740	740	751
2	742	740	740	751
3	746	740	741	752
4	746	741	743	752
5	747	741	745	752
6	751	742	745	753
7	751	742	745	753
8	752	745	747	753
9	752	745	747	753
10	753	745	749	754
11	753	745	750	754
12	753	746	750	754
13	753	746	750	754
14	754	747	752	755
15	754	747	752	755
16	755	748	752	756
17	755	748	752	756
18	755	749	755	757
19	756	749	755	757
20	756	750	755	758
Average	751.25	744.80	748.25	754.00
SD	4.47	3.32	4.89	2.03
%CV	0.60	0.45	0.65	0.27

Table 5B Weight variation of chewable tablets containing *Phyllanthus emblica* Linn. spray dried fruit extract stored under ambient condition for 3 months.

No.	Weight variation (mg)							
	A1	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
1	740	742	740	739	742	740	739	737
2	741	743	741	740	742	741	739	738
3	742	745	745	741	744	742	740	739
4	744	745	746	742	744	742	740	740
5	745	746	746	742	745	749	741	741
6	745	747	749	742	745	752	742	741
7	746	748	750	743	745	752	745	741
8	750	748	751	744	745	753	747	742
9	750	749	752	744	746	753	747	742
10	752	749	753	744	746	754	747	743
11	754	750	753	745	746	754	747	745
12	755	750	753	745	746	755	747	746
13	756	751	754	746	746	756	748	746
14	756	751	754	746	747	756	749	748
15	758	752	755	748	749	756	750	749
16	758	753	757	748	750	758	752	754
17	758	754	758	748	751	758	752	756
18	759	758	758	755	753	758	754	756
19	759	759	759	758	754	759	755	757
20	760	759	759	760	756	761	755	759
Average	751.40	749.95	751.65	746.00	747.10	752.45	746.80	746.00
SD	6.80	4.89	5.68	5.68	3.86	6.39	5.29	6.94
% CV	0.91	0.65	0.76	0.76	0.52	0.85	0.71	0.93

Table 6B Weight variation of chewable tablets containing coated *Phyllanthus emblica* Linn. fruit extract stored under ambient condition for 3 months.

No.	Weight (mg)			
	B1	**B2**	**B3**	**B4**
1	740	740	740	750
2	740	741	740	750
3	741	742	741	751
4	742	742	742	751
5	743	743	743	752
6	748	743	744	752
7	749	744	744	752
8	749	745	745	753
9	751	745	745	753
10	751	746	745	754
11	752	746	746	754
12	752	747	747	754
13	753	747	748	755
14	753	748	749	755
15	754	748	749	755
16	754	749	750	756
17	755	749	750	756
18	755	750	752	757
19	756	750	755	757
20	756	750	755	759
Average	749.70	745.75	746.50	753.80
SD	5.53	3.18	4.48	2.48
%CV	0.74	0.43	0.60	0.33

Table 7B Weight variation of chewable tablets containing *Phyllanthus emblica* Linn. spray dried fruit extract stored under ambient temperature and 75%RH for 3 months.

No.	Weight variation (mg)							
	A1	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
1	742	744	738	742	742	743	738	737
2	742	745	744	743	743	743	741	738
3	745	745	745	744	744	744	745	740
4	745	746	746	744	744	745	746	741
5	746	748	747	744	746	749	747	741
6	746	749	748	744	746	749	747	741
7	747	750	749	745	746	750	748	742
8	749	750	750	745	746	750	748	742
9	750	751	750	745	746	752	749	746
10	750	751	751	746	747	752	749	746
11	756	751	751	746	749	753	749	746
12	756	752	752	746	749	754	750	746
13	758	752	752	748	750	754	750	748
14	758	752	753	748	753	755	752	749
15	758	752	755	748	754	755	752	749
16	759	753	757	755	756	756	754	750
17	759	758	758	755	758	756	755	754
18	759	759	759	758	759	758	755	756
19	761	759	759	758	761	758	757	757
20	762	761	760	758	761	758	760	762
Average	752.40	751.40	751.20	748.10	750.00	751.70	749.60	746.55
SD	6.79	4.83	5.74	5.44	6.17	4.95	5.21	6.73
% CV	0.90	0.64	0.76	0.73	0.82	0.66	0.69	0.90

Table 8B Weight variation of chewable tablets containing coated *Phyllanthus emblica* Linn. spray extract stored under ambient temperature and 75%RH for months.

No.	Weight (mg)			
	B1	**B2**	**B3**	**B4**
1	740	741	741	749
2	741	742	742	750
3	741	742	743	750
4	743	742	743	750
5	743	743	744	751
6	748	744	744	751
7	749	745	745	752
8	749	745	745	752
9	750	746	746	753
10	751	746	747	753
11	751	747	748	754
12	751	747	749	754
13	751	748	749	754
14	752	748	752	755
15	752	749	752	755
16	753	750	752	756
17	753	750	755	756
18	754	751	755	756
19	754	751	756	757
20	755	752	756	757
Average	749.05	746.45	748.20	753.25
SD	4.78	3.41	4.95	2.53
%CV	0.64	0.46	0.66	0.34

Table 9B Weight variation of chewable tablets containing *Phyllanthus emblica* Linn. spray dried fruit extract stored under 40°C and 75%RH for 3 months.

No.	Weight variation (mg)							
	A1	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
1	741	743	741	740	740	751	740	743
2	742	744	742	740	740	751	740	745
3	743	745	744	741	741	752	742	745
4	744	745	745	741	741	754	744	746
5	744	745	745	741	741	754	744	746
6	745	747	746	743	741	755	748	747
7	745	747	747	743	742	755	749	750
8	746	750	747	744	742	755	753	750
9	747	751	749	745	743	756	753	750
10	754	753	753	745	743	756	755	750
11	754	754	755	745	743	757	756	752
12	755	755	757	747	745	757	757	752
13	756	756	757	748	745	758	757	752
14	756	757	758	749	745	759	758	753
15	758	758	759	750	746	759	758	754
16	758	758	760	750	746	759	758	756
17	759	758	760	751	747	760	759	756
18	760	760	760	752	749	760	760	758
19	761	760	762	752	752	761	760	759
20	762	761	762	756	755	761	761	762
Average	751.50	752.35	752.45	746.15	744.35	756.50	752.60	751.30
SD	7.25	6.14	7.29	4.67	4.02	3.14	7.17	5.15
% CV	0.96	0.82	0.97	0.63	0.54	0.41	0.95	0.69

Table 10B Weight variation of chewable tablets containing coated *Phyllanthus emblica* Linn. fruit extract stored under 40° C and 75%RH for 3months.

No.	Weight (mg)			
	B1	**B2**	**B3**	**B4**
1	740	740	740	750
2	741	741	740	750
3	742	741	741	751
4	745	742	742	751
5	745	742	743	752
6	751	743	745	752
7	751	743	745	752
8	752	745	745	753
9	752	745	747	754
10	753	746	747	754
11	753	746	747	754
12	754	747	748	755
13	754	747	749	755
14	755	748	749	755
15	755	748	750	756
16	755	749	750	756
17	756	749	750	756
18	756	750	752	757
19	757	751	755	757
20	757	751	755	757
Average	751.20	745.70	747.00	753.85
SD	5.48	3.48	4.45	2.35
%CV	0.73	0.47	0.60	0.31

Table 11B Hardness of chewable tablets containing *Phyllanthus emblica* Linn. spray dried fruit extract after preparation.

No.	Hardness (KP)							
	A1	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
1	10.02	14.59	8.78	10.06	12.71	11.20	8.34	5.61
2	11.51	13.15	5.79	9.98	13.21	12.21	7.71	8.19
3	7.15	15.90	10.60	10.05	13.92	10.64	10.65	8.07
4	12.14	12.06	11.89	8.96	13.59	10.41	7.31	9.22
5	8.01	15.39	11.12	9.03	11.03	11.88	9.88	8.85
6	9.98	16.15	9.66	12.04	13.65	12.68	8.05	9.95
7	12.85	14.59	11.60	11.49	12.38	12.36	6.94	7.84
8	8.80	15.59	9.03	11.15	13.55	13.53	8.61	8.92
9	10.65	15.50	11.93	10.42	12.87	12.26	9.30	9.94
10	9.26	13.15	12.04	11.15	13.41	10.03	7.84	8.47
Average	10.04	14.61	10.24	10.43	13.03	11.72	8.46	8.51
SD	1.81	1.38	1.98	1.02	0.85	1.11	1.17	1.25
%CV	18.03	9.45	19.33	9.77	6.52	9.51	13.82	14.70

Table 12B Hardness of chewable tablets containing coated *Phyllanthus emblica* Linn. fruit extract after preparation.

No.	Hardness (KP)			
	B1	**B2**	**B3**	**B4**
1	5.74	8.78	6.26	5.71
2	6.01	8.55	6.96	5.78
3	5.64	8.64	6.65	5.75
4	6.80	9.24	7.81	6.25
5	4.55	8.63	6.83	7.02
6	6.71	8.66	4.46	6.57
7	7.10	8.78	7.40	6.74
8	7.23	8.75	8.61	6.51
9	7.15	8.81	7.73	6.89
10	7.19	8.64	6.75	5.78
Average	6.41	8.75	6.95	6.30
SD	0.89	0.19	1.11	0.51
%CV	13.96	2.20	16.01	8.15

Table 13B Hardness of chewable tablets containing *Phyllanthus emblica* Linn. spray dried fruit extract stored under refrigeration for 3 months.

No.	Hardness (KP)							
	A1	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
1	12.78	13.37	7.93	9.50	8.34	9.18	10.86	6.94
2	9.24	10.01	9.45	9.54	9.71	9.31	6.27	8.10
3	9.24	10.27	10.05	9.93	9.05	9.48	7.23	11.90
4	8.77	7.78	7.24	9.23	9.20	8.43	7.58	7.63
5	6.86	11.37	4.65	10.68	11.61	10.28	5.86	10.87
6	7.51	13.32	4.33	8.95	13.08	9.29	7.23	8.35
7	7.07	12.70	6.19	11.57	9.05	9.50	7.71	7.48
8	6.65	13.03	8.24	12.85	1.45	10.99	8.64	10.40
9	7.57	12.04	6.07	9.65	9.03	10.16	8.46	8.78
10	11.93	6.38	11.28	7.10	11.40	9.58	7.14	7.96
Average	8.76	11.03	7.54	9.90	9.19	9.62	7.70	8.84
SD	2.12	2.41	2.29	1.55	3.11	0.70	1.40	1.65
% CV	24.23	21.89	30.39	15.66	33.79	7.31	18.19	18.63

Table 14B Hardness of chewable tablets containing coated *Phyllanthus emblica* Linn. fruit extract stored under refrigeration for 3 months.

No.	Hardness (KP)			
	B1	**B2**	**B3**	**B4**
1	8.45	11.36	9.10	8.12
2	10.03	11.41	8.57	9.05
3	8.84	10.36	13.00	9.27
4	9.63	10.09	9.61	8.17
5	9.16	10.70	12.30	8.38
6	10.34	10.14	10.62	9.77
7	9.07	9.75	10.89	10.12
8	10.59	10.95	8.86	10.39
9	9.22	10.11	12.10	8.72
10	9.43	10.05	12.55	8.53
Average	9.48	10.49	10.76	9.05
SD	0.68	0.58	1.66	0.81
%CV	7.13	5.54	15.46	9.00

Table 15B Hardness of chewable tablets containing *Phyllanthus emblica* Linn. spray dried fruit extract stored under ambient condition for 3 months.

No.	Hardness (KP)							
	A1	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
1	10.94	11.51	6.95	9.02	13.64	9.47	7.83	8.93
2	8.55	13.69	10.24	8.62	11.28	11.7	6.79	7.7
3	5.07	15.76	9.09	9.77	11.32	10.48	6.75	8.2
4	9.43	14.35	6.87	8.78	12.38	10.55	9.42	9.17
5	9.19	11.99	12.9	10.84	12.92	12.2	5.7	9.23
6	7.67	14.08	10.48	9.59	11.24	13.62	8.74	10.55
7	10.32	13.75	7.92	10.75	12.12	7.96	6.15	8.79
8	7.52	16.87	12.36	9.27	10.97	9.14	8.22	8.84
9	7.01	14.33	10.05	10.52	13.41	9.28	9.26	10.51
10	5.56	14.44	9.78	10.34	10.65	11.42	9.93	8.63
Average	8.13	14.08	9.66	9.75	11.99	10.58	7.88	9.06
SD	1.93	1.57	2.04	0.83	1.06	1.69	1.47	0.90
% CV	23.74	11.13	21.11	8.47	8.84	15.97	18.70	9.93

Table 16B Hardness of chewable tablets containing coated *Phyllanthus emblica* Linn. fruit extract stored under ambient condition for 3 months.

No.	Hardness (KP)			
	B1	**B2**	**B3**	**B4**
1	18.58	15.23	16.14	15.51
2	18.26	14.19	19.48	16.67
3	20.32	15.95	17.29	16.14
4	20.57	12.07	23.29	17.17
5	19.72	15.26	16.59	17.13
6	17.10	12.62	17.65	17.99
7	20.44	16.06	16.71	16.82
8	19.98	15.46	20.98	16.74
9	20.39	16.79	16.14	16.72
10	18.37	12.72	16.98	15.17
Average	19.37	14.64	18.13	16.61
SD	1.20	1.64	2.38	0.82
%CV	6.21	11.24	13.15	4.95

Table 17B Hardness of chewable tablets containing *Phyllanthus emblica* Linn. spray dried fruit extract stored under ambient temperature and 75%RH for 3 months.

No.	Hardness (KP)							
	A1	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
1	11.63	12.22	6.85	9.86	14.06	9.1	6.98	8.93
2	5.37	15.37	9.42	9.05	12.58	9.84	8.48	8.83
3	6.34	16.99	9.59	14.18	13.29	13.34	10.84	5.39
4	8.46	14.36	13.55	10.45	14.61	11.59	8.9	8.31
5	6.43	11.44	12.5	11.41	11.61	14.1	11.5	6.87
6	8.4	13.98	9.36	10.42	12.46	11.35	5.66	6.73
7	10.59	12.84	13.68	9.69	10.83	10.87	7.73	8.45
8	4.92	13.73	10.37	9.53	13.71	12.28	5.74	7.59
9	11.85	12.04	11.61	11.22	15.1	11.93	8.78	8.36
10	11.87	16.83	6.8	10.25	13.48	12.02	7.28	7.59
Average	8.59	13.98	10.37	10.61	13.17	11.64	8.19	7.71
SD	2.75	1.94	2.47	1.45	1.32	1.49	1.94	1.11
% CV	32.07	13.89	23.79	13.68	10.04	12.79	23.67	14.45

Table 18B Hardness of chewable tablets containing coated *Phyllanthus emblica* Linn. fruit extract stored under ambient temperature and 75%RH for 3 months.

No.	Hardness (KP)			
	B1	**B2**	**B3**	**B4**
1	19.09	16.82	16.66	17.22
2	19.56	17.04	21.88	15.76
3	18.81	15.14	18.75	17.30
4	18.38	17.10	18.77	16.74
5	19.84	17.20	17.91	16.74
6	18.73	16.60	24.02	16.48
7	18.74	16.81	23.78	16.75
8	18.87	17.49	20.14	17.17
9	18.38	14.98	19.75	16.20
10	19.20	17.01	17.24	16.52
Average	18.96	16.62	19.89	16.69
SD	0.47	0.86	2.59	0.48
%CV	2.49	5.16	13.00	2.87

Table 19B Hardness of chewable tablets containing *Phyllanthus emblica* Linn. spray dried fruit extract stored under 40°C and 75%RH for 3 months.

No.	Hardness (KP)							
	A1	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
1	15.87	29.03	25.02	27.26	18.9	26.42	28.16	25.33
2	15.72	30.7	24.02	26.51	18.69	26.83	26.65	26.70
3	17.08	26.38	22.66	23.45	18.85	25.03	28.91	25.64
4	15.53	27.68	22.17	27.17	18.24	26.29	26.81	23.65
5	15.59	29.61	26.90	27.03	19.24	24.56	28.64	25.77
6	15.00	31.02	25.06	24.84	19.73	27.57	26.14	23.92
7	15.45	25.85	25.23	25.37	19.71	24.76	27.45	25.50
8	15.16	32.21	25.99	25.46	20.49	25.71	25.82	22.38
9	15.70	29.43	25.76	25.82	19.02	24.77	25.68	24.15
10	15.21	29.66	23.80	24.35	18.63	26.89	25.97	22.26
Average	15.63	29.16	24.66	25.73	19.15	25.88	27.02	24.53
SD	0.58	2.02	1.49	1.28	0.66	1.07	1.20	1.50
% CV	3.70	6.91	6.04	4.99	3.45	4.12	4.44	6.10

Table 20B Hardness of chewable tablets containing coated *Phyllanthus emblica* Linn. fruit extract stored under 40°C and 75%RH for 3months.

No.	Hardness (KP)			
	B1	**B2**	**B3**	**B4**
1	5.37	13.53	13.28	4.12
2	5.53	13.05	12.06	4.38
3	5.93	12.72	11.10	3.83
4	4.77	13.11	11.07	4.33
5	5.47	13.56	11.80	3.65
6	4.92	12.23	11.37	4.27
7	5.39	12.58	12.21	4.69
8	5.04	12.84	11.83	4.90
9	5.09	12.70	11.72	4.47
10	4.65	11.86	11.72	4.18
Average	5.22	12.82	11.82	4.28
SD	0.39	0.53	0.64	0.37
%CV	7.51	4.15	5.39	8.66

Table 21B Percent friabiity of chewable tablets containing *Phyllanthus emblica* Linn. spray dried fruit extract after preparation.

Formulation	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
% Friability	0.96	0.11	0.65	0.48	0.44	0.69	1.14	0.84

Table 22B Percent friabiity of chewable tablets containing coated *Phyllanthus emblica* Linn. fruit extract after preparation.

Formulation	**B1**	**B2**	**B3**	**B4**
% Friability	1.08	0.56	0.65	1.16

Table 23B Percent friability of chewable tablets containing *Phyllanthus emblica* Linn. spray dried fruit extract stored under refrigeration for 1 month, 2 months and 3 months.

Month	**% Friability**							
	A1	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
1	1.25	0.35	0.89	0.65	0.55	0.96	1.45	0.92
2	1.47	0.28	0.78	0.62	0.65	0.92	1.52	0.95
3	1.41	0.31	0.71	0.63	0.65	0.92	1.61	0.87

Table 24B Percent friability of chewable tablets containing coated *Phyllanthus emblica* Linn. fruit extract stored under refrigeration for 1 month, 2 months and 3 months.

Month	**% Friability**			
	B1	**B2**	**B3**	**B4**
1	0.96	0.60	0.68	1.18
2	0.99	0.66	0.66	1.17
3	1.04	0.58	0.73	1.20

Table 25B Percent friability of chewable tablets containing *Phyllanthus emblica* Linn. spray dried fruit extract stored under ambient condition for 1 month, 2 months and 3 months.

Month	% Friability							
	A1	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
1	1.00	0.33	0.60	0.61	0.54	1.06	1.47	1.35
2	0.99	0.27	0.47	0.54	0.67	0.93	1.40	1.41
3	0.73	0.40	0.34	0.81	0.34	0.93	1.34	1.28

Table 26B Percent friability of chewable tablets containing coated *Phyllanthus emblica* Linn. fruit extract stored under ambient condition for 1 month, 2 months and 3 months.

Month	% Friability			
	B1	**B2**	**B3**	**B4**
1	0.86	0.48	0.60	1.10
2	0.88	0.50	0.59	1.06
3	1.04	0.58	0.62	1.12

Table 27B Percent friability of chewable tablets containing *Phyllanthus emblica* Linn. spray dried fruit extract stored under ambient temperature and 75%RH for 1 month, 2 months and 3 months.

Month	% Friability							
	A1	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
1	0.54	0.66	0.54	0.87	0.94	1.33	1.34	1.47
2	0.60	0.67	0.34	1.07	0.86	1.19	1.07	1.47
3	0.40	0.46	0.61	0.67	0.54	1.13	0.94	1.00

Table 28B Percent friability of chewable tablets containing coated *Phyllanthus emblica* Linn. fruit extract stored under ambient temperature and 75%RH for 1 month, 2 months and 3 months.

Month	%Friability			
	B1	B2	B3	B4
1	0.96	0.48	0.60	1.10
2	1.05	0.50	0.58	1.06
3	0.92	0.40	0.45	1.01

Table 29B Percent friability of chewable tablets containing *Phyllanthus emblica* Linn. spray dried fruit extract stored under 40°C and 75%RH for 1 month, 2 months and 3 months.

Month	%Friability							
	A1	A2	A3	A4	A5	A6	A7	A8
1	0.02	0.02	0.04	0.04	0.01	0.11	0.19	0.20
2	0.01	0.13	0.01	0.00	0.01	0.01	0.01	0.11
3	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00

Table 30B Percent friability of chewable tablets containing coated *Phyllanthus emblica* Linn. fruit extract stored under 40°C and 75%RH for 1 month, 2 months and 3 months.

Month	%Friability			
	B1	B2	B3	B4
1	0.96	0.63	0.66	1.18
2	0.99	0.62	0.65	1.20
3	1.04	0.58	0.62	1.17

Table 31B Percent loss on drying of uncoated and coated *Phyllanthus emblica* Linn. fruit extract; and chewable tablets containing uncoated or coated extract after preparation.

Formulation	% LOD				
	n1	n2	n3	Average	SD
A1	1.95	2.16	2.16	2.09	0.12
A2	1.82	1.84	1.81	1.82	0.02
A3	1.83	1.82	1.78	1.81	0.03
A4	1.82	1.49	1.89	1.73	0.21
A5	1.99	1.94	2.16	2.03	0.12
A6	2.01	2.07	2.04	2.04	0.03
A7	1.78	2.07	1.71	1.85	0.19
A8	1.77	1.80	1.79	1.79	0.02
B1	2.21	1.98	2.11	2.10	0.12
B2	1.83	1.96	1.86	1.88	0.07
B3	1.88	2.08	2.10	2.02	0.12
B4	2.15	2.51	2.28	2.31	0.18
Extract batch no. 08122006	1.99	2.10	2.08	2.06	0.06
Extract batch no. 28122006	1.89	1.96	2.09	1.98	0.10
Coated extract batch no. 28122006	4.39	4.61	4.76	4.59	0.19

Table 32B Percent loss on drying of uncoated and coated *Phyllanthus emblica* Linn. fruit extract; and chewable tablets containing uncoated or coated extract stored under refrigeration for 1 month, 2 months and 3 months.

Formulation	% LOD					
	1 month		2 months		3 months	
	Mean	SD	Mean	SD	Mean	SD
A1	1.81	0.16	1.83	0.06	1.87	0.02
A2	1.77	0.04	1.94	0.02	1.91	0.08
A3	1.80	0.08	1.80	0.14	1.86	0.10
A4	1.92	0.08	1.75	0.13	1.71	0.04
A5	1.78	0.04	1.80	0.11	1.80	0.06
A6	1.69	0.13	1.68	0.17	1.70	0.16
A7	1.47	0.08	1.63	0.07	1.72	0.24
A8	1.47	0.08	1.57	0.18	1.62	0.05
B1	2.12	0.15	2.08	0.07	2.04	0.08
B2	1.74	0.01	1.78	0.10	1.89	0.06
B3	1.96	0.12	1.91	0.15	2.06	0.07
B4	2.22	0.12	2.27	0.13	2.15	0.06
Extract batch no. 08122006	1.99	0.07	2.12	0.16	2.09	0.29
Extract batch no. 28122006	1.96	0.17	2.08	0.25	2.15	0.42
Coated extract batch no. 28122006	4.63	0.03	4.57	0.05	4.60	0.05

Table 33B Percent loss on drying of uncoated and coated *Phyllanthus emblica* Linn. fruit extract; and chewable tablets containing uncoated or coated extract stored under ambient condition for 1 month, 2 months and 3 months.

Formulation	% LOD					
	1 month		2 months		3 months	
	Mean	SD	Mean	SD	Mean	SD
A1	1.79	0.15	1.81	0.07	1.83	0.18
A2	1.74	0.01	1.78	0.10	1.85	0.14
A3	1.79	0.12	1.83	0.15	1.82	0.12
A4	1.77	0.12	1.86	0.13	1.86	0.10
A5	1.77	0.03	1.80	0.05	1.80	0.10
A6	1.81	0.42	1.70	0.08	1.90	0.29
A7	1.48	0.08	1.60	0.08	1.55	0.14
A8	1.37	0.04	1.51	0.07	1.61	0.05
B1	2.13	0.12	2.18	0.07	2.15	0.18
B2	1.85	0.10	1.93	0.15	1.90	0.14
B3	1.99	0.20	1.97	0.15	2.03	0.23
B4	2.25	0.12	2.32	0.23	2.27	0.15
Extract batch no. 08122006	2.07	0.24	2.03	0.16	1.96	0.09
Extract batch no. 28122006	2.09	0.36	2.08	0.21	2.04	0.17
Coated extract batch no. 28122006	4.46	0.09	4.42	0.29	4.55	0.18

Table 34B Percent loss on drying of uncoated and coated *Phyllanthus emblica* Linn. fruit extract; and chewable tablets containing uncoated or coated extract stored under ambient temperature and 75%RH for 1 month, 2 months and 3 months.

Formulation	% LOD					
	1 month		2 months		3 months	
	Mean	SD	Mean	SD	Mean	SD
A1	1.65	0.02	1.71	0.10	1.75	0.10
A2	1.72	0.05	1.74	0.11	1.75	0.11
A3	1.61	0.09	1.63	0.11	1.63	0.11
A4	1.82	0.16	1.79	0.06	1.81	0.06
A5	1.87	0.09	1.89	0.08	1.88	0.08
A6	1.93	0.28	1.56	0.09	1.68	0.09
A7	1.47	0.09	1.57	0.08	1.61	0.08
A8	1.28	0.04	1.30	0.07	1.28	0.07
B1	2.13	0.15	2.09	0.07	2.11	0.18
B2	1.96	0.09	1.85	0.10	1.87	0.14
B3	2.05	0.07	1.99	0.15	1.96	0.12
B4	2.34	0.12	2.28	0.23	2.22	0.15
Extract batch no. 08122006	1.96	0.09	2.04	0.16	2.10	0.23
Extract batch no. 28122006	1.99	0.21	1.96	0.12	2.08	0.30
Coated extract batch no. 28122006	4.48	0.06	4.51	0.12	4.53	0.16

Table 35B Percent loss on drying of uncoated and coated *Phyllanthus emblica* Linn. fruit extract; and chewable tablets containing uncoated or coated extract stored under 40°C and 75%RH for 1 month, 2 months and 3 months.

Formulation	% LOD					
	1 month		2 months		3 months	
	Mean	SD	Mean	SD	Mean	SD
A1	1.91	0.03	2.21	0.07	1.93	0.19
A2	1.69	0.23	1.75	0.16	1.91	0.10
A3	1.85	0.03	2.18	0.11	2.32	0.10
A4	1.87	0.07	2.06	0.23	2.07	0.13
A5	2.05	0.05	2.40	0.07	2.57	0.01
A6	1.95	0.07	1.81	0.60	2.14	0.06
A7	1.80	0.05	2.00	0.24	2.34	0.06
A8	1.80	0.02	1.78	0.05	2.25	0.10
B1	2.06	0.15	2.09	0.07	2.07	0.12
B2	1.79	0.10	1.78	0.10	1.89	0.14
B3	1.93	0.12	1.98	0.15	2.05	0.13
B4	2.21	0.12	2.29	0.13	2.18	0.14
Extract batch no. 08122006	2.10	0.18	2.17	0.38	2.12	0.12
Extract batch no. 28122006	2.05	0.26	2.03	0.45	2.11	0.06
Coated extract batch no. 28122006	4.66	0.10	4.55	0.10	4.65	0.15

Table 36B pH of the solutions of uncoated and coated *Phyllanthus emblica* Linn. fruit extract; and chewable tablets containing uncoated or coated extract after preparation.

Formulation	pH				
	n1	n2	n3	Average	SD
A1	2.97	2.95	2.96	2.96	0.01
A2	3.36	3.31	3.32	3.33	0.03
A3	3.15	3.13	3.13	3.14	0.01
A4	3.43	3.38	3.39	3.40	0.03
A5	3.16	3.17	3.16	3.16	0.01
A6	3.39	3.36	3.36	3.37	0.02
A7	3.21	3.22	3.22	3.22	0.01
A8	3.50	3.49	3.49	3.49	0.01
B1	3.13	3.13	3.14	3.13	0.01
B2	3.70	3.68	3.64	3.67	0.03
B3	3.39	3.30	3.32	3.34	0.05
B4	3.40	3.34	3.36	3.37	0.03
Extract batch no. 08122006	4.32	4.24	4.23	4.26	0.05
Extract batch no. 28122006	4.71	4.58	4.54	4.61	0.09
Coated extract batch no. 28122006	4.10	4.06	4.04	4.07	0.03

Table 37B pH of the solutions of uncoated and coated *Phyllanthus emblica* Linn. fruit extract; and chewable tablets containing uncoated or coated extract stored under refrigeration for 1 month, 2 months and 3 months.

Formulation	pH					
	1 month		2 months		3 months	
	Mean	SD	Mean	SD	Mean	SD
A1	3.35	0.02	3.46	0.01	3.62	0.03
A2	3.96	0.03	4.06	0.03	4.42	0.02
A3	3.40	0.01	3.49	0.01	3.65	0.02
A4	3.93	0.02	4.15	0.03	4.44	0.05
A5	3.46	0.00	3.53	0.05	3.75	0.02
A6	4.08	0.04	4.08	0.01	4.64	0.09
A7	3.50	0.01	3.60	0.01	3.73	0.01
A8	4.14	0.03	4.24	0.02	4.65	0.05
B1	3.35	0.01	3.46	0.09	3.62	0.04
B2	3.96	0.03	3.87	0.10	3.75	0.08
B3	3.40	0.03	3.45	0.05	3.39	0.09
B4	3.32	0.04	3.42	0.04	3.51	0.04
Extract batch no. 08122006	4.54	0.01	4.41	0.03	4.47	0.07
Extract batch no. 28122006	4.53	0.06	4.52	0.04	4.61	0.06
Coated extract batch no. 28122006	4.10	0.06	4.02	0.07	4.09	0.05

Table 38B pH of the solutions of uncoated and coated *Phyllanthus emblica* Linn. fruit extract; and chewable tablets containing uncoated or coated extract stored under ambient condition for 1 month, 2 months and 3 months.

Formulation	pH					
	1 month		2 months		3 months	
	Mean	SD	Mean	SD	Mean	SD
A1	3.73	0.01	3.68	0.06	3.76	0.02
A2	4.60	0.06	4.54	0.06	4.63	0.05
A3	3.78	0.03	3.60	0.01	3.74	0.03
A4	4.56	0.08	4.45	0.06	4.58	0.07
A5	3.75	0.02	3.76	0.02	3.77	0.01
A6	4.68	0.06	4.70	0.03	4.78	0.03
A7	3.78	0.02	3.74	0.02	3.76	0.02
A8	4.68	0.06	4.71	0.08	4.71	0.06
B1	3.10	0.06	3.17	0.05	3.13	0.04
B2	3.72	0.06	3.79	0.07	3.86	0.03
B3	3.54	0.07	3.48	0.08	3.39	0.08
B4	3.38	0.08	3.32	0.08	3.43	0.01
Extract batch no. 08122006	4.36	0.06	4.57	0.01	4.62	0.06
Extract batch no. 28122006	4.53	0.07	4.57	0.02	4.53	0.06
Coated extract batch no. 28122006	4.15	0.02	4.09	0.03	4.17	0.02

Table 39B pH of the solutions of uncoated and coated *Phyllanthus emblica* Linn. fruit extract; and chewable tablets containing uncoated or coated extract stored under ambient temperature and 75%RH for 1 month, 2 months and 3 months.

Formulation	pH					
	1 month		2 months		3 months	
	Mean	SD	Mean	SD	Mean	SD
A1	3.69	0.05	3.75	0.02	3.77	0.04
A2	4.58	0.09	4.75	0.07	4.62	0.01
A3	3.69	0.02	3.76	0.01	3.69	0.01
A4	4.68	0.06	4.72	0.08	4.71	0.06
A5	3.74	0.02	3.89	0.03	3.86	0.04
A6	4.82	0.06	5.01	0.07	4.95	0.08
A7	3.78	0.03	3.82	0.02	3.82	0.01
A8	4.83	0.09	4.80	0.06	4.99	0.06
B1	3.22	0.10	3.15	0.08	3.09	0.11
B2	3.87	0.12	3.97	0.06	3.65	0.13
B3	3.45	0.09	3.32	0.04	3.34	0.07
B4	3.47	0.10	3.51	0.10	3.35	0.04
Extract batch no. 08122006	5.06	0.04	4.49	0.06	4.26	0.04
Extract batch no. 28122006	4.61	0.08	5.32	0.03	4.45	0.06
Coated extract batch no. 28122006	4.23	0.05	4.06	0.12	4.16	0.08

Table 40B pH of the solutions of uncoated and coated *Phyllanthus emblica* Linn. fruit extract; and chewable tablets containing uncoated or coated extract stored under 40°C and 75%RH for 1 month, 2 months and 3 months.

Formulation	pH					
	1 month		2 months		3 months	
	Mean	SD	Mean	SD	Mean	SD
A1	3.11	0.02	3.08	0.01	3.33	0.02
A2	3.34	0.04	3.30	0.01	3.55	0.01
A3	3.14	0.03	3.20	0.03	3.37	0.01
A4	3.37	0.03	3.48	0.01	3.63	0.02
A5	3.15	0.02	3.32	0.01	3.34	0.01
A6	3.25	0.02	3.53	0.01	3.58	0.01
A7	3.15	0.02	3.31	0.01	3.39	0.01
A8	3.42	0.04	3.61	0.01	3.70	0.01
B1	3.23	0.05	3.15	0.08	3.06	0.06
B2	3.72	0.06	3.69	0.06	3.73	0.04
B3	3.42	0.08	3.44	0.05	3.39	0.03
B4	3.25	0.02	3.27	0.02	3.34	0.02
Extract batch no. 08122006	4.45	0.08	4.18	0.05	5.06	0.06
Extract batch no. 28122006	4.80	0.11	4.93	0.01	5.06	0.06
Coated extract batch no. 28122006	4.11	0.15	4.05	0.01	4.15	0.06

Table 41B Amount of total tannins and gallic acid in uncoated and coated *Phyllanthus emblica* Linn. fruit extract; and chewable tablets containing uncoated or coated extract after preparation.

Formulation	% Gallic acid		% Total Tannins	
	Mean	**SD**	**Mean**	**SD**
A1	0.55	0.01	22.77	0.68
A2	0.53	0.02	22.22	0.11
A3	0.54	0.01	22.63	0.21
A4	0.52	0.02	22.96	0.04
A5	0.50	0.02	21.06	0.86
A6	0.49	0.01	21.65	1.05
A7	0.64	0.01	20.18	0.48
A8	0.64	0.01	20.67	0.71
B1	0.79	0.02	23.06	0.52
B2	0.90	0.06	23.36	0.24
B3	0.76	0.00	22.67	0.52
B4	0.76	0.00	23.50	0.71
Extract batch no. 08122006	0.61	0.02	25.64	0.45
Extract batch no. 28122006	0.76	0.06	25.07	0.36
Coated extract batch no. 28122006	0.72	0.03	25.41	0.25

Table 42B Amount of total tannins and gallic acid in uncoated and coated *Phyllanthus emblica* Linn. fruit extract; and chewable tablets containing uncoated or coated extract stored under refrigeration for 1 month, 2 months and 3 months.

Formulation	% Gallic acid						% Total Tannins					
	1 month		2 months		3 months		1 month		2 months		3 months	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
A1	0.48	0.01	0.48	0.03	0.53	0.03	23.26	0.88	23.14	0.65	21.34	0.53
A2	0.47	0.01	0.44	0.01	0.51	0.01	23.23	0.45	22.40	0.36	21.43	0.05
A3	0.50	0.02	0.45	0.00	0.50	0.00	23.02	0.50	22.60	0.29	21.60	0.10
A4	0.49	0.00	0.45	0.00	0.50	0.01	23.90	0.26	22.25	0.80	21.71	0.14
A5	0.49	0.01	0.45	0.00	0.49	0.01	22.11	0.92	19.09	0.35	19.65	0.52
A6	0.47	0.00	0.44	0.00	0.46	0.01	22.29	2.60	17.91	1.53	18.24	0.42
A7	0.61	0.01	0.56	0.00	0.59	0.01	21.95	1.08	18.64	0.75	18.09	0.52
A8	0.61	0.00	0.58	0.01	0.59	0.01	24.18	0.70	19.83	0.26	19.05	0.37
B1	0.75	0.00	0.79	0.02	0.79	0.01	21.29	0.20	20.65	0.44	21.21	0.24
B2	0.75	0.01	0.87	0.08	0.82	0.01	22.08	0.24	21.72	0.22	21.66	0.25
B3	0.82	0.07	0.76	0.01	0.89	0.13	20.03	1.88	20.17	0.41	20.93	0.67
B4	0.92	0.01	0.77	0.01	1.01	0.00	21.54	0.66	21.07	0.43	21.54	0.38
Extract batch no. 08122006	2.06	0.17	1.94	0.03	0.60	0.12	26.48	0.49	25.21	0.03	24.34	0.14
Extract batch no. 28122006	2.62	0.37	2.32	0.10	0.80	0.06	25.55	0.56	24.46	0.53	23.11	0.77
Coated extract batch no. 28122006	1.07	0.03	0.80	0.02	0.78	0.03	23.13	0.44	22.68	0.31	22.85	0.26

Table 43B Amount of total tannins and gallic acid in uncoated and coated *Phyllanthus emblica* Linn. fruit extract; and chewable tablets containing uncoated or coated extract stored under ambient condition for 1 month, 2 months and 3 months.

Formulation	% Gallic acid						% Total Tannins					
	1 month		2 months		3 months		1 month		2 months		3 months	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
A1	0.54	0.03	0.51	0.03	0.58	0.00	23.04	0.22	23.49	0.57	23.42	0.56
A2	0.49	0.01	0.47	0.01	0.52	0.03	22.82	0.34	23.98	0.94	23.40	0.32
A3	0.50	0.01	0.48	0.01	0.55	0.00	22.44	0.49	23.68	0.21	23.42	0.17
A4	0.51	0.01	0.48	0.01	0.55	0.01	22.18	0.14	23.80	0.46	23.51	0.39
A5	0.49	0.00	0.51	0.01	0.54	0.00	17.84	0.08	21.90	1.35	22.45	1.72
A6	0.48	0.01	0.49	0.02	0.48	0.01	19.85	0.72	21.97	1.94	22.62	1.83
A7	0.62	0.01	0.55	0.01	0.61	0.04	19.19	1.95	22.82	0.29	23.73	0.17
A8	0.62	0.00	0.53	0.01	0.65	0.01	17.66	1.31	23.99	0.27	24.02	0.29
B1	0.90	0.04	0.90	0.02	0.93	0.01	21.19	0.31	22.19	0.38	21.32	0.11
B2	0.88	0.02	0.91	0.07	0.94	0.03	21.90	0.01	22.92	0.18	22.04	0.16
B3	1.07	0.01	0.83	0.01	0.96	0.03	20.97	0.42	22.13	0.17	21.21	0.34
B4	0.90	0.01	0.85	0.00	0.98	0.02	21.72	0.15	22.36	0.41	21.51	0.41
Extract batch no. 08122006	2.03	0.20	1.82	0.10	0.80	0.07	24.49	0.61	26.08	0.34	25.61	0.61
Extract batch no. 28122006	2.29	0.26	2.07	0.08	0.98	0.01	24.27	0.46	24.99	0.50	24.51	0.36
Coated extract batch no. 28122006	0.87	0.02	0.92	0.05	0.92	0.02	23.55	0.39	23.70	0.43	23.92	0.62

Table 44B Amount of total tannins and gallic acid in uncoated and coated *Phyllanthus emblica* Linn. fruit extract; and chewable tablets containing uncoated or coated extract stored under ambient temperature and 75%RH for 1 month, 2 months and 3 months.

Formulation	% Gallic acid						% Total Tannins					
	1 month		2 months		3 months		1 month		2 months		3 months	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
A1	0.53	0.03	0.49	0.03	0.56	0.03	23.70	0.59	23.02	1.57	22.82	1.57
A2	0.49	0.02	0.51	0.01	0.51	0.01	24.41	0.13	23.41	0.34	23.32	0.34
A3	0.51	0.01	0.50	0.01	0.53	0.01	24.16	0.47	23.64	0.36	22.52	0.36
A4	0.52	0.01	0.52	0.00	0.58	0.00	24.12	0.55	23.98	0.03	23.63	0.03
A5	0.51	0.02	0.51	0.01	0.56	0.01	22.73	1.26	22.32	1.06	21.09	1.06
A6	0.49	0.00	0.47	0.01	0.50	0.01	21.20	0.78	21.78	0.72	23.74	0.72
A7	0.60	0.01	0.57	0.05	0.64	0.05	20.78	0.73	22.14	0.49	22.71	0.49
A8	0.59	0.01	0.59	0.05	0.64	0.05	23.41	0.12	23.73	0.37	23.56	0.37
B1	0.87	0.03	0.91	0.05	0.98	0.05	21.15	0.13	20.28	0.21	21.38	0.26
B2	0.92	0.02	1.01	0.00	1.01	0.00	21.96	0.29	21.58	0.33	22.02	0.34
B3	0.95	0.02	1.00	0.01	1.02	0.01	20.54	0.98	20.63	0.31	20.80	0.39
B4	0.94	0.01	1.03	0.01	1.04	0.01	21.21	0.10	20.62	0.22	21.48	0.17
Extract batch no. 08122006	2.09	0.22	1.75	0.14	0.62	0.14	26.44	0.18	25.88	0.36	24.79	0.36
Extract batch no. 28122006	2.41	0.16	2.03	0.03	0.75	0.03	25.87	0.55	25.51	0.23	23.98	0.23
Coated extract batch no. 28122006	1.16	0.02	1.23	0.06	1.19	0.09	23.56	0.30	22.48	0.92	23.55	0.39

Table 45B Amount of total tannins and gallic acid in uncoated and coated *Phyllanthus emblica* Linn. fruit extract; and chewable tablets containing uncoated or coated extract stored under 40°C and 75%RH for 1 month, 2 months and 3 months.

Formulation	% Gallic acid						% Total Tannins					
	1 month		2 months		3 months		1 month		2 months		3 months	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
A1	0.79	0.02	1.31	0.01	0.60	0.03	22.12	0.18	22.45	0.22	20.56	0.20
A2	0.68	0.00	1.15	0.01	0.47	0.01	21.84	0.16	22.40	0.74	21.01	0.37
A3	0.73	0.02	1.28	0.02	0.55	0.01	22.69	0.25	21.73	0.19	20.91	0.22
A4	0.74	0.01	1.15	0.04	0.48	0.01	22.73	0.44	21.82	0.17	20.45	0.44
A5	0.71	0.01	1.30	0.00	0.51	0.00	22.02	0.85	18.06	1.23	17.17	0.55
A6	0.65	0.04	1.12	0.00	0.45	0.01	20.85	0.35	18.62	0.39	22.87	0.77
A7	0.77	0.01	1.25	0.01	0.55	0.02	19.42	1.48	16.78	0.72	21.69	0.51
A8	0.79	0.02	1.18	0.00	0.56	0.00	21.34	2.07	17.25	0.54	21.83	1.28
B1	1.59	0.11	2.13	0.10	2.49	0.01	21.72	0.47	21.32	0.25	21.77	0.16
B2	1.35	0.05	1.98	0.10	2.44	0.14	22.19	0.12	21.59	0.07	22.28	0.21
B3	1.37	0.08	1.95	0.04	2.40	0.01	20.40	1.47	20.80	0.09	21.30	0.30
B4	1.41	0.01	2.03	0.02	2.50	0.01	22.45	1.23	21.21	0.45	21.65	0.09
Extract batch no. 08122006	0.72	0.01	1.09	0.06	1.53	0.08	24.69	0.21	23.76	0.42	23.77	0.14
Extract batch no. 28122006	0.88	0.01	1.00	0.20	1.58	0.04	23.52	0.61	22.90	0.36	23.35	0.33
Coated extract batch no. 28122006	1.19	0.04	2.01	0.05	2.28	0.11	24.06	0.72	23.21	0.48	23.59	0.27

Table 46B Total aerobic bacterial count of *Phyllanthus emblica* Linn. fruit extract and chewable tablets formulations A1, A2, B1 and B4.

Sample	Dilution	Total Aerobic Microbial Count (CFU/plate)			Average (CFU/g)
		Plate 1	Plate 2	Plate 3	
Batch No. 08122006	1:10	ND	ND	ND	
	$1:10^2$	> 300	> 300	> 300	
	$1:10^3$	83	89	92	$8.8x10^4$
	$1:10^4$	7	5	6	
Batch No. 28122006	1:10	196	187	208	$2.0x10^3$
	$1:10^2$	18	22	21	
	$1:10^3$	2	2	0	
	$1:10^4$	0	0	0	
A1	1:10	ND	ND	ND	
	$1:10^2$	51	53	55	$5.3x10^3$
	$1:10^3$	8	6	3	
	$1:10^4$	0	0	0	
A2	1:10	ND	ND	ND	
	$1:10^2$	53	59	58	$5.7x10^3$
	$1:10^3$	5	4	5	
	$1:10^4$	0	0	0	
B1	1:10	ND	ND	ND	
	$1:10^2$	38	37	33	$3.6x10^3$
	$1:10^3$	7	3	3	
	$1:10^4$	1	0	0	
B4	1:10	ND	ND	ND	
	$1:10^2$	41	35	38	$3.8x10^3$
	$1:10^3$	4	2	3	
	$1:10^4$	0	0	0	

ND = No determination

* determined at 30-300 CFU/plate

Table 47B Total fungal count of *Phyllanthus emblica* Linn. fruit extract and chewable tablets formulations A1, A2, B1 and B4.

Sample	Dilution	Total fungal Counts (CFU/plate)			Average (CFU/g)
		Plate 1	Plate 2	Plate 3	
Batch No. 08122006	1:2.5	18	23	21	52
	1:5	9	9	10	
	1:10	5	4	4	
Batch No. 28122006	1:2.5	55	60	62	148
	1:5	27	28	30	
	1:10	12	13	14	
A1	1:2.5	51	49	45	121
	1:5	12	9	11	
	1:10	5	4	5	
A2	1:2.5	31	30	34	79
	1:5	18	16	19	
	1:10	10	9	10	
B1	1:2.5	8	8	7	19
	1:5	0	1	1	
	1:10	0	0	0	
B4	1:2.5	55	59	60	145
	1:5	29	28	28	
	1:10	16	15	15	

Table 48B The results of the testing for specified microorganisms of *Phyllanthus emblica* Linn. fruit extract and chewable tablets formulations A1, A2, B1 and B4.

Sample	Specified microorganism					
	Enterobacteriaceae	*Salmonella* spp.	*E. coli*	*S. aureus*	*P. aeruginosa*	*Clostridium* spp.
Batch No.0812006	absent	absent	absent	absent	absent	absent
Batch No.2812006	absent	absent	absent	absent	absent	absent
A1	absent	absent	absent	absent	absent	absent
A2	absent	absent	absent	absent	absent	absent
B1	absent	absent	absent	absent	absent	absent
B4	absent	absent	absent	absent	absent	absent

VITA

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