การพัฒนาแกรนูลฟู่และเพลเลต ผสมสารสกัดจากผลมะขามป้อม

นางสาวพัชรินทร์ กาญจนโชติดำรง

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DEVELOPMENT OF EFFERVESCENT GRANULES AND PELLETS CONTAINING *Phyllanthus emblica* L. FRUIT EXTRACT

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พัชรินทร์ กาญจนโชติดำรง : การพัฒนาแกรนูลฟู่และเพลเลต ผสมสารสกัดจากผลมะขามป้อม (DEVELOPMENT OF EFFERVESCENT GRANULES AND PELLETS CONTAINING *Phyllanthus emblica* L. FRUIT EXTRACT) อ. ที่ปรึกษา : ดร.จิตติมา ชัชวาลย์สายสินธ์, อ. ที่ปรึกษาร่วม : รศ.ดร.พจน์ กุลวานิช, 174 หน้า.

มะขามป้อม (*Phyllantus emblica* L.) เป็นพืชในวงศ์ Euphobiaceae เป็นไม้พื้นเมืองในแถบเอเชียตะวันออกเฉียงใต้ มีรายงานฤทธิ์ทางเภสัชวิทยาของมะขามป้อม เช่น ฤทธิ์ต้านอนุมูลอิสระ แก้อักเสบ ลดระดับไขมัน ป้องกันเซลล์ตับบรรเทาอาการไอ ลดไข้ และอาการเจ็บปวด เป็นต้น วัตถุประสงค์ของการศึกษานี้คือ พัฒนาแกรนูลฟู่และเพลเลตผสมสารสกัดจากผลมะขามป้อม และทดสอบคุณสมบัติทางเคมีกายภาพและความคงตัวของผลิตภัณฑ์ สารสกัดจากผลมะขามป้อมที่ใช้อยู่ในรูปผงพ่นแห้ง การทดสอบคุณสมบัติทางเคมีกายภาพและปริมาณจุลินทรีย์ของสารสกัด พบว่า สารสกัดมีการไหลที่ไม่ดี สารละลายของสารสกัดที่มีความเข้มข้น 1 เปอร์เซ็นต์ มีความเป็นกรด-ด่าง ประมาณ 3 และปริมาณเชื้อจุลินทรีย์ของสารสกัดเป็นไปตามข้อกำหนดของเภสัชตำรับประเทศไทย

แกรนูลฟู่เตรียมโดยผสมแห้งแกรนูลของสารสกัด อะซีซัลเฟมโพแทสเซียม และแมนนิทอล ร่วมกับกรดซิตริกและด่าง ศึกษาผลของตัวแปรสูตรตำรับ ได้แก่ ปริมาณของสารสกัด ปริมาณกรดซิตริก ปริมาณคอลลอยดอลซิลิกอนไดออกไซด์ และชนิดของด่าง ต่อคุณสมบัติของแกรนูลฟู่ ตามรูปแบบจุดศูนย์กลางของความถ่วง (center of gravity design) แกรนูลฟู่ที่ได้มีสีค่อนข้างเหลือง ไหลได้ดี และสามารถกระจายในน้ำภายใน 5 นาที ภายหลังการเก็บที่ 40±2 องศาเซลเซียส ความชื้นสัมพัทธ์ 75 เปอร์เซ็นต์ และ 30±2 องศาเซลเซียส ความชื้นสัมพัทธ์ 75 เปอร์เซ็นต์ หลายสูตรตำรับจะเกิดการจับตัวแน่น และ/หรือ เกิดการรวมมวลเป็นกลุ่มใหญ่ การเปลี่ยนแปลงของคุณสมบัติทางกายภาพไม่สัมพันธ์โดยตรงกับการเปลี่ยนแปลงของปริมาณแทนนินและปริมาณกรดแกลลิกในสูตรตำรับ และยังไม่ทราบสาเหตุของการเปลี่ยนแปลงนี้ การวิเคราะห์ความแปรปรวนพบว่ามีผลหลักและผลร่วมของตัวแปรสูตรตำรับ ต่อปริมาณความชื้น ปริมาณแทนนิน และกรดแกลลิก อย่างมีนัยสำคัญ ($p<0.05$)

เพลเลตผสมสารสกัด อะซีซัลเฟมโพแทสเซียม กรดซิตริก และ แมนนิทอล เตรียมโดยเทคนิคการอัดเป็นเส้นและทำให้เป็นทรงกลม โดยการทดลองรูปแบบแฟคทอเรียล (full factorial design) พบว่า ในการขึ้นรูปเพลเลต มวลเปียกที่มีสารสกัดปริมาณน้อยเท่ากับ 20 เปอร์เซ็นต์ ต้องการปริมาณน้ำมากกว่ามวลเปียกที่มีสารสกัดปริมาณมากเท่ากับ 40 เปอร์เซ็นต์ เพลเลตที่มีสารสกัดปริมาณน้อยมีรูปร่างค่อนข้างกลม ในขณะที่เพลเลตที่มีสารสกัดปริมาณมากคงเป็นรูปร่างดัมเบลล์ ภายหลังการเก็บที่ 40±2 องศาเซลเซียส ความชื้นสัมพัทธ์ 75 เปอร์เซ็นต์ และ 30±2 องศาเซลเซียส ความชื้นสัมพัทธ์ 75 เปอร์เซ็นต์ เพลเลตที่มีสารสกัดปริมาณมากมีการเปลี่ยนสีที่ชัดเจน พบว่ามีผลหลักและผลร่วมของปริมาณสารสกัด ปริมาณอะซีซัลเฟมโพแทสเซียม และปริมาณกรดซิตริก ต่อปริมาณกรดแกลลิก อย่างมีนัยสำคัญ ($p<0.05$) ปริมาณของสารเหล่านี้ยกเว้นปริมาณสารสกัดมีผลต่อปริมาณแทนนินอย่างมีนัยสำคัญ ($p<0.05$) ด้วย

ผลการทดลองแสดงให้เห็นว่ามีความเป็นไปได้ในการพัฒนาแกรนูลฟู่และเพลเลตผสมสารสกัดจากผลมะขามป้อม โดยความสำเร็จในการพัฒนาส่วนหนึ่งขึ้นกับคุณสมบัติของสารสกัด ดังที่สะท้อนให้เห็นในความคงตัวทางกายภาพของแกรนูลฟู่หลายสูตรที่มีปริมาณสารสกัด 30 เปอร์เซ็นต์ และ เพลเลตที่มีสารสกัด 20 เปอร์เซ็นต์ ทั้งนี้ยังคงมีความจำเป็นในการศึกษาผลกระทบของสารสกัด และตัวแปรสูตรตำรับ ต่อความคงตัวของผลิตภัณฑ์ต่อไป

ภาควิชา.....เภสัชอุตสาหกรรม..... ลายมือชื่อนิสิต.....พัชรินทร์ กาญจนโชติดำรง.....

สาขาวิชา.....เภสัชอุตสาหกรรม..... ลายมือชื่ออาจารย์ที่ปรึกษา.....จิตติมา ชัชวาลย์สายสินธ์

ปีการศึกษา.....2550..... ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

4776586833 : MAJOR MANUFACTURING PHARMACY
KEY WORD : *Phyllanthus emblica* / EFFERVESCENT GRANULES / PELLETS / EXTRUSION-SPHERONIZATION / STABILITY / PHYSICOCHEMICAL PROPERTIES

PATCHARIN KARNJANACHOTDUMRONG: DEVELOPMENT OF EFFERVESCENT GRANULES AND PELLETS CONTAINING *Phyllanthus emblica* L. FRUIT EXTRACT. THESIS ADVISOR : JITTIMA CHATCHAWALSAISIN, Ph.D., THESIS COADVISOR : ASSOC. PROF. POJ KULVANICH, Ph.D., 174 pp.

Ma-kham-pom (*Phyllantus emblica* L.), a plant of the Euphobiaceae, is a deciduous tree native to Southeastern Asia. Its pharmacological activities such as antioxidant, anti-inflammatory, hypolipidaemic, hepatoprotective, antitussive, antipyretic and analgesic activities have been reported. The objectives of the present study were to develop effervescent granules and pellets containing *Phyllanthus emblica* fruit extract, and investigate the physicochemical properties and stability of the products. The *Phyllanthus emblica* fruit extract was used in the form of spray dried powder. The physicochemical properties and microbial limit of the extract were determined. The results showed that the extract had poor flowability and its 1% solution showed pH around 3. The microbial limit of the extract complied to Thai pharmacopoeia.

The effervescent granules were prepared by dry mixing the granules of the extract, acesulfame potassium and mannitol, with citric acid and alkalines. The effects of formulation variables, i.e. levels of the extract, levels of citric acid, levels of colloidal silicon dioxide, types of alkaline, on the properties of effervescent granules were studied according to the center of gravity design. The products were yellowish, free-flowing and could disperse in water within 5 min. After storage at 40°C, 75% relative humidity and 30°C, 75% relative humidity, in many cases, caking and/or formation of large agglomerates occurred. Change in the physical property was not directly related to change in the total tannins and gallic acid contents in the formulation; and the cause of this has not been identified. The analysis of variance showed that there were significant main and interaction effects of the levels of formulation variables on loss on drying, and the total tannins and gallic acid contents ($p<0.05$).

Pellets containing the extract, acesulfame potassium, citric acid and mannitol were prepared by extrusion-spheronization process, based on full factorial designed experiment. It was found that to form pellets, the wet mass of low level, 20%, of the extract required greater amount of water than the wet mass of high level, 40%, of the extract. The pellets produced from the low level of the extract were round, while produced from the high level of the extract remained dumbbell-shape. After storage at 40°C, 75% relative humidity and 30°C, 75% relative humidity, change in color of pellets was clearly observed for the pellets containing the high level of the extract. There was significant main and interaction effects of the levels the extract, acesulfame potassium and citric acid on loss on drying and gallic acid content ($p<0.05$). These variables, except for the level of the extract, also significantly influenced on total tannins content ($p<0.05$).

The results signify that there is possibility of developing effervescent granules and pellets containing *Phyllanthus emblica* fruit extract. The potential for successful development partly relies on the properties of the extract as reflected in physical stability of many effervescent granules containing 30% extract and pellets containing 20% extract. The impact of the extract properties and effect of formulation variables on the product stability still need to be further investigated.

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LIST OF ABBREVIATIONS

%	Percentage
μg	Microgram (s)
μl	Microliter (s)
μm	Micrometer (s)
^{O}C	Degree celcius (centrigrade)
^{O}F	Degree fahrenheit (centrigrade)
BGA	Brinlliant green agar medium
BSA	Bismuth sulfite agar medium
CA	Cetrimide agar medium
CFU	Colony forming unit
cm	Centrimeter (s)
cm^3	Cubic centrimeter (s)
EF	Effervescent granules
et al.	*et alii*, 'and others'
g	Gram (s)
GAE	Gallic acid equivalence
h	Hour (s)
HCl	Hydrochloric acid
HPLC	High performance liquid chromatographic
H_2SO_4	Sulfuric acid
i.p.	Intra-peritoneal
LB	Fluid lactose medium
LOD	Loss on drying
kg	Kilogram (s)
kv	Kilovolt (s)
nm	Nanometer (S)
McA	MacConkey agar medium
mg	Milligram (s)
min	Minute (s)
ml	Milliliter (s)

mm	Millimeter (s)
MSA	Mannitol-salt agar medium
MW	Molecular weight
PE	Pellets
pH	The negative logarithm of the hydrogen ion concentration
r^2	Coefficient of determination
RH	Relative humidity
rpm	Revolutions per minute
RSD	Relative standard deviation
SD	Standard deviation
SDA	Sabouraud dextrose agar medium
sec.	Second
SEM	Scanning electron microscopy
TSA	Soybean-casein digest agar medium
TSB	Fluid soybean-casein digest medium
UV	Ultraviolet
w/v	Weight by volume
w/w	Weight by weight
XLD	Xylose-lysine-desoxycholate agar medium

CHAPTER I

INTRODUCTION

Phyllanthus emblica L. (also called *Emblica officinalis* Gaerth., a member of the family Euphorbiaceae) is commonly known as indian grooseberry or amla. In Thailand, it is called as Ma-kam-pom. *Phyllanthus emblica* L. is a small or medium-sized deciduous tree with smooth grayish-brown bark, small greenish-yellow flowers and light greenish yellow fruits. The fruit's taste is bitter-sour. The tree is distributed in tropical South-Eastern Asia, such as India, Srilanka, Nepal, Malaya, Laos, Burma, Vietnam, Southern China, Pakistan, Bangladesh and Thailand (Morton, 1987; Williamson, 2002).

Phyllanthus emblica L. has been used as a valuable ingredient in tropical South-Eastern Asia. The fruit is the most commonly used plant part. The juice pressed from fruits is drunk to relieve the bowels of costiveness and to soothe inflamed eyes. The fruits are used to stop diarrhea and colic. The bark and root are astringent. The leaves and fruits are used to soothe inflammation and to combat fever. A paste of the dried fruit powder is also applied to the hair and skin as a substitute for soap (Wiart, 2006; Williamson, 2002).

Pharmacological activities of *Phyllanthus emblica* L. have been investigated both *in vitro* and *in vivo* by many groups of reseachers. Kumar et al. (2006) studied antioxidant activity of the free and bound phenolic extracts of *Phyllanthus emblica* fruits. Bhattacharya et al. (2002) investigated antioxidant activity of fresh juice of *Phyllanthus emblica* fruits against ischemia-reperfusion (IRI)-induced oxidation stress in rat heart. Scartezzini et al. (2006) studied vitamin C content and antioxidant activity of *Phyllanthus emblica* fruits. Ram et al. (2002) studied the immunomodulatory properties of *Phyllanthus emblica* fruits using chromium (VI) as an immunosuppressive agent. Perianayagam et al. (2004) investigated the anti-pyretic and analgesic activity of *Phyllanthus emblica* fruit. Nosalova et al. (2003) tested the antitussive activity of *Phyllanthus emblica* fruit in cats. Sairam et al. (2002) studied the effect of methanolic extract of *Phyllanthus emblica* fruit on the ulcer protective potential. Anila and Vijayalakshmi (2002) investigated reduction of lipid level by using the extraction of

flavonoids of *Phyllanthus emblica* L. Mathur et al. (1996) evaluated the lipid lowering and antiatherosclerotic effects of *Phyllanthus emblica* fresh juice in cholesterol-fed rabbits. Jose and Kuttan (2000) showed that the extract of *Phyllanthus emblica* fruit inhibited the hepatotoxicity produced by carbon tetrachloride administration in rats. Jeena et al. (1999) studied effect of *Phyllanthus emblica* L. on N-nitrosodiethylamine induced hepatocarcinogenesis in rats.

Phyllanthus emblica fruits are the richest source of vitamin C that is approximately 20 times of orange (Disclaimer, 2002). Major constituents of *Phyllanthus emblica* fruit are also low molecular weight hydrolyzable tannin group (molecular weight < 1000) such as emblicanin A, emblicanin B, pedunculagin and punigluconin. These compounds have the potent vitamin C-like activity (Ghosal et al., 1996). Techadamrongsin and Dechatiwongse-Na-Ayudhya (1997) suggested that specifications of the crude drug of *Phyllanthus emblica* L. are not more than 4% of ash content, not more than 1% of acid-insoluble ash content, not more than 9% of loss on drying, not less than 26% of water-soluble extractive, not less than 16% of ethanol-soluble extractive and not less than 20%w/w of tannin content.

Phyllanthus emblica L. is usually used by mixing with other ingredients to produce a product. The product containing *Phyllanthus emblica* extract has manufactured in many forms such as gel, liquid, semi-soild, powder, tablet and capsule for oral (http://www.allayurveda.com/db/salableproducts.asp?currentPage=2[2008, Jan 31]). In Thailand, *Phyllanthus emblica* fruits usually used for cough relief such as cough syrup of Chophaya abhaibhubejhr Hospital, Yoki cough syrup of Siribuncha Co., Ltd. and herbal pill of OuayUn's. Moreover, *Phyllanthus emblica* L. is component in cosmetic products such as StriVectin-SD face and eye creams (http://www.makemeheal.com/mmh/ product.do?id=15936[2008, Jan 31]), acne whitening cream of Pan cosmetic (http://www.pancosmetic.com/AcneWhite/product.html[2008, Jan 31]), anti-aging of Pharmacare international Co., Ltd. (http://www.premacare.co.th /antiaging2_th.htm[2008, Jan 31]), Ratna herbal soap (http://www.herbnepal.com.np/productdetail. php?sn=3[2008, Jan 31]) and scalp treatment of Chophaya abhaibhubejhr Hospital. Pisansalhidikam (2000) found that the pills containing *Phyllanthus emblica* pickle, icing sugar and honey were most accepted and it contained 44.9 mg of vitamin C per 100 g of pill. Pills

containing *Phyllanthus emblica* powder, palm sugar and honey had higher vitamin content than the other formula and it contained 481 mg of vitamin C per 100 g of pill. Mahattanapokai (2004) showed that 1 and 3%w/w *Phyllanthus emblica* extract creams were stable when it was tightly packed in a package and stored at lower than 45^{O}C. Temdee et al. (2007) studied the development of *Phyllanthus emblica* fruits and maltitol syrup lozenge product that reduces tooth decay.

Moisture content, temperature and pH have influence on stability of hydrolyzable tannins and ascorbic acid. Methakhup et al. (2005) found that *Emblica officinalis* fruits flake dried by low-pressure superheated steam had decreased vitamin C content and color change less than those dried by vacuum. Moura et al. (1994) showed that the degradation rate of ascorbic acid was a minimum rate at pH 2.5 to 3.0. Sukkiattibhai et al. (2005) and Udomsom et al. (2005) found that *Phyllanthus emblica* extract was stable at buffer pH 5.5. Ghosal et al. (1996) suggested that low molecular weight hydrolyzable tannin group such as emblicanin A, emblicanin B, pedunculagin and punigluconin were degraded by hydrolysis process.

Several studies have suggested the active ingredients of *Phyllanthus emblica* L. that should be analysed. Raghu et al. (2007) analysed the ascorbic content of *Emblica officinalis* fruits. Scartezzini et al. (2006) analysed the ascorbic content and total polyphenol content of *Emblica officinalis* fruits. Kumar et al. (2006) analysed the free and bound phenolic of *Emblica officinalis* fruits. Leewongpan and Laoruangsinchai (2004) studied validated HPLC determination of gallic acid in *Phyllanthus emblica* fruit extract.

Cough symptom is reflex for expelling irritating substances such as dust, pollen, accumulated fluids and inflammatory cell from the upper airways. The type of cough is divided to productive cough and unproductive cough refered from cough symptom with or without mucus. Dry coughs are treated with antitussives that suppress the cough center, while productive coughs are treated with expectorants or mucolytic agents that loosen mucus from the respiratory tract. As Thai traditional use, antitussive activity of *Phyllanthus emblica* L. has been of interest (วิทย์ เที่ยงบูรณธรรม, 2531). Nosalova (2003) also reported that the antitussive activity of *Phyllanthus emblica* fruit extract in cats. In present

study, effervescent granules and pellets were developed to be *Phyllanthus emblica* L. alternative products.

Effervescent salts are granules or coarse to very coarse powders containing a medicinal agent in effervescent base. Effervescent base is combined between acid and base. After adding water into effervescent salts, acid and base of effervescent base react to liberate carbon dioxide. Acid source for the effervescent reaction can be divided from three main sources: acids, acid anhydrides and acid salts. Akaline sources used most often in effervescent salts are both the bicarbonate and carbonate forms. Advantage of effervescent solution is taste masking of drug; however, disadvantage of effervescent products are hygroscopic and lead to the premature effervescent reaction (Lindberg and Hansson, 2002). Conversion of the surface of sodium bicarbonate particle to 2-10% sodium carbonate by heat has been found to be an effective stabilizing agent because the anhydrous sodium carbonate on surfacce of particle absorbs the free water to produce stable hydrated forms as water scavenger (Gergely et al., 1999).

Pelletization is process to make spherical granules or pellets from powder. The spherical particles or pellets are of interest due to good flow, low dusting, uniform size distribution, low friability, high hardness, ease of coating, and reproducible packing. Several methods of pellet formation such as balling, globulation, solution or suspension layering of core, melt pelletisation or extrusion/spheronization are available. Extrusion/spheronization are usually used as a method to prepare pelletized product because advantage of this method is producing drug-loaded spheres at a high level of active ingredient without producing an excessively large particle and more effcient than other techniques for producing pellets (Ghebre-Sellassie and Knoch, 2002). Extrusion-spheronization is a multiple-step process of preparation of spherical particles, including dry mixing, wet granulation, extrusion, spheronization and drying. The materials are dry mixed and granulated to the wet mass with binder liquid. The wet mass was passed a extruder to produce the extrudate. Then, the extrudate was transfered into a spheronizer to produce pellets (Erkoboni, 2003).

The objectives of the study:

- To develop formulations of effervescent granules and pellets containing *Phyllanthus emblica* fruit extract.
- To evaluate physicochemical properties of effervescent granules and pellets containing *Phyllanthus emblica* fruit extract.
- To study the stability of effervescent granules and pellets containing *Phyllanthus emblica* fruit extract

CHAPTER II

LITERATURE REVIEW

1. *Phyllanthus Emblica* extract

Phyllanthus emblica L. (also called *Emblica officinalis* Gaerth.) is a member of the family Euphorbiaceae. Common names of this tree is also called Emblica myrobalan or Indian gooseberry in English; Amla in Hindi; Ma-kam-pom in Thai; Mak-kham-pom in Laos; Melaka, Asam melaka, or Amlaka in Malaya; Amalaki in Sanskrit; Kam lam or Kam lam ko in Cambodia; Bong ngot in Southern Vietnam; Chu me in North Vietnam and Nelli in the Philippines (Morton, 1987; Williamson, 2002).

1.1 Botanical description

Phyllanthus emblica L. is a small or medium-sized deciduous tree, reaching 8-15 m in height. The smooth bark is a grayish-brown. Its leaves are simple, small, linear oblong shape and 8-12 mm length and 2.5-5 mm width in size. The phyllotaxy of leaves is alternate, looking like pinnate. Its flowers are small, greenish-yellow, unisexual with monoecious plant, forming in axillary clusters and start appearing in January to April (เมธินี ตาพุมาศสวัสดิ์, 254; http://www.dnp.go.th/EPAC/Herb/21makhampom1.htm[2008, Jan 14]). The fruits are nearly spherical, smooth surface, light greenish yellow when ripe, with 6 vertical stripes or furrows and contain three loculi each containing two trigonous seeds (Williamson, 2002). Normally, fruit is 1-2 cm of diameter and ripen from October to May. Its taste is bitter-sour (ก่องกานดา ชยามฤต, 2541; สมจิต พงศ์พงัน และ สุภาพ ภู่ประเสริฐ, 2534).

Figure 1 Characteristics of *Phyllanthus emblica* L. (พร้อมจิต ศรลัมพ์ และคณะ, 2535; เมธินี ตาพุฒาศ สวัสดิ์, 2542)

1.2 Distribution

The native of *Phyllanthus emblica* L. is in tropical South-Eastern Asia, such as India, Srilanka, Nepal, Malaya, Laos, Burma, Vietnam, Southern China, Pakistan and Bangladesh. In Thailand, it is found in the mixed deciduous forests, deciduous dipterocarp forests and open hill evergreen forests. This tree can grow equally well on all soil type and often propagate by seeds (เมธินี ตาพุมาศสวัสดิ์, 2549; วิทย์ เที่ยงบูรณธรรม, 2531; Morton, 1987).

1.3 Phytochemistry

Phyllanthus emblica L. has received attention due to its phytochemical and biological activities. The several compounds of this tree have been isolated and identified as listed in table 1.

Table 1 The classes of chemical constituents reported in *Phyllantus emblica* L.

Class	Compound	Occurrence	Reference
Alkaloids	Phyllantine Phyllantidine	Leaves, fruit, and tissue cultures	Khanna and Bansal, 1975
	Zeatin Zeatin nucleotide Zeatin riboside	Leaves and fruit	Ram and Rao, 1976
Benzenoids	Chebulic acid Chebulinic acid Chebulagic acid Gallic acid	Leaves	Theresa et al., 1965, 1967
	Ellagic acid	Leaves Fruit	Theresa et al. 1965 Hui and Sung, 1968 Subramanian et al., 1971 Desai et al., 1977

Benzenoid	β-glucogallin	Leaves Fruit	Theresa et al. 1967 Srivastava and Ranjan, 1967
	Amlaic acid	Fruit	Theresa et al. 1967
	Corilagin 3-6-di-O-galloyl-glucose Ethyl gallate	Fruit	Srivastava and Ranjan, 1967
	1,6-di-O-galloyl-β-D-glucose 1-di-O-galloyl-β-D-glucose Putranjivain A Digallic acid	Fruit	El-Mekkawy et al., 1995
	Phyllemblic acid Emblicol Music (=galactaric) acid	Fruit	Pillay and Iyer, 1958 Basa and Srinivasulu, 1987
	Emblicanin A Emblicanin B Pedunculagin Punigluconin	fruit	Ghosal, 1996
	Phyllanemblicuns A-F	Fruit, leave and branches	Zhang et al., 2001
Furanolactone	Ascorbic acid	Leaves Fruit	Basa and Srinivasulu, 1987 Damoradan and Srinivasan, 1935 Shah and Hamid, 1968
Diterpene	Gibberellin A-1 Gibberellin A-3 Gibberellin A-4 Gibberellin A-7 Gibberellin A-9		Ram and Raja, 1978

Triterpene	Lupeol	Leaves Fruit	Hui and Sung, 1968 Desai et al., 1977
Flavonoid	Leucodelphinidin Kaempherol Kaempherol-3-glucoside Rutin Quercetin	Leaves Leaves Leaves	Laumas and Seshadri, 1958 Subramanian et al., 1971 Yrjonen et al., unpublished results
	Kaempherol-3-O--β-D-glucoside Quercetin-3-O--β-D-glucoside	Fruit	El-Mekkawy et al., 1995
Sterol	β-sitosterol	Leaves	Hui and Sung, 1968
Carbohydrate	Acidic and neutral poly-saccharides Glucose	Fruit Leaves	Nizzamuddin et al., 1982 Theresa et al. 1967
Fatty acids	Arachidic acid Behenic acids	seed oil	Ihantola-Vormisto et al., 1997

It has been reported that *Phyllanthus emblica* fruits are the richest source of vitamin C or ascorbic acid, containing ascorbic acid up to 720 mg/100 g of fresh pulp and 921 mg/100 ml of pressed juice. This is approximately 20 times more than that found in orange (Disclaimer, 2002). Major constituents of *Phyllanthus emblica* which is of interest are low molecular weight hydrolyzable 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, 1996).

Emblicanin A is a yellowish-grey 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, 1996) as shown in figure 3(A).

Emblicanin B was a tan colored hygroscopic powder. Its chemical name is 2,3,4,6-bis-(S)-hexahydroxydiphenoyl-2-keto-glucono-δ-lactone and its molecular formula is $C_{34}H_{20}O_{22}.2H_2O$. On boiling with water, it is partially hydrolysed and produces one mole hexahydroxydiphenic acid per mole of emblicanin B and hexahydroxydiphenic acid changes its structure to ellagic acid (Ghosal, 1996) as shown in figure 3(B).

Punigluconin is a grey amorphous hygroscopic powder. Its chemical name is 2,3-di-O-galloyl-4,6-(S)-hexahydroxydiphenoyl gluconic acid and its molecular formula is $C_{34}H_{26}O_{23}.2H_2O$. Punigluconin (22 mg) in 1 N H_2SO_4 solution (5 ml) for 4 hours give gallic acid (4 mg) and ellagic acid (3 mg) (Ghosal, 1996).

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

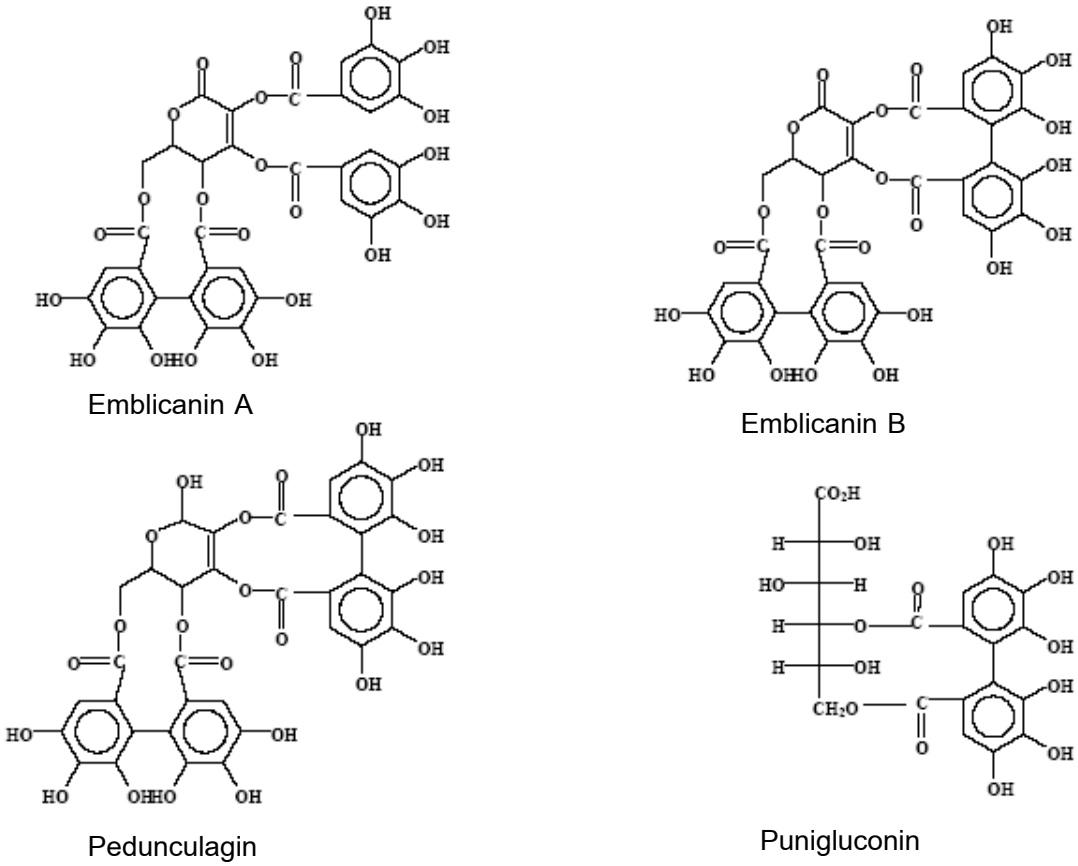


Figure 2 Structures of emblicanin A, emblicanin B, pedunculagin and punigluconin (http://www.dwe ckdata.com/Published_papers/Emblica_officinalis.pdf[2008, Jan 23])

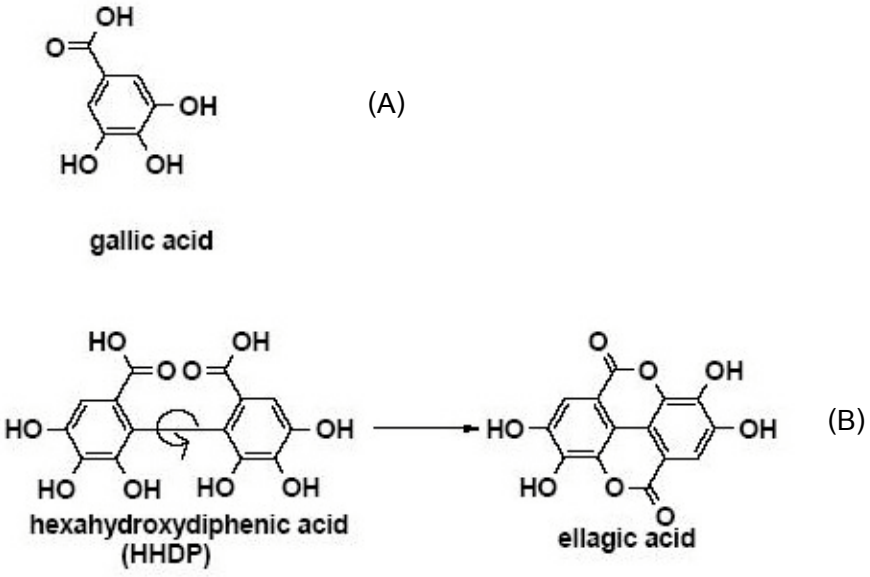


Figure 3 Structure of gallic acid (A) and structure of hexahydroxydiphenic acid to change to ellagic acid (B) (Hagerman, 2002)

1.4 Traditional medicine

Phyllanthus emblica L. has been used as a valuable ingredient in tropical South-Eastern Asia from immemorial time. In Burma, the juice pressed from fruits is drunk to relieve the bowels of costiveness and to soothe inflamed eyes. The bark and root are astringent. In China, the roots are used to lower body temperature and to expel impurities. The leaves and fruits are used to soothe inflammation and to combat fever. In Indonesia, a decoction of the fruits is drunk to check bloody flux, soothe inflammation and combat fever. In Malaysia, a decoction of the leaves is used to soothe inflammation and to combat fever. In Vietnam, the leaves are used to combat fever. The fruits are used to stop diarrhea and colic. In India, the leaves and the fruits are used to soothe inflammation and to combat fever. The fruits are used to invigorate the liver. A paste of the dried fruit powder is also applied to the hair and skin as a substitute for soap. The powdered root bark mixed with honey to treat mouth ulcers. The flowers are used by Asians living in Britain to soothe sores, stop dysentery, promote urination, relieve the bowels of costiveness, soothe inflammation and treat scurvy (Wiart, 2006; Williamson, 2002). In

Thailand, the powdered dried bark is used to dress on bloodly sore and hematoma. The water boiled with bark is to treat dysentery. The fresh leaves are pounded to powder to cover on rash of the skin having lymphatic, inflammatory skin and anal abscess. The fruits are eaten to nourish body, relieve the thirst symptom, promote urination, increase laxative, mucolytic and combat cough, fever and scurvy. The fresh fruits are made the fruit wine to combat jaundice, fever, hiccough, vomit and to increase digestion. The pressed *Phyllanthus emblica* fruits juice mixed with lemon juice to treat dysentery from bacteria. The fresh fruits mixed with honey to treat anthelmintic. The powdered dried fruits mixed with hot water is to treat gonorrhea, diarrhea, dysentery and to soothe inflamed eyes. The powdered fresh or dried seeds mixed with a hot water to treat fever, vomit, eye disease, diabetes, asthma and bronchitis. The water boiled with dried roots is to cure leprosy, hypertension and diarrhea. The fresh roots are pounded to powder to relieve centipede bites (วิทย์ เที่ยงบูรณธรรม, 2531).

1.5 Pharmacological activities

1.5.1 Antioxidant activity

Kumar et al. (2006) found that the free and bound phenolic extracts of *Phyllanthus emblica* fruits has antioxidant activity. Free and bound phenolic acids were isolated by differential extraction procedure. Antioxidant activity was evaluated by reducing power assays, 1,1-diphenyl-2-picryl hydrazyl (DPPH) scavenging ability and DNA protection against oxidative damage. The studies suggested that gallic acid and tannic acid were identified as the major antioxidant components in phenolic fraction of *Phyllanthus emblica*.

Bhattacharya et al. (2002) investigated an emblicanin A (37%) and emblicanin B (33%) enriched fraction of fresh juice of *Phyllanthus emblica* fruits for antioxidation activity against ischemia-reperfusion (IRI)-induced oxidation stress in rat heart by using vitamin E as the standard antioxidant agent. The extract dose of 50 and 100 mg/kg body weight, orally twice daily for 14 days could prevent IRI-induced effect. The study confirms the antioxidant effect of *Phyllanthus emblica* and indicates that *Phyllanthus emblica* may have a cardioprotective effect.

Scartezzini et al. (2006) studied vitamin C content and antioxidant activity of *Phyllanthus emblica* fruits from India, ayurvedic samples of *Phyllanthus emblica* from Italy and the dry extract of *Phyllanthus emblica* from Merck. All samples of *Phyllanthus emblica* were first analysed for the quantitative determination of ascorbic acid content and then diluted in order to have an exact concentration of ascorbic acid in the range 0.01-0.5 mg/ml. Pure ascorbic acid was also diluted to the same concentration. Antioxidant activity of both *Phyllanthus emblica* samples and pure ascorbic acid solution were evaluated by 1,1-diphenyl-2-picryl hydrazyl (DPPH) test and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) test. The data showed that all extracts exhibited an antioxidant activity even at the lowest concentration tested (0.01 mg/ml). A comparison between antioxidant activity of ascorbic acid standard solution and the three different *Phyllanthus emblica* products, measured at the same concentration of ascorbic acid. In DPPH test, ayurvedic samples of *Phyllanthus emblica* and dried *Phyllanthus emblica* fruits have higher antioxidant activity than the other samples. In ABTS test, ayurvedic samples of *Phyllanthus emblica*, dried *Phyllanthus emblica* fruits and the Merck extract have higher antioxidant activity than the pure ascorbic acid. It was also found that the antioxidant activity of *Phyllanthus emblica* is due to ascorbic acid for 45-70%. The results of both tests also show that the antioxidant activity does not depend only vitamin C content but also depend with other compound in *Phyllanthus emblica*.

1.5.2 Immunodulating properties

Ram et al. (2002) studied the immunomodulatory properties of the fruits extract with 90% ethanol of *Phyllanthus emblica* using chromium (VI) as an immunosuppressive agent, in immuno-compromised states with the emphasis on lymphocytes. *Phyllanthus emblica* extract significantly inhibited chromium (Cr)-induced free radical production and restored the antioxidant state back to control level. This extract also inhibited apoptosis and DNA fragmentation induced by Cr; resulting in, inbibition of both lipopolysaccharide and concanavalin-A-stimulated lymphocyte proliferation by suppressing Cr.

1.5.3 Anti-pyretic and analgesic activity

Perianayagam et al. (2004) investigated the anti-pyretic and analgesic activity of ethanol and aqueous extracts of *Phyllanthus emblica* fruit in several experimental models. A single oral dose of ethanol and aqueous extracts (500 mg/kg, i.p.) showed significant reduction in brewer's yeast induced hyperthermia in rats and inhibition on acetic acid-induced writhing response in the analgesic test in mice. Both the extracts did not show significant analgesic activity in the tail-immersion test.

1.5.4 Antitussive activity

Nosalova et al. (2003) tested the antitussive activity of ethanol extract of *Phyllanthus emblica* fruit in conscious cats by mechanical stimulation of the laryngopharyngeal and tracheobronchial mucous areas of airways. The results of the extract dose of 50 mg/kg body weight decreased significant the number and frequency of cough from both irritated areas of the airways but it did not decrease significantly the intensity of cough. At a extract dose of 200 mg/kg body weight, the results of the cough suppressive was more pronounced in decreasing the number, frequency and intensity of cough. For comparison of the *Phyllanthus emblica* extract at the dose of 200 mg/kg body weight, norcotic antitussive drug, codeine, at the dose of 10 mg/kg body weight and non-norcotic antitussive drug, dropropizine, at the dose of 100 mg/kg body weight, the antitussive activity of *Phyllanthus emblica* is less effective than codeine, but more effective than dropropizine.

1.5.5 Anti-ulcer activity

Bandyopadhyay et al. (2000) reported that the butanol extract of the water fraction of *Phyllanthus emblica* fruit at the dose of 100 mg/kg body weight, orally administered to rats for 10 days, was found to enhance secretion of gastric mucus and hexosamine ($p < 0.001$) in the indomethacin induced ulceration of rats.

Sairam et al. (2002) studied the methanolic extract of *Phyllanthus emblica* fruit in different acute gastric ulcer models in rats induced by aspirin, ethanol, cold restraint stress and pyloric ligation and healing effect in chronic gastric ulcer models in rats induced by acetic acid. The dose of 10-50 mg/kg administered orally, twice daily for 5 days showed dose-dependent ulcer protective effects in all the acute ulcer models and

significantly ulcer healing effect in dose of 20 mg/kg after 5 days treatment. Further study on gastric mucosal factors showed that was significantly decrease the offensive factor like acid and pepsin and increased the defensive factors like mucin secretion, cellular mucus and life span of mucosal cells.

Al-Rehaily et al. (2002) reported that the ethanol extract of *Phyllanthus emblica* fruit at the dose of 250 and 500 mg/kg body weight administered orally, significantly inhibited the development of gastric lesions in different models in rats, including pylorus ligation, indomethacin, hypothermic restraint stress-induced gastric ulcer and necrotizing agents (80% ethanol, 0.2 M NaOH and 25% NaCl).

Moreover et al. (2005) showed anti-ulcer and anti-oxidant activity of Pepticare, a herbomineral formula contained *Glycyrrhiza glabra*, *Emblica officinalis* and *Tinospora cordifolia*.

1.5.6 Hypolipidaemic activity

Anila and Vijayalakshmi (2002) investigated reduction of lipid level by using the extraction of flavonoids of *Phyllanthus emblica* at a dose of 10 mg/kg body weight administered orally per day, in rats induced hyperlipidemia. After 90 days duration of the study, the results showed a significant decrease in cholesterol, triglycerides, phospholipids and free fatty acid levels in serum and tissues.

Mathur et al. (1996) evaluated the lipid lowering and antiatherosclerotic effects of *Phyllanthus emblica* fresh juice in cholesterol-fed rabbits (rendered hyperlipidaemic by atherogenic diet and cholesterol feeding). *Phyllanthus emblica* fresh juice was administered at a dose of 5 ml/kg body weight per rabbit per day for 60 days. Serum cholesterol, Triglyceride (TG), phospholipid and Low-density lipid (LDL) levels were lowered by 82%, 66%, 77% and 90%, respectively and the tissue lipid levels showed a significant reduction following *Phyllanthus emblica* fresh juice administration.

1.5.7 Hepatoprotective activity

Jose and Kuttan (2000) showed that the aqueous extract of *Phyllanthus emblica* fruit inhibited the hepatotoxicity produced by acute and chronic carbon tetrachloride administration in rats as seen from the decreased levels of serum and liver lipid peroxides,

glutamate-pyruvate transaminase, and alkaline phosphatase. The extract could also inhibit the induction of fibrosis in chronic carbon tetrachloride administration in rats.

1.5.8 Irradiation protection

Singh et al. (2006) found that oral administration of *Phyllanthus emblica* fruit extract before exposure to gamma radiation was effective in protecting mice against the hematological and biochemical modulation in peripheral blood. A significant increase in the red blood cells, white blood cells, hemoglobin and hematocrit values was observed in the animals pretreated with *Phyllanthus emblica* extract as compared to the value observed in the irradiated group.

1.5.9 Antimicrobial activity

Aqueous infusion and decoction of *Emblica officinalis* exhibited potent antibacterial activity against *Escherichia coli, Klebsiella pneumoniae, Klebsiella ozaenae, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B* and Serratia marcescens, but did not show any antibacterial activity against Gram negative urinary pathogens (Saeed and Tariq, 2007). Mayachiew and Devahastin (in press) evaluted the microbial activities of the 95% ethanol extract of *Phyllanthus emblica* fruit against *Staphylococcus aureus*. The minimum inhibitory concentration (MIC) values was found to be 13.97 mg/ml and the minimum biocidal concentration (MBC) values was 13.97 mg/ml.

1.5.10 Anticarcinogenic activity

Jeena et al. (1999) studied effect of *Phyllanthus emblica* on N-nitrosodiethylamine induced hepatocarcinogenesis in rats. Treatment of extracts significantly reduced γ-glutamyl transpeptidase in serum and tissues of tumor bearing animals and glutathione in tissue. The results indicated that the extract offered protection against chemical carcinogenesis.

1.6 Specification, extaction and analysis of *Phyllanthus emblica*

Phyllanthus emblica fruits have been in forms of fresh fruit, dried fruit and the fruit extract. For traditional medicine, *Phyllanthus emblica* fruits were usually used in form of crude drug of powdered dried fruit. Techadamrongsin and Dechatiwongse-Na-Ayudhya (1997) proposed that the appropriate specifications of the crude drug of *Phyllanthus emblica* were not more than 4% of ash content, not more than 1% of acid-insoluble ash content, not more than 9% of loss on drying, not less than 26% of water-soluble extractive, not less than 16% of ethanol-soluble extractive and not less than 20%w/w of tannin content.

Ghosal (1996) isolated hydrolyzable tannins by comprehensive chromatographic such as column, TLC, HPLC, HPTLC. Low molecular weight hydrolyzable tannins group (molecular weight < 1000) such as emblicanin A, emblicanin B, pedunculagin and punigluconin were found. In Thailand, Buranasudja et al. (2005) reported isolation of major constituents of *Phyllanthus emblica* by column chromatography and HPLC technique. In their study, the compound at low molecular weight (molecular weight = 788) was extracted.

Quantity of ascorbic acid and tannin in *Phyllanthus emblica* were often analysed by many groups of researchers. Raghu et al. (2007) analysed the ascorbic content of *Emblica officinalis* fruits by different analytical methods such as 2,4-dinitrophenylhydrazine method, indophenol-xylene method, enzymatic method and liquid chromatography with fluorescence detection.

2,4-dinitrophenylhydrazine method determined vitamin C based on the oxidation of ascorbic acid to dehydroascorbic acid and coupling with 2,4-dinitrophenylhydrazine under controlled conditions to give red coloured ozazones.

Indophenol-xylene method determined vitamin C based on decolouration of 2,6-dicholrophenol indophenol by ascorbic acid.

Enzymatic method determined ascorbic acid in the sample after treatment with ascorbic acid oxidase in phosphate buffer.

Scartezzini et al. (2006) analysed the ascorbic content by HPLC method and total polyphenol content by the Folin-Ciocalteu colorimetric method. Kumar et al. (2006) analysed the free and bound phenolic by HPLC method.

Folin-Ciocalteu method was the best method presently available for the total polyphenol content determination in dry wines, plant extracts, brandies and similar product. This method involve oxidation of the phenol by a yellow molybdotungstophosphoric heteropolyanion reagent (or called Folin-Ciocalteu reagent) and colorimetric measurement of the resultant molybdotungstophosphate blue (Slinkard and Singleton, 1977). Color development in the Folin-Ciocalteu method was relatively stable after final colorimetric process approximately 50 minutes. High levels of fructose, sulphite and ascorbic acid may interfere with the assay. It was recommended that these factors of samples be tested in model solution (http://ourworld.compuserve.com /homepages/andrew_lea/tanmeths.htm[2005, August 10]).

Leewongpan and Laoruangsinchai (2004) studied validated HPLC determination of gallic acid in *Phyllanthus emblica*. They found that the best condition of HPLC method was column C18 (150 x 4.6 mm, 5 μm) with mobile phase containing methanol : 0.3% trifluoroacetic acid (8 : 92) at a flow rate of 1 ml/min. The gallic acid was analysed at wavelength 270 nm.

1.7 Stability

Methakhup et al. (2005) investigated vacuum drying and low-pressure superheated stem drying (LPSSD) condition on quality of indian gooseberry flake. Conditions of drying for testing were vacuum or low-pressure superheated stem dryer at temperature of 65 and 75 ^{O}C and absolute pressures of 7, 10 and 13 kPa. The vacuum drying took shorter time to dry the product than LPSSD. Drying in the vacuum system at temperature of 75 ^{O}C and absolute pressures of 7 kPa had similar level of ascorbic acid retention compared to LPSSD at every condition. LPSSD could retain the color better than the vaccum drying system, because the degree of ascorbic acid and chlorophyll degradation of LPSSD was much lower than the vaccum drying system.

Moura et al. (1994) studied the degradation rate of ascorbic acid at different pH value (2.5-5.0) by adjusted with HCl 37% for 2 hours. It was showed that the rate of oxidation was pH dependent, showing a maximum at pH 4.0 and a minimum at pH 2.5 to 3.0.

Sukkiattibhai et al. (2005) found that the *Phyllanthus emblica* extract at 0.05 M acetate buffer pH 5.5 after testing 240 minutes had change of gallic acid lower than 0.05M HCl-KCl buffer pH 2.0. In addition, Udomsom et al. (2005) showed that the *Phyllanthus emblica* extract at phosphate buffer pH 5.5 had stable polyphenol content higher than citric acid-citrate buffer pH 3.5, phosphate buffer pH 7.4 and phosphate buffer pH 9.0.

Ghosal et al. (1996) suggested that low molecular weight hydrolyzable tannin group such as emblicanin A, emblicanin B, punigluconin and pedunculagin were degraded by hydrolysis process. Partial hydrolysis of emblicanin A with tannase produces two moles of gallic acids per one mole of emblicanin A. On boiling with water, emblicanin B is partially hydrolysed and produces one mole hexahydroxydiphenic acid per mole of emblicanin B and hexahydroxydiphenic acid changes its structure to ellagic acid. Punigluconin (22 mg) in 1 N H_2SO_4 solution (5 ml) for 4 h. give gallic acid (4 mg) and ellagic acid (3 mg). Acid hydrolysis of pedunculagin produced a mixture of ellagic acid and hexahydroxydiphenic acid.

1.8 Product containing *Phyllanthus emblica*

Phyllanthus emblica was composition of many products such as gelly, liquid, semi-soild, powder, tablet or capsule for oral (http://www.allayurveda.com/db/salable products.asp?currentPage=2[2008, Jan 31]). Abundance™ Aller-7® Plus for relief of allergy symptoms is tablet containing seven herbal extracts such as *Phyllanthus emblica* (fruit) extract, *Terminalia chebula* (fruit) extract, *Terminalia bellerica* (fruit) extract, *Albizia lebbeck* (bark) extract, *Zingiber officinale* (root) extract, *Piper longum* (fruit) extract and *Piper nigrum* (fruit) extract (http://www.abundancehealth.com/ aller7_plus.aspx [2008, Jan 31]). Cosmetics product has *Phyllanthus emblica* as ingredient, for example, StriVectin-SD face and eye creams (http://www.makemeheal. com/mmh/product.do?id=15936[2008, Jan 31]), acne whitening cream of Pan cosmetic (http://www.pancosmetic.com/AcneWhite/product.html[2008, Jan 31]), anti-aging of Pharmacare international Co., Ltd. (http://www.premacare.co.th/antiaging2_th.htm[2008, Jan 31]), Ratna herbal soap (http://www.herbnepal.com.np/productdetail.php?sn=3[2008, Jan 31]) and scalp treatment of Chophaya abhaibhubejhr Hospital.

Mahattanapokai (2004) studied the preparation of *Emblica officinalis* extract cream. It was found that the extract of *Emblica officinalis* dried fruits with ethyl acetate was the highest antioxidant activity and it was chosen to prepare *Emblica officinalis* extract cream. *Phyllanthus emblica* extract creams of concentration 1 and 3% w/w were stable when it was tightly packed in a package and stored at lower than 45^{O}C for 3 months. In vivo study, *Phyllanthus emblica* extract creams of concentration 3% w/w appeared to be effective in improving skin lightening.

In Thailand, *Phyllanthus emblica* Linn. usually used for cough relief product. The hundred mililiters of cough syrup of Chophaya abhaibhubejhr Hospital contained 60 ml of concentration of 40% of *Phyllanthus emblica* extract. Yoki cough syrup of siribuncha Co., Ltd. is a traditional drug containing *Phyllanthus emblica*, licorice and orange fruit peel. Herbal pill with apricot, orange or lemon flavor of OuayUn containing platycodon grand florum, licorice, *Phyllanthus emblica*, menthol and honey.

Pisansalhidikam (2000) studied the development of a vitamin C pill from *Phyllanthus emblica* fruits. The fruits were processed in to three products: *Phyllanthus emblica* powder, *Phyllanthus emblica* pickle and *Phyllanthus emblica* juice, to use as raw material of pill. It was found that the pills containing *Phyllanthus emblica* pickle, icing sugar and honey were the most accepted and it contained 44.9 mg of vitamin C per 100 g of pill. Pills containing *Phyllanthus emblica* powder, palm sugar and honey had higher vitamin C content than the other formula and it contained 481 mg of vitamin C per 100 g of pill. The pills stored in air-tight container for 1 month and at room temperature had changed of darken color, became of sticky pill and decreased of vitamin C content, especially in those stored at room temperature. Vitamin C of the pills containing *Phyllanthus emblica* pickle, icing sugar and honey were 27.8 mg per 100 g of pill at 5-10^{O}C for 1 month and 15.1 mg per 100 g of pill at room temperature. Vitamin C of the pills containing *Phyllanthus emblica* powder, palm sugar and honey were 486 mg per 100 g of pill at 5-10^{O}C for 1 month and 338 mg per 100 g of pill at room temperature.

Moreover, Temdee et al. (2007) studied the development of *Phyllanthus emblica* fruits and maltitol syrup lozenge product that reduce tooth decay. Total tannins of *Phyllanthus emblica* fruits are determined before prepare products. Lozenge products were prepared by process of hard candy and pastilles. Components of hard candy were maltitol syrup, glucose syrup, *Phyllanthus emblica* extract (fresh fruits or pickle fruits), licorice extract, salt and ascorbic acid. Components of pastilles were maltitol syrup,

glucose syrup, *Phyllanthus emblica* extract (fresh fruits or pickle fruits), licorice extract, gelatin, arabic gum, salt, orange flavor and ascorbic acid. It was found that maltitol syrup as sweetening agent was higher accepted than maltitol syrup mixed with glucose syrup as sweetening agent. And *Phyllanthus emblica* extract from pickle fruits was more accepted than *Phyllanthus emblica* extract from fresh fruits.

Figure 4 Products containing *Phyllanthus emblic* (http://www.allayurveda.com/db/salable products.asp?currentPage=2 [2008, Jan 31]; http://www.herbnepal.com.np/product detail.php?sn=3[2008, Jan 31]; http://www.abundance health.com/aller7_plus.aspx [2008, Jan 31])

2 Effervescent granules

Effervescent salts are granules or coarse to very coarse powders containing a medicinal agent in effervescent base. After addition of water into effervescent salts, the acid and alkali of effervescent base react to liberate carbon dioxide. Advantage of effervescent solution is taste masking of drug. On the other hand, disadvantage of effervescent products are hygroscopic and lead to the premature effervescent reaction.

2.1 Raw material

The properties of raw material are important to products. Raw materials with low moisture content and easily wet will be choosen to increase the solubility and stability of effervescence (Lindberg and Hansson, 2002).

Acid source for the effervescent reaction can be classified from three main sources: acids, acid anhydrides and acid salts. The acids are the most commonly used because they are produced from nature and are usually used as food additive. Acids most commonly used are such as citric acid, tartaric acid, malic acid, fumaric acid, ascorbic acid, acetylsalicylic acid and succinic acid. Citric acid is usually used in effervescent preparations. Powdered forms of citric acid are also commercially available, in a monohydrate grade and an anhydrous grade. Citric acid monohydrate melt at 100 ^{O}C and become anhydrous at 135 ^{O}C. At relative humidity (RH) between approximately 65% and 75%, citric acid monohydrate begins to absorb an amount of moisture. At the same condition, citric acid anhydrous is converted to monohydrate form; as a result, moisture content is stable as shown in figure 5 (Lindberg and Hansson, 2002). Effervescent salts are usually prepared from a combination of citric acid and tartaric acid rather than using a single acid because citric acid alone brings about a sticky mixture that is difficult to granulate, while tartaric acid alone results in losing granule firmness (Ansal, 1969). Tartaric acid is more soluble than citric acid and also more hygroscopic. It is as strong an acid as citric acid, but tartaric acid must be used in more quantity than citric acid in acid-base reactions. At < 65% RH, tartaric acid absorbs insignificantly amount of moisture as sorption isotherms in figure 5.

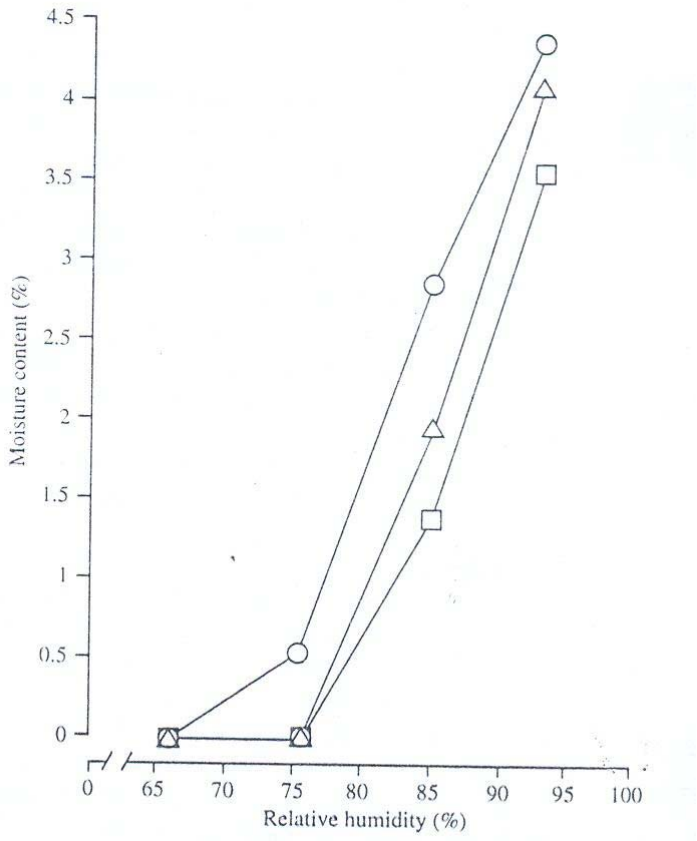


Figure 5 Sorption isotherms of some hygroscopic acids. Key: x axis = relative humidity, %; y axis = moisture content, %; O = citric acid monohydrate; Δ = anhydrous citric acid; □ = tartaric acid (Lindberg and Hansson, 2002).

The other acid sources, acid anhydrides, are hydrolyzed to the corresponding acid, when mixed with water. The corresponding acid can react with the carbon dioxide source to occur effervescence. Finally, acid salts are useful in the effervescent formula such as sodium dihydrogen phosphate, disodium dihydrogen pyrophosphate and acid citrate salts (Lindberg and Hansson, 2002; Mohrle, 2002).

Carbonate sources used most often in effervescent salts are both the bicarbonate and carbonate forms. Sodium bicarbonate is the first choice in effervescent systems. It has the carbon dioxide yield approximately 52% by weight. Sodium bicarbonate begins to dissociate into sodium carbonate, carbon dioxide and water about 50 ^{O}C and completely converts into anhydrous sodium carbonate on heating to 250-300 ^{O}C for a short time (Mohrle, 2002). Sodium bicarbonate can convert to sodium carbonate about 9% at 100 ^{O}C for 45 min (Amela et al., 1996).

Binders are materials that help to hold other materials together. Type of binder should be a water-soluble grade otherwise the binder will retard the disintegration/dissolution of effervescence. Binders from natural, such as cellulose gums, gelatin, and starch paste, are not used due to high residual water content. Polyvinylpyrrolidone (PVP) is a common binder to produce an effective effervescence. The materials are either added in dry form or dissolved in water, ethanol, isopropanol or other solvents as the granulating fluid (Mohrle, 2002). However, citric acid, partly dissolved in ethanol or isopropanol and evaporated, is function as a binder. In order to compress ascorbic acid with sodium bicarbonate, polyvinylpyrrolidone (PVP) or polyvinylpyrrolidone-poly (vinyl acetate)-copolymer are not used as a binder because they led to a change of color of ascorbic acid granule (Lindberg and Hansson, 2002).

Diluent of effervescence is normally little need for effervescent product because the materials of effervescent reaction are added in large quantities. The properties of diluent of effervescence should be good soluble and particle size similar to the other ingredients in product (Mohrle, 2002).

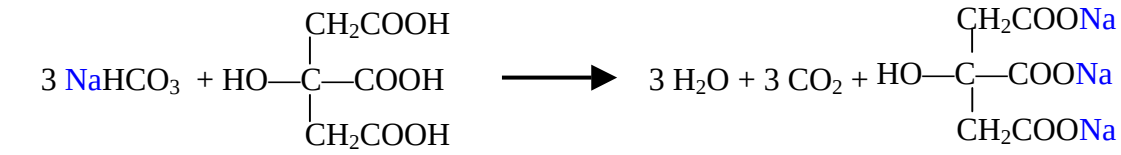
A perfect lubricant for effervescent products must nontoxic, tasteless and very soluble. The lubricant is one of the most important for the production of effervescent tablets on high speed equipment. Many substances are efffective lubricants but inhibit tablet disintegration at the same concentration. When concentration is lowered; as a result, tablet disintegration is increased but the lubricating efficiency of material for tableting is decreased. Stearate groups, such as magnesium stearate or calcium stearate, which are lubricant in conventional tablets, have a problem in effervescent tablets because they will leave a cloudy solution due to their water insoluble. The lubricant used in effervescent formulations should combine hydrophobic and hydrophilic properties that lead to both good lubrication and a short disintegration time (Mohrle, 2002; Lindberg and Hansson, 2002). Sodium sterate and sodium oleate are water soluble in low concentrations, but they have the soapy taste. Powdered sodium benzoate and micronized polyethylene glycol 8000 are effective water soluble lubricant. It has been found that the addition of sodium benzoate promotes tablet disintegration rather than an inhibiting effect (Khanuja et al., 2004). Roscheisen and Schmidt (1995) studied preparation and optimization of L-leucine as lubricant for effervescent tablet formulations. Rotthauser et

al (1998) also studied optimization of an effervescent tablet formulation containing spray dried L-leucine and polyethylene glycol 6000 as lubricants. To avoid having tablets stick to the punch faces, polytetrafluorethylene or polyurethane have been applied to the punch faces (Sieganus et al., 1963).

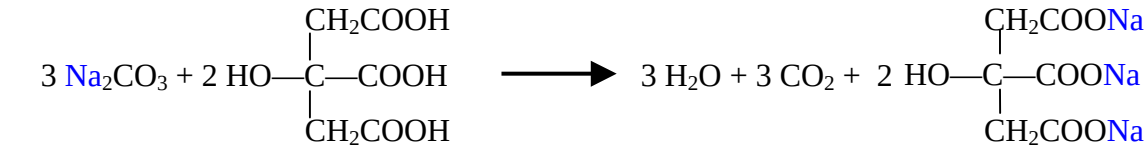
Effervescence may contain ingredients other than the previously presented. For example, using sweeteners, flavors and color led to the attractiveness of product, using surfactant increase the wetting and dissolution rate of drugs, using antifoaming agents reduced the formation of foam, using glidants to increase flowability, using disintegrants to increase the disintegration time and using antiadherents protected the punch surface (Mohrle, 2002).

2.2 The effervescent reaction

Acid-base reactions between citric acid and sodium bicarbonate or sodium carbonate will start after addition of effervescence into water. One mole of citric acid anhydrous are completely neutralized with three mole of sodium bicarbonate, while two moles of citric acid anhydrous are completely neutralized with three moles of sodium carbonate. The reaction equation is shown as follows:



Sodium bicarbonate (MW = 84.01) Citric acid anhydrous (MW = 192.12)



Sodium carbonate (MW = 106) Citric acid anhydrous (MW = 192.12)

2.3 Processing

In processing of effervescent products, environment conditions and equipment are required. A maximum of 25% relative humidity at a controlled room temperature of 25 ^{O}C (72 ^{O}F) or less is usually satisfactory to prevent the absorption of moisture from the air, and lead to product stability (Lindberg and Hansson, 2002). The processing equipment, such as mixers, granulators, drying equipment and mill, should be made from stainless steel or other material resistant to acids (Ansal, 1969).

Preparation of effervescent salts have three general methods, the wet method, the dry method and the fusion method. The wet method is mixing of the dry ingredients with a granulating fluid to produce a damp mass. The acid and carbonate parts can be granulated separately or together with water, ethanol, isopropanol or other solvents. Then, the granules are prepared and dried in dryer (Ansal, 1969; Lindberg and Hansson, 2002).

The dry method is suitable for materials that cannot be wet to granule. Dry granulation is prepared by slugging or roller compaction. The acidic and basic components may be dry granulated separately or together. In the wet and dry method, all materials may be anhydrous (Ansal, 1969; Lindberg and Hansson, 2002).

The fusion method employs heat to liberate water of crystallization from hydrous citric acid to bind the mixture. The citric acid crystal are powdered through a number 60 sieve and then mixed with the other powders. The mixture is placed in an oven at 93-104 ^{O}C. Next, it is removed from oven and passed through a sieve to produce granules. The granules are immediately dried at a temperature not exceeding 54 ^{O}C (Ansal, 1969; Lindberg and Hansson, 2002).

2.4 Stability

Finely anhydrous sodium carbonate about 10% w/w of formulation has been found to be an effective stabilizing agent. It is theorized that the anhydrous sodium carbonate absorbs the free water to produce stable hydrated forms (Gergely et al., 1999).

Usually, sodium bicarbonate in formulation is heated to convert the surface of sodium bicarbonate particle to 2-10% sodium carbonate. The addition of sodium carbonate did not improve stability by itself. The stabilizing effect results from a uniform distribution of carbonate on the surface of bicarbonate, that forms a barrier to react with acid in formulation. The particle of carbonate from heated sodium carbonate is finer than added crystalline sodium carbonate (Usui and Carstensen, 1985). Furthermore, double salts might be better scavenger than the carbonate itself. The moisture scavenging effect of potassium carbonate was examined (Well et al., 1997).

3 Extrusion-Spheronization

Extrusion-spheronization is a multiple-step process of preparation of spherical particles, which are commonly referred to as spheres or pellets. The spheres are of interest due to good flow, low dusting, uniform size distribution, low friability, high hardness, ease of coating, and reproducible packing.

3.1 Applications

In pharmaceutical, extrusion/spheronization are usually used as a method to prepare pelletized product. Major advantage of this method is producing drug-loaded spheres at high level of active ingredient without producing an excessively large particle. And, the process is more efficient than other techniques for producing pellets (Ghebre-Sellassie and Knoch, 2002). The pellets can be used for both immediate-release or modified-release applications. They offer the common therapeutic advantages found with multiparticulate drug delivery system, such as a reduced risk of dose dumping in modified-release products, and less gastrointestinal irritation. Two and more actives that are incompatible or have different release profiles can easily be combined in the same dosage unit. Small pellets can be used to limit drug migration for low-dose actives. The active ingredients and excipient can be modified to improve physical properties and downstream. As an example, a low density and finely of active can be pelletized to increase density, to improve flow and to limit dusting. Functional coatings can be applied

easily and effectively. If, the coated pellets are to be compressed into tablets, it will require sufficient strength to withstand the forces of compression (อำพล ไมตรีเวช, 2538; Erkoboni, 2003; Sellassie and Knoch, 2002).

3.2 Equipment and process parameters

Extrusion-spheronization is a process requiring at least five units of operation including dry mixing, wet granulation, extrusion, spheronization and drying. For controlled-release applications, a coating step may also be necessary (Erkoboni, 2003).

3.1.1 Dry mixing

The first step of preparing pellets, the materials are dry mixed to achieve a homogeneous powder dispersion. The uniformity of the dry mix has a significant effect on the quality of the granulation and the spheres produced. An uneven distribution of materials in different properties such as size and solubility can result in localized overwetting during the granulation step. The more soluble and finely divided components can dissolve and become parts of the granulating fluid. The unevenly wetted particle surfaces will result in pellets with a broad size distribution. Size and shape uniformity of pellets are very much dependent on the uniform distribution of dry mix and the composition of the granulating fluid (Ojile et al., 1982).

3.1.2 Granulation

In the second step, the homogeneous powder is wet granulated to produce the wet mass that is plastic or deformable. Mixer or granulator with continuous type such as planetary mixers, vertical or horizontal high shear mixers and sigma blade mixers is generally used to prepare a wet mass for compression (Erkoboni, 2003).

Granulation variables including granulating fluid level and wet mass mixing time have a significant effect on the size of the pellet produced. At relatively low granulating fluid levels, longer mixing times will distribute the water more effectively and result in more cohesive granule surfaces. This results in a slight increase in the size of the pellet

produced. On the other hand, when higher fluid levels are used, longer mixing times will distribute the water effectively within the pore structure of the particles, reducing or eliminating overwet surface while ensuring a sufficiently plastic mass. The distribution of water and the reduction of surface water occur in the particle having a smaller mean size and narrower distribution (Erkoboni, 2003).

The type of granulation liquids result in the formulation of pellet. Dreu et al. (2005) suggested that the surface tension (γ_L) of product, relative permittivity (ε_R) of the granulation liquid and the cosine of content angle (θ) of granulation liquid on pellets solid has been introduced in order to explain the mechanism of this phenomenon. Tensile strength and disintegration times of pellets increase with increase in the proposed factor $\gamma_L \times \cos(\theta) \times \varepsilon_R$, while friability, average pore diameter and porosity decreases. Boutell et al. (2002) found that the type and level of liquid were an important factor in term of the force necessary to extrude, resulting in pellets size, size range, roundness and porosity of pellets.

Moreover, the rising temperature from using high-energy mixers such as high-shear mixers can result in a greater than acceptable level of evaporation or in an increase in the solubility of some of the solids. It may be necessary to use a jacket to guard against heat buildup (Baert et al., 1991).

3.1.3 Extrusion

The third step is the extrusion step, the wet mass is extruded through dies of extruder to form small cylindrical particle having uniform diameter. The extrudate particles break at similar lengths under their own weight. The extrudate must have enough plasticity to deform but not so much as to adhere to other particles when rolled in the sphernizer (Erkoboni, 2003).

Extruders can be divided into three classes based on their feed mechanisms as screw-fed extruders, gravity-fed extruders and ram extruders as shown in figure 6. Screw-fed extruders have screws that rotate along the horizontal axis and transfer the material horizontally. They may be axial, dome or radial screw extruder. Gravity-fed extruders include the cylinder, gear and radial types. The screw and gravity-fed extruders are used

for development and manufacturing in pharmaceuticals (Erkoboni, 2003; Ghebre-Sellassie and Knoch, 2002).

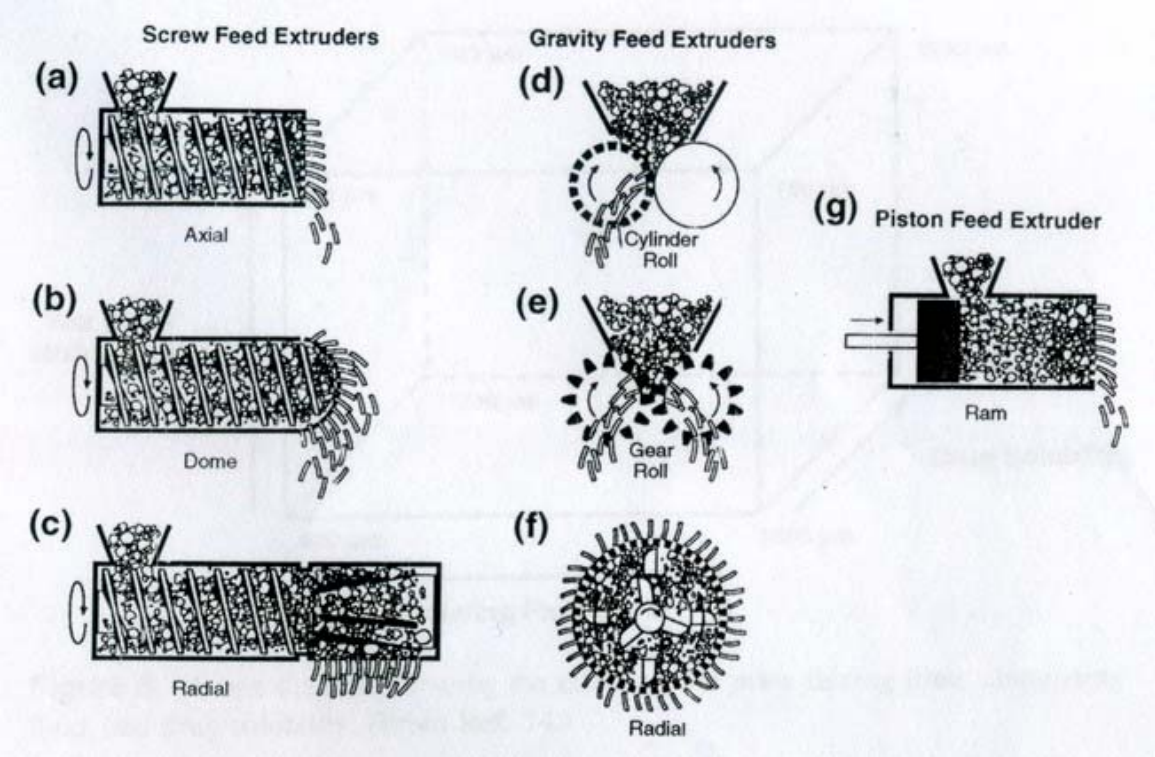


Figure 6 Schematic diagrams of extruder type used in extrusion/spheronization (Erkoboni, 2003).

The piston-fed or ram extruder is primarily used in research because it is designed to the flow characterization of wet masses through a die. The force profile produced as the mass flows through a die has been divided into three stages: compression, steady state flow and forced flow. The three stages are shown in the force versus displacement profile in figure 7. The compression stage is where the materials are consolidated under slight pressure. Better formulation have a minimal compression stage with a rapid increase to a maximum force. The steady state flow stage is where the pressure required to maintain flow is constant. Low state flow or an evidence of moisture exiting the die prior to the extrudate generally indicated an overwet mass. High steady state forces bring about an extrudate with surface irregularities. The irregularities are commonly referred to as sharkskin. While significant surface irregularities are undesirable, some irregularities can be beneficial. They assist in the extrudate breaking up into appropriate-sized, rod-shaped particles during the initial stage of the spheronization process. Last stage, forced flow is

where an increase in force required to maintain flow. This condition is typically due to an insufficiently plastic mass (Erkoboni, 2003).

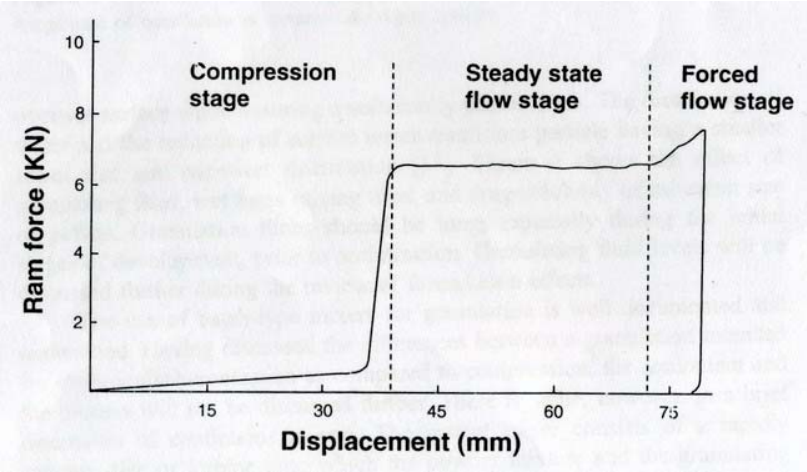


Figure 7 A force displacement profile for a microcrystalline cellulose/lactose/water mixture showing the three stages of extrusion on a ram extruder, compression,steady-state flow and forced flow (ram speed 4 mm/s, die diameter 1.5 mm, R/L ratio 12 (Erkoboni, 2003).

3.1.4 Spheronization

The forth step is the spheronization step. The extrudate or rod-shaped particle are transferd into a spheronizer and drawn to the walls of spheronizer due to centrifugal forces. The extrudate is breaks into smaller, more uniform pieces. Under ideal conditions, the length of each piece is approximately equal to the diameter the attrition and the rapid movement of the bottom plate or disk. The differential in particle velocity as the pieces move outward to the walls, begin to climb the wall and fall back onto the rotating bed results in a rope-like formation as shown in figure 8. Then, this piece is rounded off into spherical particles (Reynolds, 1970).

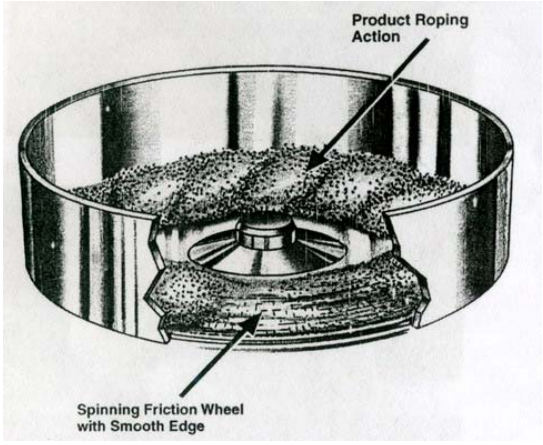


Figure 8 A graphic representation of the characteristic rope-like formation in a spheronizer bowl during operation (Erkoboni, 2003).

The rounding of the extrudate into spheres is dependent on frictional forces. The forces are generated by particle-particle and particle-equipment interactions. So, the working parts of spheronizer is a bowl having fixed sidewalls with a rapidly rotating bottom plate or disk. Disk have two geometric patterns, a cross-hatched pattern with the groves running at right angles to one another and a radial pattern with the groves running radially from the center. The two varieties are shown in figure 9 (Erkoboni, 2003). Disk having a radial pattern is believed that it has more efficiency than a cross-hatched pattern because the grooved surface of disk running radially from the center result in well energy-transferring in system. But, disadvantage of disk having a radial pattern is decreasing of efficiency when increasing of distance between particle and disk center. Width of groove of disk have a effect on pellet size, for example, 1-mm of pellet size should used 2-mm of grooved width of disk (อำพล ไมตรีเวช, 2538).

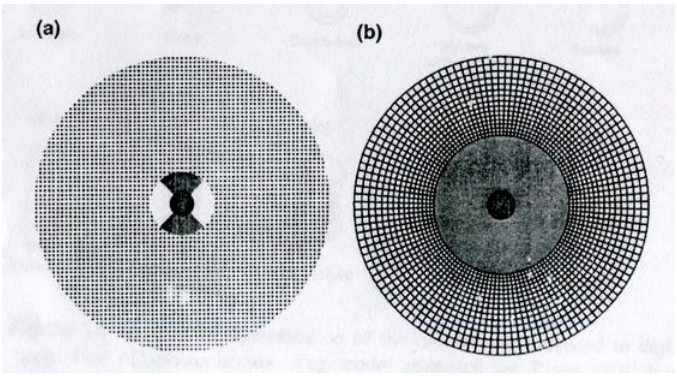


Figure 9 Spheronizer disks having two geometric patterns: (a) a cross-hatched pattern with the groves running at right angles to one another, and (b) a radial pattern with the groves running radially from the center (Erkoboni, 2003).

During the spheronization step, the transformation from cylinder-shape extrudate to a sphere occur in various stage. Two models have been proposed to describe the mechanism as shown graphically in figure 10. The first model proposed by Baert et al. (1993) suggested that the cylindrical particles (figure 10-1a) are deformed into a bent rope-shaped particle (figure 10-1b), and then form a dumbbell with a twisted middle (figure 10-1c). The twisting action causes the dumbbell to break into two spherical particles with a flat side having a hollow cavity (figure 10-1d). Next, continued action in the spheronizer causes the particles to round off into spheres (figure 10-1e). The second model proposed by Rowe (1985) found that the cylindrical particles (figure 10-2a) are first rounded off into cylindrical particles with rounded edges (figure 10-2b), then form dumbbell-shaped particles (figure 10-2c), ellipsoids (figure 10-2d), and finally spheres (figure 10-2e). However, the exact mechanism is likely composition-dependent. If the extrudate is overwet, particle growth will occur, leading to a broad size distribution. In contrast, if the extrudate is underwet, extrudate will not have enough plasticity to further round off in the spheronizer, leading to the formation of dumbbells (Erkoboni, 2003).

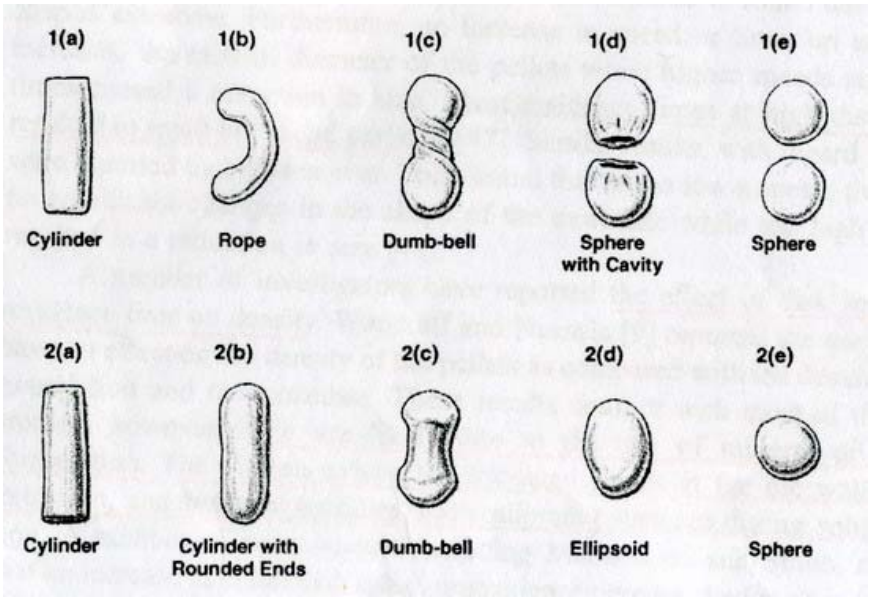


Figure 10 A graphic representation of the two models proposed to describe the mechanism of spheronization: (1) Baert et al.'s model (1993) and (2) Rowe's model (1985) (Erkoboni, 2003).

3.1.5 Drying

In the final step, the spherical particles are dried by dryers such as tray dryers or fluidized bed dryers. Tray drying is the slowest of fluid removal. This drying method is simple but it may increase migration and recrystallization of drug. Fluidized bed dryers is a more rapid drying rate because of the higher air volumes and the higher inlet temperatures. The more rapid rate in a fluid bed will likely mininize the effects of migration (Erkoboni, 2003).

Bashaiwoldu et al. (2004) found that the different drying techniques produced pellets of different structural and mechanical properties. Pellets dried by freeze-drying and fluid-bed drying were higher porous and greater mean diameter. On the other hand, pellets dried by oven or desiccation with silica-gel were lower porous.

4 Effervescent granules and pellets Additives

4.1 Mannitol

Mannitol is a white, odorless, crystalline powder, or free-flowing granule. It has a sweet taste, approximately as sweet as glucose and half as sweet as sucrose and a cooling sensation in the mouth. Mannitol is primarily used as a diluent in tablet formulations. It is not hygroscopic and hence may be used with moisture-sensitive active ingradients. Mannitol is commonly used as an excipient in chewable tablet formulation because of its negative heat of solution, sweetness and mouth feel. One part of mannitol can solve in 5.5 parts of water, 3.7 parts of water at 50 ^{O}C, 18 parts of glycerin, 83 parts of 95% ethanol or 100 parts of propan-2-ol. Mannitol is incompatible with xylitol infusion and may from complexes with some metals such as aluminum, copper and iron (Rowe et al., 2003).

4.2 Citric acid anhydrous

Citric acid anhydrous is a odorless, colorless crystals or a white crystalline powder. One part of citric acid can solve in 1 part of water and 1 part of ethanol (95%). Citric acid, powdered forms are used in the preparation of effervescence. Citric acid anhydrous, fine granular 51N is one of the fineness of citric acid anhydrous used for effervescent tablet. It has size distribution between 149-595 μm. It is suggest to prevent caking at temperatures above 40 ^{O}C (Rowe et al., 2003).

4.3 Acesulfame potassium

Acesulfame potassium is used as a sweetening agent in cosmetics, food, beverage products and pharmaceutical preparations. The approximate sweentening powder is 180-200 times that of sucrose. It is a colorless to white-colored, odorless, crystalline powder. One part of acesulfame potassium can solve in 7.1 parts of water at 0 ^{O}C, 3.7 parts of water at 50 ^{O}C, 0.77 parts of water at 100 ^{O}C, 100 parts of 50% v/v ethanol or 1000 parts

of ethanol. In aqueous solution, pH 3.0-3.5 at 20 ^{O}C, it does not reduce in sweetness which was observed approximately 2 years (Rowe et al., 2003).

4.4 Povidone

Povidone is a fine, white to creamy-white colored, odorless or almost odorless, hygroscopic powder. Povidones with K-values ≤ 30 are produced by spray drying and occur as spheres. Povidone K-90 and higher K-value povidones are produced by drum drying and occur as plates. Concentration of povidone of uses is suggest that 10-25% for carrier for drugs, 0-5% for dispersing agent or suspending agent, 2-10% for eye drops and 0.5-5% for tablet binder, tablet diluent or coating agent. Povidone is very hygroscopic, amounts of moisture being significant absorbed at low relative humidities.

Povidone is freely soluble in acids, chloroform, ethanol, ketones, methanol and water. The viscosity of povidone solution depends on both the concentration and the K-value. For example, 10% w/v aqueous povidone (Kollidon) K-28/32 solution at 20 ^{O}C has dynamic viscosity as 5.5-8.5, 5% w/v povidone (Kollidon) K-30 solution in ethanol at 25 ^{O}C has dynamic viscosity as 3.4 mPas and 5% w/v povidone (Kollidon) K-30 solution in propan-2-ol at 25 ^{O}C has dynamic viscosity as 4.7 mPas (Rowe et al., 2003).

4.5 Sodium bicarbonate

Sodium bicarbonate is an odorless, white, crystalline powder with a saline, slightly alkaline taste. It is generally used in pharmaceutical formulation as a source of carbon dioxide in effervescent system and widely used to produce or maintain an alkaline pH in a preparation. Concentration of sodium bicarbonate of uses is suggest that 10-40% for buffer in tablets, 25-50% for effervescent tablets and 1.39% for isotonic injection/infusion. For a freshly prepared 0.1 M aqueous solution at 25 ^{O}C, its pH is 8.3 and its alkalinity increase on standing, agitation or heating. The moisture content is less than 1% w/w at below 80% relative humidity. Sodium bicarbonate rapidly absorbs moisture and start to decompose with loss of carbon dioxide at above 8.5% relative humidity (Rowe et al., 2003).

4.6 Sodium carbonate

Sodium carbonate, also known as soda ash, is commercially available as an anhydrous form and a monohydrate or a decahydrate. All forms are very soluble in water. The anhydrous form is hygroscopic and convert to monohydrate form after absorbing moisture.

4.7 Effer-soda™ 12

Effer-soda™ is sodium bicarbonate which is modified its surface to convert the surface of sodium bicarbonate to sodium carbonate approximate 8-12%, which is called a desiccant skin, as shown in figure 11. Effer-soda™ is suggested by SPI Pharma™ that the sodium carbonate outer layer protects the sodium bicarbonate core by absorbing moisture to form a hydrate salt (sodium sesquicarbonate). Sodium sesquicarbonate which is stable up to 70 ^{O}C is the key of temperature stability to prevent effervescent reaction at ambient and elevated temperature storage conditions (SPI Pharma™, 2003).

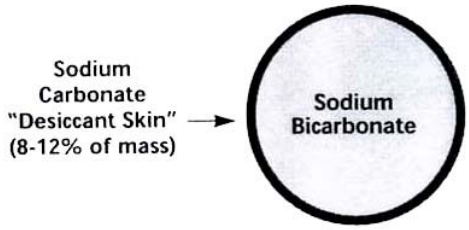


Figure 11 Characteristic of Effer-soda™ particle (SPI Pharma™ group, 2003).

4.8 Colloidal silicon dioxide

Colloidal silicon dioxide is a submicroscopic fumed silica with a particle size of about 15 mm. It is a light, loose, bluish-white-colored, odorless, tasteless, nongritty amorphous powder. Its small particle size and large specific surface area change the flow characteristics of dry powder to improve the flow properties. Colloidal silicon dioxide has functional category such as adsorbent, anticaking agent, emulsion stabilizer, glidant,

suspending agent, tablet disintegrant, thermal stabilizer and viscosity-increasing agent. Recommended concentrations for uses are, for example, 0.5-2.0% for aerosols, 1.0-5.0% for emulsion stabilizer, 0.1-0.5% for glidant and 2.0-10.0% for suspending and thickening agent. Its solubility is practically insoluble in organic solvents, water and acid except hydrofluoric acid and soluble in hot solutions of alkali hydrixide. Colloidal silicon dioxide have hydroscopic surface that greatly minimize their hygroscopicity (Rowe et al., 2003).

4.9 Microcrystalline cellulose

Microcrystalline cellulose (MCC) is a white, odorless, tasteless, crystalline powder composed of porous particles. Nominal mean particle size of MCC pH-101 is 50 μm. Amount of MCC pH-101 is retained on sieve mesh size number 60 as ≤ 1.0% and sieve mesh size number 200 as ≤ 30.0%. Moisture content of MCC pH-101 is ≤ 5.0%. Concentration of uses is suggested that 5-20% for antiadherent, 5-15% for tablet disintegrant and 20-90% for adsorbent, capsule diluent or tablet diluent (Rowe et al., 2003).

CHAPTER III

EXPERIMENTAL

Material

The following material and commercial sources were used.

1. Active Ingredient:

Phyllanthus Emblica extract (Lot no. 081206G used for effervescent granule formulation and Lot no. 171106E used for pellet formulation, Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand)

2. Standard materials:

Gallic acid (Lot no. 456715/1 62804019, % purity = 99.6, % LOD = 9.9, Fluka Chemie GmbH, Buchs, Switzerland)

3. Effervescent granules and pellets Diluents:

- Mannitol (Batch no. 06101002, Shandong jiejing group, Shandong, China, distributed by Asia Drug & Chemical LTD., Bangkok, Thailand)
- Citric acid anhydrous, fine granular 51N (Lot no. 255245 for effervescent granule formulation and Lot no. 256087 for pellet formulation, DSM Citric Acid (Wuxi) Ltd., Jiangsu, China, distributed by Adinop Co.,LTD., Bangkok, Thailand)
- Acesulfame potassium, Vitasweet® Ace K (Batch No. 0508088, VitaSweet Co., LTD., Beijing, China, distributed by Rama Production, Bangkok, Thailand)

- Povidone K30 USP (Plasdone K29/32, Lot no. 05500141059, distributed by Maxway Co.,LTD., Bangkok, Thailand)
- Sodium bicarbonate (Ajax Finechem, NSW, Australia)
- Sodium carbonate (Ajax Finechem, NSW, Australia)
- Effer-soda^{TM} 12 (Lot No. 05H066, SPI Pharma, DC, UK, distributed by Erawan Chemical Company, Bangkok, Thailand)
- Colloidal silicon dioxide (Aerosil® HDK N20, Lot no. VA70093, Wacker Chemie AG , Munchen, Germany, distributed by Maxway Co.,LTD., Bangkok, Thailand)
- Microcrystalline cellulose 101, Comprecel® (Lot No. 70126, Mingtai Chemical Co.,LTD., Taoyuan Hsien, Taiwan, distributed by Maxway Co.,LTD., Thailand)

4. Chemical

- Methanol HPLC (Batch no. 0607432, Fisher Scientific, Leicestershire, UK)
- Methanol Analytical reagent grade (Batch no. 0623782, Fisher Scientific, Leicestershire, UK)
- Trifluoroacetic acid (Lot no. 60900, Fluka Chemie GmbH, Buchs, Switzerland)
- Folin-Ciocalteu's phenol reagent (Lot no. 026K0008, Sigma, MO, USA)
- Sodium chloride (Lot no. 040574, J.T.Baker, Phillipsburg, USA)
- Sabouraud Dextrose Agar (Lot no. 5283, Britania, Buenos Aires, Argentina)
- Tryptic Soy Agar (Lot no. 6192377, Becton Dickinson and Company, NJ, USA)
- Tryptic Soy broth (Lot no. VM817759, Merck, Darmsfadt, Germany)
- Mannitol Salt Agar (Lot no. 458.3, Britania, Buenos Aires,Argentina)
- Cetrimide Agar Base (Lot no. 4268698, Dickinson and Company, NJ, USA)
- Lactose broth (Lot no. V189561, Merck, Darmsfadt, Germany)
- Selenite cystine enrichment broth (Lot no. V183509, Merck, Darmsfadt, Germany)
- Tetrathionate broth (Lot no. V127882, Merck, Darmsfadt, Germany)

- Brilliant green phenol red lactose sucrose agar (BPLS agar) (Lot no. V728032, Merck, Darmsfadt, Germany)
- Xylose Lysine Desoxycholate Agar (Lot no. 1000IODJYA, BBL, NJ, USA)
- Bismuth Sulfite Agar (Lot no. V744718, Merck, Darmsfadt, Germany)
- MacConkey Agar (Lot no. V960465, Merck, Darmsfadt, Germany)
- Cooked-Meat Medium (Lot no. 887988, Oxoid, Hampshire, England)
- Glycerol (Lot no. 6AD2026613, Carlo erba, Rodano, Milano)
- Liquid paraffin (Lot no. 502235, Srichand United Dispensary Co.,LTD, Bangkok, Thailand)
- Iodine (Lot no. 31089826, Merck, Darmsfadt, Germany)
- Potassium iodide (May & Baker Laboratory Chemicals, Battersea, England)
- Brilliant green (Lot no. 2065340, BDH Chemical, Poole, England)
- Sheep blood (Department of Animal Husbandry, Faculty of Veterinary Science, Chulalongkorn University, Thailand)

Equipment

- Moisture analyzer (Model HR83,Mettler Toledo, Schwerzenbach, Switzerland)
- Sieve shaker with 100 mm diameter of sieves (Model FT-150M, Filtra, Badalona, Barcelona)
- Sieve shaker with 200 mm diameter of sieves (Model FT-200M, Filtra, Badalona, Barcelona)
- pH meter (Model 201, ORION, Boston, USA)
- Texture analyser (Model TA-XT plus, Stable Micro System, Godalming, UK)
- Spectrophotometer (Model V-530, Jasco, Tokyo, Japan)
- High performance liquid chromatography system as follows:
    - Shimadzu liquid chromatograph (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)
    - Column oven (Model CTD-10AS, Shimadzu, Kyoto, Japan)
    - Shimadzu diode array detector (Model SPD-M10A vp, Shimadzu, Kyoto, Japan)
    - Shimadzu UV-Vis detector (Model SPD-10A, Shimadzu, Kyoto, Japan)
    - Shimadzu system controller (Model SCL-10A vp, Shimadzu, Kyoto, Japan)
    - Shimadzu software (Model Class VP, Shimadzu, Kyoto, Japan)
- Alltima C18 column, 4.6 x 150 mm, 5µ (Lot no. 0509001018, AllTech, IL, USA, distributed by Ligand scientific Co.,LTD., Bangkok, Thailand)
- Balance (No. MT-045, Mettler Toledo, Schwerzenbach, Switzerland)
- Analytical Balance (Model A200S, Sartorius, Goettingen, Germany)
- Ultrasonic Cleaner (Type TP680DH, Elma, Singen, Germany)
- Planetary mixer (Kitchen Aid Model 5K5SS, St. Josoph, Michigan, USA)
- Cubic-shape mixer (Model AR 400, ERWEKA® GmbH, Heusenstamn, Germany)
- Single screw extruder (Model EXKS-1, Fuji Paudal Co.,Ltd, Osaka, Japan)
- Spheronizer (Type S320, Aeromatic-fielder, Hampshire, England)
- Hot air Oven (Model Tv40uL 998001, Memmert, Munich, Germany)

- Ultrapycnometer 1000 (Quantachrome, NY, USA)
- Laser diffraction particle sizer (Mastersizer S long bed Ver. 2.11, Malvern, Worcestershire, UK)
- Scanning electron microscope (Model S-2500, Hitachi, Tokyo, Japan) for morphology analysis of *Phyllanthus Emblica* extract
- Scanning electron microscope (Model JSM-5410LV, Joel Ltd., Tokyo, Japan) for morphology analysis of pellets
- Petri dish with 9-cm diameter
- Glass funnel with 1.5-cm orifice
- Graduate cylinder 25 ml (Duran®, Wertheim, Germany)
- Desiccator with outlet, 250-mm diameter (Duran®, Wertheim, Germany)
- Syringe filter, Nylon (Lot no. 49006, Vertical™, Bangkok, Thailand, distributed by Ligand scientific Co.,LTD., Bangkok, Thailand)
- Filter papers, 110-mm diameter (Lot no. G1857835G, Whatman®, Maidstone, England)
- 2 ml vial camber (Lot no. 293040015125, Sun-sri, TN, USA, distributed by Ligand scientific Co.,LTD., Bangkok, Thailand)
- Anaerobic indicator (Lot no. 13792001, Oxoid, Hampshire, England)
- Anaerobic system envelopes, Gaspak™ (Lot no. 5290603, Becton Dickinson and Company, NJ, USA)
- Friabilator (Type TA3, ERWEKA® GmbH, Heusenstamn, Germany)
- Steromicroscope (Model ML9300, Meiji, Tokyo, Japan) linked with Digital camera (EOS100, Cannon, Tokyo, Japan)

Method

1 Preparation of *Phyllanthus Emblica* extract

Phyllanthus Emblica extract was prepared by the Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University. Firstly, Selected the *Phyllanthus Emblica* fruits, not become rotten, were cleaned by water. Secondly, 1.5 kg of *Phyllanthus Emblica* fruits were mixed with 1 litre 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 litre of the distilled water was added to the soild part and crushed again by grinder for 5 minutes. The liquid part was separated again. Next, both the liquid parts were mixed and filtered by polyester cloth again. Finally, the liquid parts were dried by spray dry technique to fine yellow powders. One-kilogram of *Phyllanthus Emblica* extract lot. 171106E was extracted from 23.7 kg of *Phyllanthus Emblica* fruits and 1 kg of *Phyllanthus Emblica* extract lot no. 081206G was extracted from 25 kg of *Phyllanthus Emblica* fruits.

2 Characterization of *Phyllanthus Emblica* extract and excipients

2.1 Morphology study

The *Phyllanthus Emblica* extract was prepared by gold sputtering technique and viewed using a scanning electron microscope (SEM). Size, shape and surface topography were determined using magnification of 1,500x, 15 kv. and 3,500x, 15 kv.

2.2 Loss on drying

Two grams of *Phyllanthus Emblica* extract was heated at 105 ^{O}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.

The moisture constants of the excipients used in the formulation were also measured and presented here.

2.3 pH

The *Phyllanthus Emblica* extract was dissolved/dispersed to prepare 1% w/v *Phyllanthus Emblica* extract at ambient temperature. The pH of the liquid was measured. The value of pH was average from three replicates.

2.4 Bulk density, Tapped density and Percent compressibility

Five grams of *Phyllanthus Emblica* extract were filled into a 25-ml graduate cylinder. The bulk volume was recorded and the bulk density was calculated as the following equation :

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

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

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

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 and B = bulk density

The flowability can be identified from the Carr's compressibility index as shown in table 2

Table 2 Classification of flowability by Carr's compressibility index (Davies, 2001)

Carr's Index (%)	Flow
5-12	Free Flowing
12-16	Good
18-21	Fair
23-33	Poor
35-38	Very poor
>40	Extremely Poor

2.5 Flow rate

Twenty grams of *Phyllanthus Emblica* extract were fillled in a glass funnel with 1.5-cm orifice. The glass funnel was fixed on the clamp at 6.5-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.

2.6 Apparent density

Apparent density was determined by gas displacement technique. The *Phyllanthus Emblica* extract was dried at 45 ^{O}C overnight and weighed. They were filled to a micro cell and analysed by Ultrapycnometer 1000 in Helium gas (99.999%) at 23.4-23.5 ^{O}C. The value of apparent density, in term of g/cm^3, was average from five determinations of one sample.

The apparent densities of the excipients used in the formulation were also measured and presented here.

2.7 Particle Size Distribution

2.7.1 Sieve analysis

The sieve shaker (power = 9 and cycle = 9) with sieve mesh size number 40 (0.425 mm), 60 (0.250 mm), 80 (0.180 mm) and 100 (0.150 mm) was used. Five grams of *Phyllanthus Emblica* extract were accurately weighed and put on the top of sieve. Then, the set of sieves was shaked for 15 minutes. Powders remained on each sieve size were weighed and calculated in percentage of weight distribution.

2.7.2 Laser diffraction technique

Size distributions of *Phyllanthus Emblica* extract and excipients in the formulations were determined by Mastersizer S long bed Ver. 2.11. The particle reflective index was 1.5330 and dispersant reflective index was 1.4000. The *Phyllanthus Emblica* extract was added slowly and dispered in isopropyl myristate until obscuration value of measurement increased to 15-20%. The mean diameter by volume was average from three determinations.

2.8 Total tannins analysis

Total tannins were measured by Folin-Ciocalteau method. (Slinkard and Singleton, 1977) The method was proceeded as the following :

2.8.1 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, 60, 80 and 100 μg/ml) which were used as standard solutions.

2.8.2 Sample preparation

Twenty-five milligrams of *Phyllanthus Emblica* extract were weighed into a 250-ml volumetric flask, added with water approximately 125-ml and sonicated for 15 minutes. Then, the volumetric flask was filled up to volume with water and mixed well

(concentration of the solution = 100 μg/ml). The solution was filtered through a Whatman® no. 1 filter. Total tannins were determined in three replicates.

2.8.3 Diluted solution of Folin-Ciocalteau reagent

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

2.8.4 Colorimetric reaction

One milligram of standard solution, sample solution or water (reagent blank) was transferred into a 15-ml glass tube. Five milliliters of diluted Folin-Ciocalteau reagent were added and mixed. After waiting for 3-8 minutes, 4-ml of 7.5% sodium carbonate anhydrous solution were added and mixed by vortex mixer. This glass tubes stayed for 2 hours at ambient temperature in the dark cabinet. Then, absorbance of the solution was measured by spectrometer at 747 nm.

2.8.5 Calculation

The absorbance and concentrations of the gallic acid standard solutions were graphically plotted and the coefficient of determination (R^2) was calculated. The total tannins concentration of sample solution were calculated from the linear equation of the standard curve and presented were in terms of gallic acid equivalence (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.

2.9 Gallic acid analysis

The percentage of gallic acid in *Phyllanthus Emblica* 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 column	:	Alltima® C18 column (4.6x150 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	:	1.0 ml/min
Detection	:	UV detector at 270 nm
Injection volume	:	20 μl
Temperature	:	Ambient
Run Time	:	12 min

2.9.1 Validation of HPLC method

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

2.9.1.1 Linearity

Gallic acid standard solutions with various concentrations ranging from 0.25 to 7.5 μg/ml were prepared and analyzed. The linear equation of the curve obtained by plotting the peak areas versus the concentrations of each standard solution was calculated using the least square method. The regression coefficient was more than 0.999.

2.9.1.2 Accuracy

Five mixtured solutions containing of varied contents gallic acid (0.5, 1.0, 2.5, 4.0 and 5.0 μg/ml) and constant level of *Phyllanthus Emblica* extract (7.5 μg/ml) were

prepared and analyzed on different days. Accuracy was calculated as the percentage of recovery as the following equation :

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

Before % recovery was calculated, peak area of each of the solutions was substracted with peak area of *Phyllanthus Emblica* extract. Peak area of the extract was obtained from Y-intercept value of the linear graph plotted between peak area of the mixtured solution versus varied gallic acid concentrations.

2.9.1.3 Precision

A Within Run Precision

Mixtured solution containing of gallic acid 1.0 μg/ml) and *Phyllanthus Emblica* extract 7.5 μg/ml was prepared as described in section 2.9.1.2. Six replicated injections of this solution were analyzed in the same day. Percentages of relative standard deviation (% RSD) were calculated for determination of the precision. The % RSD values were not more than 2%.

B Between Run Precision

The between run precision was determined the solution that were prepared described in section 2.9.1.3.A and analyzed on different day for three days. Percentages of relative standard deviation (% RSD) were calculated for determination of the precision. The % RSD values of each days were not more than 2%.

2.9.1.4 Specificity

The specificity of the method was determined by comparison of HPLC chromatograms of gallic acid solution, placebo solution and gallic acid mixed with

placebo solution. The peak of gallic acid should have no interference from extraneous components and be well resolved from them.

The placebo solution was the solution of the excipients in EF3 formulation or PE7 formulation. EF3 formulation was selected for specificity test of effervescent granules because this formula was the center of the design. PE7 formulation was selected for specificity test of pellets because this formula had acesulfame potassium and citric acid at high level and had mannitol in formula. Gallic acid solution at a concentration of 1 μg/ml, placebo solution at a concentration of the total excipients of 500 μg/ml and gallic acid at a concentration of 1 μg/ml mixed with placebo solution at a concentration of the total excipients of 500 μg/ml were prepared and evaluted by using the chromatographic conditions as described in section 2.9.

2.9.1.5 System suitability

Gallic acid solution was prepared to obtain the final concentration 1 μg/ml described in section 2.9.2.1. Six replicated injections of this solution were analyzed. The % RSD values were not more than 2%. Tailing factor were not more than 2.0.

2.9.2 Assay for *Phyllanthus Emblica* extract

2.9.2.1 Standard preparation

Twenty-five milligrams of gallic acid were weighed into a 50-ml volumetric flask, dissolved in methanol and diluted to volume (concentration of the solution = 500 μg/ml). Next, 5 ml of the solution were pipetted filled into a 50-ml volumetric flask and diluted to volume with 0.3% trifluoroacetic acid (concentration of the solution = 50 μg/ml). Then, 1 ml of the solution was filled into a 50-ml volumetric flask and diluted to volume with mobile phase (concentration of the solution = 1 μg/ml). The final solution was filtered through 0.45 μm filter paper and injected into HPLC column.

2.9.2.2 Sample solution preparation

Twenty-five milligrams of *Phyllanthus Emblica* extract was weighed into a 50-ml volumetric flask. Methanol was added approximately 25 ml. Next, this volumetric flask was sonicated for 5 minutes and diluted to volume (concentration of the solution = 500 μg/ml). Then, 5 ml of the solution was filled into a 25-ml volumetric flask and diluted to volume with 0.3% trifluoroacetic acid (concentration of the solution = 100 μg/ml). The solution was filtered through 0.45 μm filter paper and injected into HPLC column. Each sample was determined in three replicates.

2.9.2.3 Calculation

The percent of gallic acid was calculated from peak area as the following equation.

$$\% \text{ Gallic acid} = \frac{PAsp}{PAstd} \times \frac{Wt\ std}{50} \times \frac{5}{50} \times \frac{1}{50} \times \frac{50}{Wt\ sp} \times \frac{25}{5} \times \%\ purity \times \frac{(100 - \%\ LOD)}{100}$$

PAstd = Peak area of gallic acid standard solution
PAsp = Peak area of extract solution
Wt std = Weight of gallic acid
Wt sp = Weight of extract
% purity = The gallic acid content in standard
% LOD = The moisture content in gallic acid standard

2.10 Microbial Contamination Test

Phyllanthus Emblica extract was detected for the amount of microbial contamination according to the method described in Thai Pharmacopoeia (Thai pharmacopoeia committee, 1987) and inspected for the limit of microbial contamination in product containing crude drug for internal use, according to Thai pharmacopoeia (Thai pharmacopoeia committee, 1997) as shown in table 3. And morphologic characteristics of *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus* and *Clostridium* spp. were showed as table 4-8.

Table 3 Limit of microbial contamination (Thai pharmacopoeia committee, 1997)

Total aerobic microbial count	Not more than 5.0×10^5
Yeasts and moulds count	Not more than 5.0×10^3
Enterobacterial count	Not more than 5.0×10^3
Escherichia coli count	Not more than 50 in sample 1 g or 1 ml
Staphylococcus aureus	Absent in sample 1 g or 1 ml
Salmonella spp.	Absent in sample 10 g or 10 ml
Clostridium spp.	Absent in sample 10 g or 10 ml

Table 4 Morphologic characteristics of *Salmonella* species on selective agar media (Thai pharmacopoeia committee, 1987)

Medium	Description of colony
Brinlliant green agar medium	Small, transparent, colourless or pink to white opaque (frequently surrounded by pink to red zone)
Xylose-lysine-desoxycholate agar medium	Red, with or without black centres
Bismuth sulfite agar medium	Black or green

Table 5 Morphology and diagnostic characteristics of *Pseudomonas aeruginosa* on Selective agar Media (Thai pharmacopoeia committee, 1987)

Medium	Cetrimide agar medium	Pseudomonas agar medium for detection of fluorescin	Pseudomonas agar medium for detection of pyocyanin
Characteristic colonial morphology	Generally greenish	Generally colourless to yellowish	Generally greenish
Fluorescence in UV light	Greenish	Yellowish	Blue
Oxidase test	Positive	Positive	Positive
Gram stain	Negative rods	Negative rods	Negative rods

Table 6 Morphologic characteristics of *Escherichia coli* on MacConkey agar medium (Thai pharmacopoeia committee, 1987)

Characteristic colonial morphology	Brick-red; may have surrounding zone of precipitated bile
Gram stain	Negative rods (cocco-bacilli)

Table 7 Morphologic characteristics of *Staphylococcus aureus* on selective agar media (Thai pharmacopoeia committee, 1987)

Selective medium	Mannitol-salt agar medium	Baird-Parker agar medium	Vogel-Johnson agar medium
Colonial morphology characteristics	Yellow colonies surrounded by yellow zone	Black, shiny colonies surrounded by clear zones 2 to 5 mm	Black surrounded by yellow zones
Gram stain	Positive cocci (in clusters)	Positive cocci (in clusters)	Positive cocci (in clusters)

Table 8 Characteristics of *Clostridium* Species on 5% defibrinated blood agar medium (Thai pharmacopoeia committee, 1987)

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

3 Formulation and preparation of *Phyllanthus emblica* extract products

3.1 Effervescent granules containing *Phyllanthus emblica* extract

3.1.1 Formulation of effervescent granules

Formulation variables may affect characteristics such as appearance, moisture, flowability and stability of product. The influence of three formulation variables were studied according to the center of gravity design (Podczeck, 1996).

The quantities of *Phyllanthus emblica* extract, colloidal silicon dioxide and citric acid anhydrous were studied. *Phyllanthus emblica* extract is hygroscopic and its content in the formulation might affect stability and properties of products. The extract contents in the formulation were designed in the range of 5-40%. Colloidal silicon dioxide has functional category such as adsorbent and glidant. It has been used for enhance flowability and moisture scavenger (Durig and Fassihi, 1993). Recommended quantity is 0.1-0.5% for glident (Rowe et al., 2003). The quantity, 15-35% of citric acid anhydrous was varied due to consideration of desired formulation pH which may affect product stability. Thus, formulation EF3 was the centre of the design as shown in table 9.

Two levels of the three formulation variables, 10% and 30% of *Phyllanthus emblica* extract, 0.2% and 0.4% of colloidal silicon dioxide, 20% and 30% of citric acid, were chosen for test of interaction (EF14-21). Formulation EF22 was a control formulation for colloidal silicon dioxide in the formulation.

Table 9 Formulation of Effervescent granules (Batch size = 400 g)

No.	*Phyllanthus emblica* extract (%)	Colloidal silicon dioxide (%)	Citric acid anhydrous (%)	Sodium bicarbonate (%)	Acesulfame potassium (%)	Povidone K29/32 (%)	Mannitol (%)
EF1	5	0.3	25	19.7	1	0.5	48.5
EF2	10	0.3	25	19.7	1	0.5	43.5
EF3*	20	0.3	25	19.7	1	0.5	33.5
EF4	30	0.3	25	19.7	1	0.5	23.5
EF5	40	0.3	25	19.7	1	0.5	13.5
EF6	20	0.1	25	19.7	1	0.5	33.7
EF7	20	0.2	25	19.7	1	0.5	33.6
EF8	20	0.4	25	19.7	1	0.5	33.4
EF9	20	0.5	25	19.7	1	0.5	33.3
EF10	20	0.3	15	19.7	1	0.5	43.5
EF11	20	0.3	20	19.7	1	0.5	38.5
EF12	20	0.3	30	19.7	1	0.5	28.5
EF13	20	0.3	35	19.7	1	0.5	23.5
EF14	10	0.2	20	19.7	1	0.5	48.6
EF15	30	0.4	30	19.7	1	0.5	18.4
EF16	30	0.2	20	19.7	1	0.5	28.6
EF17	10	0.4	20	19.7	1	0.5	48.4
EF18	10	0.2	30	19.7	1	0.5	38.6
EF19	30	0.4	20	19.7	1	0.5	28.4
EF20	30	0.2	30	19.7	1	0.5	18.6
EF21	10	0.4	30	19.7	1	0.5	38.4
EF22**	20	0	25	19.7	1	0.5	33.8

* center of the design

** A control experiment for extra information.

In addition, the effect of the alkaline type in the formulation was of interest. Sodium bicarbonate combined with sodium carbonate, or Effer-soda®, the surface of which is modified to be sodium carbonate, were studied with varied levels of citric acid (EF23-29).

Table 10 Formulation of effervescent granules (continue) (Batch size = 400 g).

No.	*Phyllanthus emblica* extract (%)	Colloidal silicon dioxide (%)	Citric acid anhydrous (%)	Effer-soda® (%)	Sodium bicarbonate (%)	Sodium carbonate (%)	Acesulfame potassium (%)	Povidone K29/32 (%)	Mannitol (%)
EF23	20	0.3	15	18.2	-	-	1	0.5	45
EF24	20	0.3	20	18.2	-	-	1	0.5	40
EF25	20	0.3	25	18.2	-	-	1	0.5	35
EF26	20	0.3	30	18.2	-	-	1	0.5	30
EF27	20	0.3	35	18.2	-	-	1	0.5	25
EF28	20	0.3	15	-	15.7	2.5	1	0.5	45
EF29	20	0.3	20	-	15.7	2.5	1	0.5	40
EF30	20	0.3	25	-	15.7	2.5	1	0.5	35
EF31	20	0.3	30	-	15.7	2.5	1	0.5	30
EF32	20	0.3	35	-	15.7	2.5	1	0.5	25

3.1.2 Preparation of effervescent granules

Weighed amounts of the *Phyllanthus emblica* extract, acesulfame potassium and mannitol were mixed using mortar and pestle by geometric dilution method. Then, povidone K29/32 solution in isopropyl alcohol was added. The mass was further mixed and passed through a 20-mesh sieve. After that, the granules were dried in a hot air oven at 50 ^{O}C for 2 hours. The dried granule was passed through a 40-mesh sieve and weighed. Finally, weighed amount of citric acid anhydrous, aerosil, sodium bicarbonate, sodium carbonate or Effer-soda® were mixed with the dried granules in a cubic-shape mixer for 5 minutes. Ten grams of product were filled in amber glass bottle and aluminium cap for further test.

3.2 Pellets containing *Phyllanthus emblica* extract.

3.2.1 Formulation of pellets

The influence of three formulation variables, *Phyllanthus emblica* extract, acesulfame potassium and citric acid anhydrous, were studied according to full factorial design as shown in table11.

All formulations contained 50% of microcrystalline cellulose because it was found in the preliminary study that the formulation with microcrystalline cellulose of less than 50% was difficult to prepare pellets. The *Phyllanthus emblica* extract contents in the formulation were designed in the quantities of 20% or 40%. Acesulfame potassium in the quantities of 2% or 4% and citric acid anhydrous the quantities of 3% or 6% were used for flavoring agent in the formulations. Mannitol was used as a filler to 100%. Mannitol is not hygroscopic and has a cooling sensation in the mouth (Rowe et al., 2003).

Thus, formulation PE9 was the central point of experiment (n=3) of the level of *Phyllanthus emblica* extract, acesulfame potassium and citric acid anhydrous.

Table 11 Formulation of pellets (Batch size = 300 g).

No.	*Phyllanthus emblica* extract (%)	Acesulfame potassium (%)	Citric acid anhydrous (%)	Microcrystalline cellulose (%)	Mannitol (%)
PE1	20	2	3	50	25
PE2	40	2	3	50	5
PE3	20	4	3	50	23
PE4	40	4	3	50	3
PE5	20	2	6	50	22
PE6	40	2	6	50	2
PE7	20	4	6	50	20
PE8	40	4	6	50	0
PE9	30	3	4.5	50	12.5

3.2.2 Preparation of pellets

Phyllanthus emblica extract, acesulfame potassium, citric acid anhydrous, microcrystalline cellulose and mannitol were weighed in a planetary mixer by geometric dilution method. The powders were blended in the mixer and mixed at the lowest speed for 15 minutes. Then, binder liquid was added slowly into the mixture and mixing continued for 10 minutes. Required quantity of binder liquid was recorded. After that, the wet mass was extruded through a 1-mm diameter die of extruder. The extrudate was spheronized at 700 rpm for 5 minutes on a 30 cm diameter spheronizer fitted with a plate with radial grooves. Pellets were dried in a hot air oven at 50 ^{O}C for 20 hours. Ten grams of product were filled in amber glass bottle and aluminium cap for further test.

4 Characterization of *Phyllanthus emblica* extract products.

The products, i.e. effervescent granules and pellets were characterized for physical appearance (section 4.1), loss on drying (section 4.3), pH (section 4.4), bulk density, tapped density and percent compressibility (section 4.5), flow rate (section 4.6), angle of repose (section 4.7), apparent density (section 4.8), amount of total tannins (section 4.14) and amount of gallic acid (section 4.15). The disintegration time (section 4.9) of effervescent granules were also measured. In addition, morphology (section 4.2), particle size distribution (section 4.10), sphericity (section 4.11), hardness (section 4.12), friability (section 4.13) were determined for pellets.

4.1 Physical appearance

The apperance of products i.e. effervescent granules and pellets was visually observed.

4.2 Morphology

The pellets were prepared by gold sputtering technique and viewed using a scanning electron microscope (SEM). Shape, surface topography and cross-sectioned for

internal texture of pellets were determined using magnification of 15x, 15 kv. and 75x, 15 kv.

4.3 Loss on drying

The products, i.e. effervescent granules and pellets were examined using the method as described in section 2.2.

4.4 pH

The products, i.e. effervescent granules and pellets were examined using the method as described in section 2.3.

4.5 Bulk density, Tapped density and Percent compressibility

The products, i.e. effervescent granules and pellets were examined using the method as described in section 2.4.

4.6 Flow rate

The products, i.e. effervescent granules and pellets were examined using the method as described in section 2.5.

4.7 Angle of repose

The angle of repose for the effervescent granules and pellets were determined by the funnel method. The angle of repose was measured from a heap carefully built up by dropping 20-grams of granules through a glass funnel with 1.5-cm orifice to the horizontal surface lied with a graph paper. The height (H) and the radius (R) of the heap granules were recored in centimeter. The angle of repose was average from three replicates. Each the angle of repose (α) was calculated from the following equation :

$$\alpha = \tan^{-1} H/R$$

The flowability was finded by using table 12.

Table 12 Relationship between the angle of repose and flowability (Nagel and Peck, 2003)

Angle of repose (θ)	flowability
$\leq 38^{O}$	Good
$38 - 42^{O}$	Fair
$\geq 42^{O}$	Poor

4.8 Apparent density

The products, i.e. effervescent granules and pellets were examined using the method as described in section 2.6.

4.9 Disintegration

Disintegration test was modified from that described in BP 2005. Effervescent granules of 2.5-g were added into water 50-ml. When the evolution of gas around the individual grains ceases, the granules have disintegrated, being either dissolved or dispersed in the water. According to BP 2005, it should dissolved or dispersed within 5 minutes. The actual time taken for the granules to dissolve or disperse was recorded and the average value was obtained from three replicates.

4.10 Particle size distribution

Size distributions of the pellets were determined by sieve analysis. The sieve shaker (power = 5 and cycle = 5) with sieve mesh size number 12 (1.7 mm), 14 (1.4 mm), 16 (1.18 mm), 18 (1.00 mm), 20 (0.85 mm), 25 (0.71 mm) and 35 (0.50 mm) was used. Pellets were accurately weighed and put on the top of sieve. Then, the sieves were shaked for 15 minutes. Pellets weighted on each sieve size were calculated in percentage of distribution.

4.11 Sphericity

The sphericity of pellets were determined by using image analyzer. One hundred sample pellets of each formulation were analyzed by software program Image Pro Plus® of Image analyzer. Longest diameter or Feret miximum (R_1) and smallest diameter or Feret miximum (R_2) of pellets were divided to perform the aspect ratio, i.e. ratio between major axis and minor axis of ellipse equivalent to object that referred to the sphericity of pellets. Another parameter used to define the sphericity of pellets in this study was the value of roundness which was derived from perimeter devided by 4π area of the projected image.

4.12 Hardness

Hardness of fifty pellet was determined by texture analyser with 6-mm diameter cylinder stainless. Texture analyser settings were test model as compression, 0.01 mm/sec of pre-test speed, 0.01 mm/sec of test speed, 10 mm/sec of post-test speed and target mode as 50% of strain.

4.13 Friability

Two grams of pellets passed through a 14 mesh (1.4 mm) sieve and retained on a 25 mesh (0.71 mm) sieve were filled with five 5-mm diameter metal spheres into the PVC container. The container was rotated at 25 rpm for 4 minutes. Then, pellets finer than 35 mesh (0.50 mm) was sieved off. The results average from three determinations were reported in term of percentage of weight loss.

4.14 Amount of Total tannins

Standard preparation, the other reagent preparation and procedure as described in section 2.8

4.14.1 Sample preparation of effervescent granules

Weighed amount of effervescent granules equivalent to 25-mg of *Phyllanthus Emblica* extract was filled into a 250-ml volumetric flask, added with water of approximately 125-ml and sonicated for 15 minutes. Then, the volumetric flask was filled up to volume with water and mixed. Solution was filtered through a Whatman® no. 1 filter. Each sample was determined in three replicates.

4.14.2 Sample preparation of pellets

Weighed amount of pellets equivalent to 25-mg of *Phyllanthus Emblica* extract was filled into a 250-ml volumetric flask, added water of approximately 125-ml and sonicated for 15 minutes. Then, the volumetric flask was filled up to volume with water and mixed. Solution was filtered through a Whatman® no. 1 filter. Each sample was determined in three replicates.

4.14.3 Calculation

$$\% \text{ Total tannins} = \frac{\text{GAE concentration } (\mu g/ml) \text{ of samples} \times 100}{\text{concentration of products } (\mu g/ml)}$$

GAE = total tannins in terms of gallic acid equivalence.

4.15 Amount of Gallic acid

HPLC chromatographic conditions and standard preparation as described in section 2.9.2

4.15.1 Sample preparation of effervescent granules

Weighed amount of effervescent granules equivalent to 25-mg of *Phyllanthus Emblica* extract was filled into a 50-ml volumetric flask. Methanol of approximately 25-ml was added. Next, This volumetric flask was sonicated for 10 minutes and the solution

was diluted to volume. Then, 5-ml of the solution was pipetted into a 25-ml volumetric flask and diluted to volume with 0.3% trifluoroacetic acid. The solution was filtered through 0.45 μm filter paper and injected into HPLC column. Each sample was determined in three replicates.

4.15.2 Sample preparation of pellets

Weighed amount of pellets equivalent to 25-mg of *Phyllanthus Emblica* extract was filled into a 50-ml volumetric flask. Mobile phase of approximately 25-ml was added. Then, this volumetric flask was sonicated for 10 minutes and the solution was diluted to volume. After that, 5-ml of the solution was pipetted into a 25-ml volumetric flask and diluted to volume with mobile phase. The solution was filtered through 0.45 μm filter paper and injected into HPLC column. Each sample was determined in three replicates.

4.15.3 Calculation of products

The amount of gallic acid in 100 g products was calculated from peak area as the following equation.

$$\% \text{ Gallic acid} = \frac{PAsp}{PAstd} \times \frac{Wt\ std}{50} \times \frac{5}{50} \times \frac{1}{50} \times \frac{50}{Wt\ sp} \times \frac{25}{5} \times \%\ purity \times \frac{(100 - \%LOD)}{100}$$

PAstd = Peak area of gallic acid standard solution
PAsp = Peak area of products solution
Wt std = Weight of gallic acid
Wt sp = Weight of products
% purity = The gallic acid content in standard
% LOD = The moisture content in gallic acid standard

5 Stability of *Phyllanthus emblica* extract products

5.1 Stability of effervescent granules

Effervescent granules containing *Phyllanthus emblica* extract stored in amber glass bottles fitted with aluminium caps were placed inside a stability chamber at 40±2 ^{O}C 75% RH and 30±2 ^{O}C 75% RH for 3 months. Two glass bottles filled with effervescent granules were randomly sampled at time intervals of 1, 2 and 3 months and analysed for physical appearance, loss on drying, pH, amount of total tannins and amount of gallic acid.

5.2 Stability of pellets

Pellets containing *Phyllanthus emblica* extract stored in amber glass bottles fitted with aluminium caps were placed inside a stability chamber at 40±2 ^{O}C 75% RH and 30±2 ^{O}C 75% RH for 3 months. One glass bottle filled with pellets were randomly sampled at time interval of 1, 2 and 3 months and analysed for physical appearance, loss on drying, amount of total tannins and amount of gallic acid.

6 Statistical analysis

The results before and after stored stability study of effervescent granules and pellets were the statistical analysis that was carried out by analysis of variance using statistical package for social sciences, SPSS 13.0, SPSS UK Ltd.

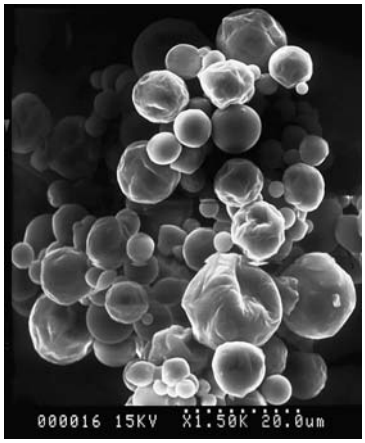
CHAPTER IV

RESULT AND DISCUSSION

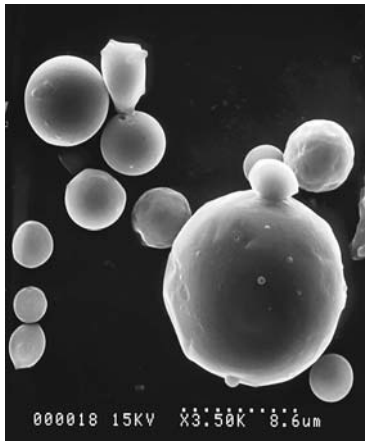
1. Characterization of *Phyllanthus Emblica* extract and excipients

1.1 Morphology study

Phyllanthus Emblica extract (spray dried) was examined using scanning electron microscope. The shape and surface topography of *Phyllanthus Emblica* extract of lot no. 171106E and lot no. 081206G were mostly the sphere particles with smooth surface as presented in figure 12.

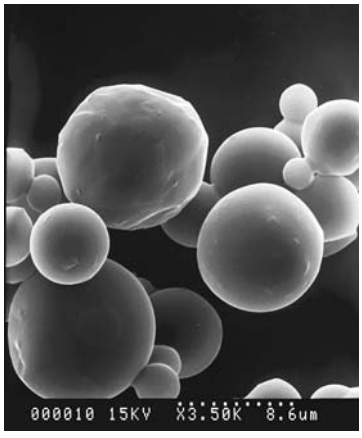


(a) Lot no. 171106 E; 1,500x



(b) Lot no. 171106 E; 3,500x

(c) Lot no. 081206 G; 1,500x



(b) Lot no. 081206 G; 3,500x

Figure 12 Scanning electron micrographs of *Phyllanthus Emblica* extract at magnification of 1,500x and 3,500x.

1.2 Loss on drying

Loss on drying of *Phyllanthus Emblica* extract lot no. 171106E used for preparation of pellets and lot no. 081206G used for preparation of effervescent granules were 2.95% and 2.35%, respectively as shown in table 13.

Loss on drying of citric acid anhydrous, sodium carbonate, acesulfam potassium, colloidal sillicon dioxide, mannitol and microcrystalline cellulose were 0.19%, 0.64%, 0.13%, 1.58%, 0.11% and 4.55%, respectively as shown in table 14. Loss on drying of sodium bicarbonate and Effer-soda® could not be determined because sodium bicarbonate begins to dissociate into sodium carbonate, carbon dioxide and water about 50 ^{O}C. They used analytical time more than 20 min. and had loss on drying more than 10%.

1.3 pH

Phyllanthus Emblica extract were acidic compound because pH of 1% w/v *Phyllanthus Emblica* extract lot no. 171106E and lot no. 081206G were 2.97 and 3.02, respectively as shown in table 13.

1.4 Flow rate

Flow rate of *Phyllanthus Emblica* extract could not be measured because sample could not flow through a glass funnel. Thus, *Phyllanthus Emblica* extracts were considered as poor flowability.

1.5 Bulk density, Tapped density and Percent compressibility

Bulk density, tapped density and % compressibility of *Phyllanthus Emblica* extract lot no. 171106E were 0.40 g/cm^3, 0.50 g/cm^3 and 19.91%, respectively. Bulk density, tapped density and % compressibility of *Phyllanthus Emblica* extract lot no. 081206G were 0.44 g/cm^3, 0.52 g/cm^3 and 14.38%, respectively as shown in table 13.

From Carr's compressibility index in table 2, *Phyllanthus Emblica* extract lot no. 081206G was classified as fair flow characteristic level and *Phyllanthus Emblica* extract

lot no. 171106E was classified as good flow characteristic level. This results were differed with result of flow rate. It may be from static charge of *Phyllanthus Emblica* extract; as a result, the extract could not passed a funnel glass.

1.6 Apparent density

Apparent density of *Phyllanthus Emblica* extract lot no. 171106E and lot no. 081206G by gas displacement technique were found to 1.57 g/cm^3 and 1.48 g/cm^3, respectively as shown in table 13.

Apparent density of citric acid anhydrous, sodium bicarbonate, sodium carbonate, Effer-soda®, acesulfame potassium, colloidal sillicon dioxide, mannitol and microcrystalline cellulose were 1.64 g/cm^3, 2.20 g/cm^3, 2.20 g/cm^3, 2.18 g/cm^3, 1.81 g/cm^3, 3.35 g/cm^3, 1.47 g/cm^3 and 1.57 g/cm^3 as shown in table 14.

Table 13 Physicochemical properties of *Phyllanthus Emblica* extract

Lot no.	Mean (SD)	
	171106 E	081206G
% Loss on drying (n=3)	2.95 (0.22)	2.35 (0.14)
pH (n=3)	2.97 (0.02)	3.02 (0.02)
Bulk density (g/cm^3) (n=3)	0.4 (0.0)	0.4 (0.0)
Tapped density (g/cm^3) (n=3)	0.5 (0.0)	0.5 (0.0)
% Compressibility (n=3)	19.9 (3.2)	14.4 (4.6)
Apparent density (g/cm^3) (n=5)	1.57 (0.00)	1.48 (0.00)
The volume mean diameter (μm) (n=3)	28.02 (0.52)	36.02 (2.55)
% Total tannins (n=3)	26.92 (0.41)	24.54 (0.19)
% Gallic acids (n=3)	0.69 (0.01)	1.24 (0.00)

Table 14 Physicochemical properties of excipients

	Mean (SD)		
	% Loss on drying (n=3)	Apparent density (g/cm^3) (n=5)	The volume mean diameter (μm) (n=3)
Citric acid anhydrous	0.19 (0.03)	1.64 (0.00)	367.70 (3.89)
Sodium bicarbonate	>10	2.20 (0.01)	223.05 (1.65)
Sodium carbonate	0.64 (0.03)	2.20 (0.01)	308.49 (3.81)
Effer-soda®	>10	2.18 (0.01)	251.33 (2.73)
Acesulfame potassium	0.13 (0.03)	1.81 (0.00)	402.61 (6.87)
Colloidal silicon dioxide	1.58 (0.34)	3.35 (0.11)	53.14 (1.15)
Mannitol	0.11 (0.04)	1.47 (0.00)	230.43 (3.87)
Microcrystalline cellulose	4.55 (0.14)	1.57 (0.00)	64.06 (1.06)

1.7 Particle Size Distribution

1.7.1 Sieve analysis

Phyllanthus Emblica extract lot no. 081206G was of larger particle size and narrower size distribution than *Phyllanthus Emblica* extract lot no. 171106E as displayed in figure 13 and table 15. The highest percent of size distribution of *Phyllanthus Emblica* extract lot no. 171106E (32.72%) and lot no. 081206G (54.82%) was in the particle size range between to 250 μm - 425 μm and >425 μm, respectively.

Table 15 Size distribution data of *Phyllanthus Emblica* extract by using sieve analysis

Particle size (μm) / Lot no.	% Weight on sieve				
	< 150	**150** –180	**180** –250	**250** –425	>**425**
171106 E	23.39	10.46	16.31	32.72	16.45
081206 G	1.85	3.11	10.83	30.38	54.82

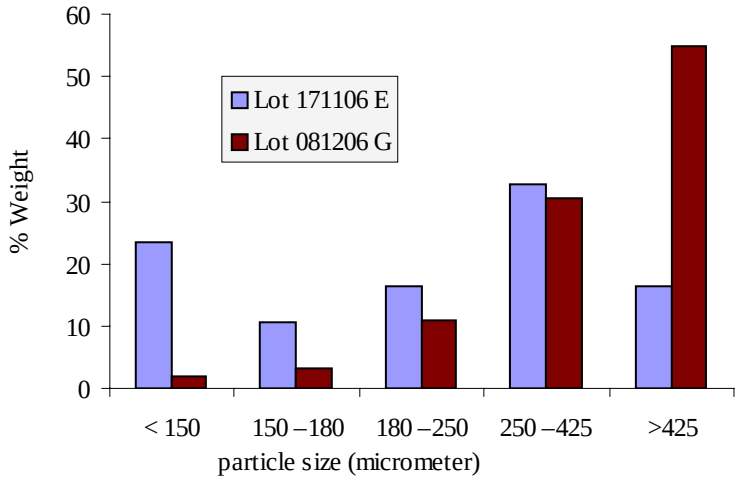


Figure 13 Size distribution of *Phyllanthus Emblica* extract

1.7.2 Laser diffraction technique

Both *Phyllanthus Emblica* extracts had size distribution at the same pattern by distributed to 2 groups as figure 14 The volume mean diameter of *Phyllanthus Emblica* extract lot no. 171106E and lot no. 081206G were found to be 28.02 μm and 36.02 μm, respectively.

The volume mean diameter of citric acid anhydrous, sodium bicarbonate, sodium carbonate, Effer-soda®, acesulfame potassium, colloidal sillicon dioxide, mannitol and microcrystalline cellulose were 367.70 μm, 223.05 μm, 308.49 μm, 251.33 μm, 402.61 μ m, 53.14 μm, 230.43 μm and 64.06 μm.

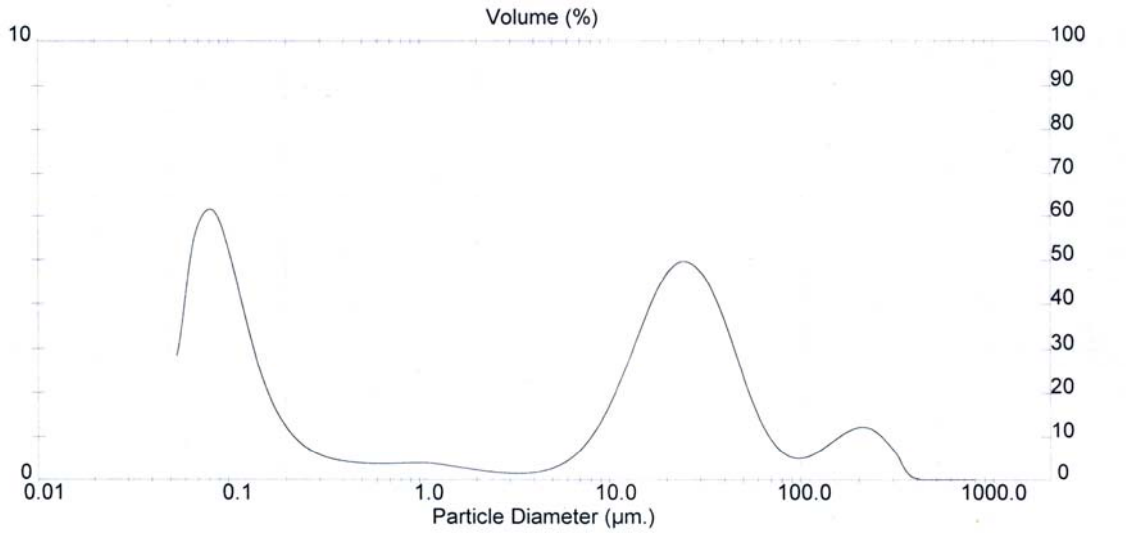


Figure 14 Size distribution of *Phyllanthus Emblica* extract lot no. 171106E

1.8 Total tannin analysis

Total tannins of *Phyllanthus Emblica* extract lot no. 171106E and lot no. 081206G by Folin-Ciocalteau method were 26.92% and 24.54%, respectively as shown in table 13. Both *Phyllanthus emblica* extracts had tannin content more than 20%, they complied to specifications of the crude drug of Techadamrongsin and Dechatiwongse-Na-Ayudhya (1997)

1.9 Gallic acid analysis

1.9.1 Validation of HPLC method

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

1.9.1.1 Linearity

The linear equation of the curve obtained by plotting the peak area at each level prepared versus the concentrations of each standard was shown in figure 44 and table 40

(appendix B). The standard concentration that gave linear standard curve was in the range of 0.25 to 7.5 μg/ml. The regression coefficient (R^2) for standard curve were > 0.9999. This result showed a good linearity of peak area and standard concentration.

1.9.1.2 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 ranges of percentage of recovery of *Phyllanthus Emblica* extract on 3 days were 99.79-102.69%, 101.78-102.39% and 100.70-103.01% as shown in table 41-43 (appendix B). And the percentage of relative standard deviation (% RSD) of percentage of recovery on 3 days was 0.16-0.95, 0.29-1.13 and 0.16-1.21. The % RSD were less than 2%, so it indicated the good accuracy of this method.

1.9.1.3 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 was expressed as the percentage of relative standard deviation (% RSD) and the data were shown in table 44 (appendix B). The % RSD of mixtured solution on 3 days was in the range of 0.53 to 0.97. The low % RSD (<2%) indicated the good precision of this method.

1.9.1.4 Specificity

The chromatogram was presented in figure 45-50 (appendix B). The excipients in the formulation did not interfere with the peak of gallic acid.

1.9.1.5 System suitability

The % RSD of gallic acid solution was 1.16 and its tailing factor was 1.25. The low % RSD (<2%) and tailing factor (<2.0) indicated the good system suiability of this method as shown in table 45 (appendix B).

1.9.2 Assay for emblica extract

The amount of gallic acid in 100g *Phyllanthus Emblica* extract lot no. 171106E and lot no. 081206G by HPLC were 0.69% and 1.24%, respectively as shown in table 13.

1.10 Microbial Contamination Test

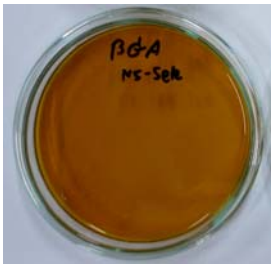
Total aerobic microbial count of *Phyllanthus Emblica* extract lot no. 171106E and lot no. 081206G were 9.3 x 10^3 cfu/g and 5.5 x 10^2 cfu/g, respectively. Total yeast and moulds count of *Phyllanthus Emblica* extract lot no. 171106E was 5 CFU/g and lot no. 081206G was absent. The specified micro-organisms test for *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* species and *Clostridium* species were done. The results of extract with positive control were as shown in table 16 and figure 15-18.

Thus, the both extracts had microbial contamination within the regulation limits of Thai pharmacopoeia (Thai pharmacopoeia committee, 1987), the limit of microbial contamination in product containing crude drug for internal use, as shown in table 3.

Table 16 Microbial contamination of *Phyllanthus Emblica* extract

Sample	Total microbial count (average of cfu/g)		Specified micro-organisms				
	Bacteria	Fungi	*E. coli*	*S. aureus*	*Ps. aeruginosa*	*Salmonella* spp.	*Clostridium* spp.
171106 E	9.3 x 10^3	5	absent	absent	absent	absent	absent
081206 G	5.5 x 10^2	absent	absent	absent	absent	absent	absent

(a) Positive control of *Salmonella* Species on BGA

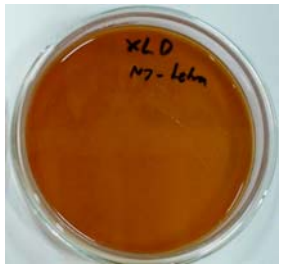


(b) The extract lot no. 171106E on BGA

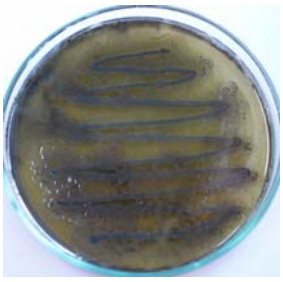
(c) The extract lot no. 081206G on BGA

(d) Positive control of *Salmonella* Species on XLD

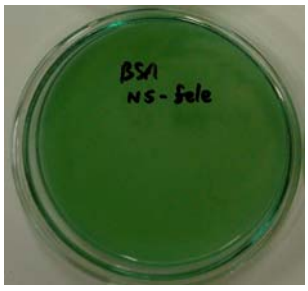
(e) The extract lot no. 171106E on XLD



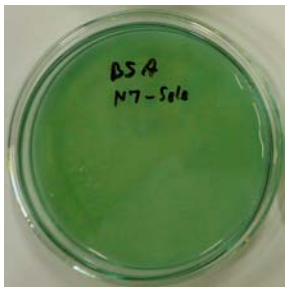
(f) The extract lot no. 081206G on XLD



(g) Positive control of *Salmonella* Species on BSA



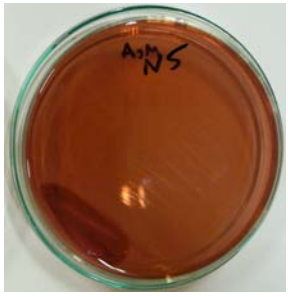
(h) The extract lot no. 171106E on BSA



(i) The extract lot no. 081206G on BSA

Figure 15 Result of *Salmonella* Species test on selective agar media

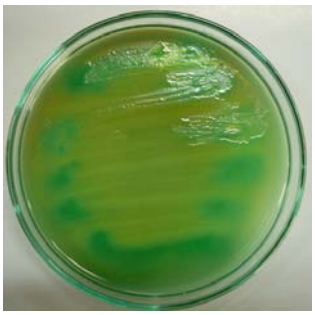
(a) Positive control of *Escherichia coli* on McA



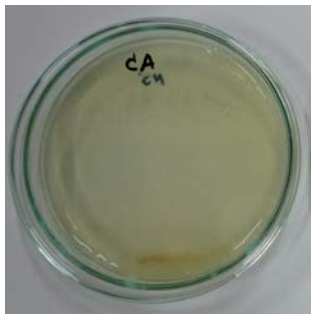
(b) The extract lot no. 171106E on McA

(c) The extract lot no. 081206G on McA

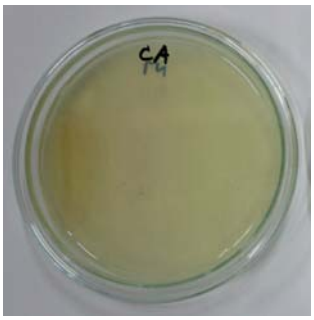
Figure 16 Result of *Escherichia coli* test on MacConkey agar



(a) Positive control of *Ps. aeruginosa* on CA

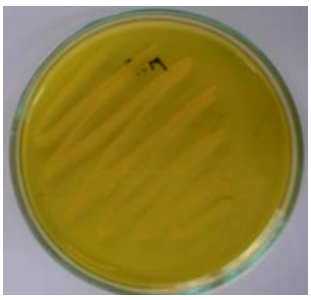


(b) The extract lot no. 171106E on CA

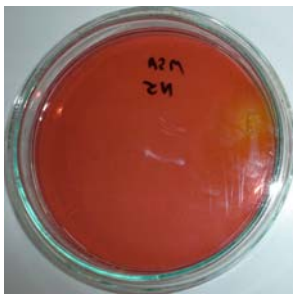


(c) The extract lot no. 081206G on CA

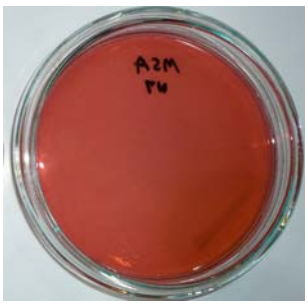
Figure 17 Result of *Pseudomonas aeruginosa* on cetrimide agar



(a) Positive control of *S. aureus* on MSA



(b) The extract lot no. 171106E on MSA*



(c) The extract lot no. 081206G on MSA

* This colony was not *S. aureus* because its gram strain was positive rod

Figure 18 Result of *Staphylococcus aureus* on mannitol-salt agar

2. Effervescent granules containing *Phyllanthus emblica* extract

2.1 Preparation of effervescent granules containing *Phyllanthus emblica* extract

In this study, *Phyllanthus emblica* extract, acesulfame potassium and mannitol were prepared to granule before dry mixing with acid and alkalines because granules from extract combined with acid were sticky. Alkalines should be not combined with extract to prepare granules because extract was not stable in alkalinity.

The products of 32 formulations looked similar. They were yellowish granules. However, when they were added to water, the color of solutions were different according to their pH.

After addition of granules into water, EF10 containing 15% of citric acid and 19.7% of sodium bicabonate was brownish solution; on the other hand, EF3 containing 25% of citric acid and 19.7% of sodium bicabonate and EF13 containing 35% of citric acid and 19.7% of sodium bicabonate was yellowish solution as shown in figure 19. The occurred bubble of 32 formulations was large volume and stable. This finding agreed with the results of Wipasawad and Seetao (1995) that herbal (roselle, bael fruit and tamarind) effervescent granules formed stable bubble.

2.2 Characterization of effervescent granules containing *Phyllanthus emblica* extract

2.2.1 Physical appearance

The apperance of granules containing *Phyllanthus emblica* extract was obserbed. *Phyllanthus emblica* extract was hygroscopic, fine yellow powder; as a result, effervescent granules were yellow color. The color of products were brownish, if the amounts of *Phyllanthus emblica* extract in the formulations were higher.

(a) EF 3 dispersion, after adding water at 30 sce

(a) EF 10 dispersion, after adding water at 30 sce

(a) EF 13 dispersion, after adding water at 30 sce

(b) EF 3 dispersion, after adding water at 5 min

(b) EF 10 dispersion, after adding water at 5 min

(b) EF 13 dispersion, after adding water at 5 min

Figure 19 Color of effervescent granules dispersions

2.2.2 Loss on drying

Effervescent granules were determined loss on drying by a moisture analyzer. They had loss on drying between 0.57%-1.82% as shown in table 17. EF 14 containing 10% of *Phyllanthus emblica* extract, 0.2% of colloidal silicon dioxide, 20% of citric acid and base type as sodium bicarbonate showed of lowest LOD. EF19 containing 30% of *Phyllanthus emblica* extract, 0.4% of colloidal silicon dioxide, 20% of citric acid and base type as sodium bicarbonate was of highest LOD.

2.2.3 pH

Solution of effervescent granules had pH value between 3.91-5.75 as shown in table 18. EF 26 contanining 20% of *Phyllanthus emblica* extract, 30% of citric acid and 18.2% of Effer-soda® was of lowest pH. EF28 contanining 20% of *Phyllanthus emblica* extract, 15% of citric acid, 15.7% sodoum bicarbonate and 2.5% of sodium carbonate was of highest pH.

Quantity of citric acid anhydrous was major factor to pH solutions. The formulations contanining high level of citric acid anhydrous had lower pH value than the formulations contanining low level of citric acid anhydrous.

The color of EF10 solution (pH=5.55) was not stable. The color of solution at pH less than 4.5 looked stable. This results differed with the result of Sukkiattibhai et al. (2005) and Udomsom et al. (2005) that the *Phyllanthus emblica* extract at 0.05 M acetate buffer and phosphate buffer pH 5.5 had stable of polyphenol and gallic acid. Whereas, this results looked similarity with the result of Moura et al. (1994) that the degradation rate of ascorbic acid is a minimum at pH 2.5 to 3.0. So, the color change of solutions may be effected from type of buffer or components in formulations.

2.2.4 Bulk density, Tapped density and Percent compressibility

Effervescent granules had bulk density, tapped density and % compressibility between 0.5 g/cm^3 - 0.6 g/cm^3, 0.6 g/cm^3 - 0.7 g/cm^3 and 9.5% - 20.6%, respectively as shown in table 19-21. EF8 containing 20% of *Phyllanthus emblica* extract, 0.4% of colloidal silicon dioxide, 25% of citric acid and base type as sodium bicarbonate, and EF19 containing 30% of *Phyllanthus emblica* extract, 0.4% of colloidal silicon dioxide, 20% of citric acid and base type as sodium bicarbonate were of lowest % compressibility. EF23 containing 20% of *Phyllanthus emblica* extract, 0.3% of colloidal silicon dioxide, 15% of citric acid and base type as Effer-soda® was of highest % compressibility.

From Carr's compressibility index in table 2, Effervescent granules were classified as good to free flowing characteristic level

Table 17 % Loss on drying of effervescent granules (n=3)

No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)
EF1	1.14 (0.02)	EF9	1.43 (0.05)	EF17	1.49 (0.07)	EF25	0.76 (0.05)
EF2	0.69 (0.09)	EF10	0.81 (0.02)	EF18	0.60 (0.01)	EF26	0.70 (0.06)
EF3	1.45 (0.11)	EF11	1.17 (0.12)	EF19	1.82 (0.12)	EF27	0.78 (0.03)
EF4	1.68 (0.05)	EF12	1.62 (0.09)	EF20	1.34 (0.06)	EF28	1.42 (0.02)
EF5	1.51 (0.11)	EF13	1.13 (0.08)	EF21	1.52 (0.02)	EF29	1.69 (0.04)
EF6	1.25 (0.03)	EF14	0.57 (0.03)	EF22	0.78 (0.07)	EF30	0.69 (0.06)
EF7	1.57 (0.02)	EF15	1.67 (0.05)	EF23	0.82 (0.03)	EF31	1.01 (0.03)
EF8	1.05 (0.04)	EF16	1.17 (0.04)	EF24	0.78 (0.05)	EF32	0.95 (0.02)

Table 18 pH of effervescent granules (n=3)

No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)
EF1	4.74 (0.21)	EF9	4.69 (0.18)	EF17	5.58 (0.66)	EF25	4.07 (0.48)
EF2	4.82 (0.13)	EF10	5.55 (0.37)	EF18	4.58 (0.24)	EF26	3.91 (0.09)
EF3	4.44 (0.09)	EF11	4.88 (0.15)	EF19	4.64 (0.18)	EF27	4.18 (0.11)
EF4	4.12 (0.04)	EF12	4.13 (0.19)	EF20	4.14 (0.07)	EF28	5.75 (0.11)
EF5	4.25 (0.06)	EF13	4.17 (0.11)	EF21	4.24 (0.10)	EF29	4.75 (0.02)
EF6	4.19 (0.35)	EF14	5.15 (0.12)	EF22	4.49 (0.08)	EF30	4.38 (0.06)
EF7	4.69 (0.17)	EF15	4.07 (0.05)	EF23	5.39 (0.15)	EF31	4.44 (0.13)
EF8	4.30 (0.07)	EF16	4.89 (0.19)	EF24	4.87 (0.24)	EF32	4.14 (0.12)

Table 19 Bulk density (g/cm^3) of effervescent granules (n=3)

No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)
EF1	0.5 (0.0)	EF9	0.6 (0.0)	EF17	0.5 (0.0)	EF25	0.6 (0.0)
EF2	0.6 (0.0)	EF10	0.5 (0.0)	EF18	0.6 (0.0)	EF26	0.6 (0.0)
EF3	0.6 (0.0)	EF11	0.6 (0.0)	EF19	0.6 (0.0)	EF27	0.6 (0.0)
EF4	0.6 (0.0)	EF12	0.6 (0.0)	EF20	0.6 (0.0)	EF28	0.6 (0.0)
EF5	0.6 (0.0)	EF13	0.6 (0.0)	EF21	0.6 (0.0)	EF29	0.6 (0.0)
EF6	0.5 (0.0)	EF14	0.6 (0.0)	EF22	0.6 (0.0)	EF30	0.6 (0.0)
EF7	0.6 (0.0)	EF15	0.6 (0.0)	EF23	0.5 (0.0)	EF31	0.6 (0.0)
EF8	0.6 (0.0)	EF16	0.6 (0.0)	EF24	0.6 (0.1)	EF32	0.6 (0.0)

Table 20 Tapped density (g/cm^3) of effervescent granules (n=3)

No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)
EF1	0.6 (0.0)	EF9	0.7 (0.0)	EF17	0.6 (0.0)	EF25	0.7 (0.0)
EF2	0.7 (0.0)	EF10	0.6 (0.0)	EF18	0.7 (0.0)	EF26	0.7 (0.0)
EF3	0.7 (0.0)	EF11	0.6 (0.0)	EF19	0.7 (0.0)	EF27	0.7 (0.0)
EF4	0.7 (0.0)	EF12	0.7 (0.0)	EF20	0.7 (0.0)	EF28	0.7 (0.0)
EF5	0.7 (0.0)	EF13	0.7 (0.0)	EF21	0.7 (0.0)	EF29	0.7 (0.0)
EF6	0.6 (0.0)	EF14	0.7 (0.0)	EF22	0.7 (0.0)	EF30	0.7 (0.0)
EF7	0.7 (0.0)	EF15	0.7 (0.0)	EF23	0.6 (0.1)	EF31	0.7 (0.0)
EF8	0.7 (0.0)	EF16	0.7 (0.0)	EF24	0.7 (0.1)	EF32	0.7 (0.0)

Table 21 % Compressibility of effervescent granules (n=3)

No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)
EF1	16.7 (0.0)	EF9	14.3 (0.0)	EF17	16.7 (0.0)	EF25	15.1 (1.4)
EF2	14.3 (0.0)	EF10	16.7 (0.0)	EF18	14.3 (0.0)	EF26	14.3 (0.0)
EF3	14.3 (0.0)	EF11	11.1 (9.6)	EF19	9.5 (8.2)	EF27	14.3 (0.0)
EF4	14.3 (0.0)	EF12	14.3 (0.0)	EF20	14.3 (0.0)	EF28	19.0 (8.2)
EF5	19.0 (8.2)	EF13	14.3 (0.0)	EF21	14.3 (0.0)	EF29	14.3 (0.0)
EF6	15.1 (14.4)	EF14	15.1 (1.4)	EF22	14.3 (0.0)	EF30	14.3 (0.0)
EF7	15.1 (1.4)	EF15	14.3 (0.0)	EF23	20.6 (6.9)	EF31	14.3 (0.0)
EF8	9.5 (8.2)	EF16	15.9 (1.4)	EF24	15.1 (1.4)	EF32	14.3 (0.0)

2.2.5 Flow rate

All effervescent granules could flow pass the funnel orifice of a glass funnel. Effervescent granules had flow rate between 15.7 g/sec - 22.5 g/sec as shown in table 22. EF1 containing 5% of *Phyllanthus emblica* extract, 25% of citric acid and base type as sodium bicarbonate was of lowest flow rate and EF26 containing 10% of *Phyllanthus emblica* extract, 30% of citric acid and base type as Effer-soda® was of highest flow rate.

2.2.6 Angle of repose

Effervescent granules had angle of repose between 21.7^{O}-29.1^{O} as shown in table 23. EF5 containing 40% of *Phyllanthus emblica* extract was of lowest angle of repose and EF1 containing 5% was of highest angle of repose. So, quantity of *Phyllanthus emblica* extract may affect to angle of repose.

From table of relationship between the angle of repose and flowability in table 12, effervescent granules were classified to good flowability as well as result from carr's compressibility index on section 2.2.4. and flow rate on section 2.2.5.

Table 22 Flow rate (g/sec) of effervescent granules (n=3)

No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)
EF1	15.7 (1.0)	EF9	19.1 (1.5)	EF17	17.9 (1.0)	EF25	19.3 (0.6)
EF2	19.3 (1.4)	EF10	18.3 (1.8)	EF18	21.6 (0.2)	EF26	22.5 (0.9)
EF3	18.7 (1.2)	EF11	19.7 (3.7)	EF19	20.8 (1.2)	EF27	20.6 (0.9)
EF4	18.5 (1.7)	EF12	21.2 (0.9)	EF20	21.4 (0.3)	EF28	19.9 (0.9)
EF5	20.3 (0.2)	EF13	22.0 (1.9)	EF21	18.4 (0.7)	EF29	19.8 (0.4)
EF6	20.1 (2.1)	EF14	20.5 (0.9)	EF22	21.0 (0.8)	EF30	20.1 (0.2)
EF7	17.5 (1.8)	EF15	19.5 (0.6)	EF23	18.9 (0.5)	EF31	20.8 (1.6)
EF8	19.1 (1.0)	EF16	19.2 (0.9)	EF24	18.5 (0.6)	EF32	21.3 (1.1)

Table 23 Angle of repose (^{O}) of effervescent granules (n=3)

No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)
EF1	29.1 (1.1)	EF9	25.3 (0.8)	EF17	25.8 (0.5)	EF25	22.3 (1.1)
EF2	27.2 (0.5)	EF10	23.9 (0.3)	EF18	22.1 (0.3)	EF26	24.3 (1.4)
EF3	26.6 (0.3)	EF11	25.6 (0.6)	EF19	23.7 (0.3)	EF27	25.2 (0.5)
EF4	24.3 (0.2)	EF12	25.0 (1.5)	EF20	23.2 (0.3)	EF28	24.6 (0.2)
EF5	21.7 (2.1)	EF13	25.0 (0.5)	EF21	25.8 (0.7)	EF29	24.6 (0.2)
EF6	26.3 (1.1)	EF14	25.7 (0.2)	EF22	23.5 (1.1)	EF30	25.1 (0.2)
EF7	26.3 (0.5)	EF15	23.7 (1.1)	EF23	24.3 (0.4)	EF31	24.9 (0.6)
EF8	24.9 (1.1)	EF16	24.3 (1.4)	EF24	23.8 (1.0)	EF32	23.8 (1.8)

2.2.7 Apparent density

Effervescent granules had apparent density between 1.57 g/cm^3 -1.75 g/cm^3 as shown in table 24. EF1 containing 5% of *Phyllanthus emblica* extract, 0.3% of colloidal silicon dioxide, 25% of citric acid and base type as sodium bicarbonate was of lowest

apparent density. EF23 containing 20% of *Phyllanthus emblica* extract, 0.3% of colloidal silicon dioxide, 15% of citric acid and base type as Effer-soda® was of highest apparent density. Apparent density of effevescent granules varied slightly.

The apparent density of components affected on the apparent density of the products.

2.2.8 Disintegration time

Effervescent granules had disintegration time between 1.83 min - 3.76 min as shown in table 25. EF26 containing 20% of *Phyllanthus emblica* extract, 0.3% of colloidal silicon dioxide, 30% of citric acid and base type as Effer-soda® was of lowest disintegration time. EF14 containing 10% of *Phyllanthus emblica* extract, 0.2% of colloidal silicon dioxide, 20% of citric acid and base type as sodium bicarbonate was of highest disintegration time.

All formulation could dissolved or dispersed within 5 minutes, complying to BP 2005.

2.2.9 Amount of Total tannins

Total tannins were determined by Folin-Ciocalteau method. Effervescent granules had total tannins between 0.87%-8.96% as shown in table 26. EF1 containing 5% *Phyllanthus emblica* extract was of lowest %total tannins and EF5 containing 40% *Phyllanthus emblica* extract was of highest %total tannins.

The total tannins content was dependent on the quantity of *Phyllanthus emblica* extract in the formulations.

2.2.10 Amount of Gallic acid

The percentage of gallic acid was measured by high performance liquid chromatographic method. Effervescent granules had gallic acid between 0.05% - 0.48% as shown in table 27. EF1 containing 5% *Phyllanthus emblica* extract was of lowest gallic acid content and EF5 containing 40% *Phyllanthus emblica* extract was of highest gallic acid content.

The gallic acid content related on the quantity of *Phyllanthus emblica* extract.

Table 24 Apparent density (g/cm^3) of effervescent granules (n=5)

No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)
EF1	1.57 (0.00)	EF9	1.60 (0.00)	EF17	1.69 (0.00)	EF25	1.66 (0.00)
EF2	1.70 (0.00)	EF10	1.62 (0.00)	EF18	1.74 (0.00)	EF26	1.71 (0.00)
EF3	1.67 (0.00)	EF11	1.58 (0.01)	EF19	1.72 (0.00)	EF27	1.60 (0.00)
EF4	1.71 (0.01)	EF12	1.70 (0.00)	EF20	1.65 (0.00)	EF28	1.72 (0.01)
EF5	1.65 (0.00)	EF13	1.68 (0.00)	EF21	1.74 (0.00)	EF29	1.72 (0.00)
EF6	1.74 (0.00)	EF14	1.60 (0.00)	EF22	1.72 (0.00)	EF30	1.67 (0.00)
EF7	1.69 (0.00)	EF15	1.66 (0.00)	EF23	1.75 (0.00)	EF31	1.69 (0.00)
EF8	1.65 (0.00)	EF16	1.73 (0.01)	EF24	1.71 (0.00)	EF32	1.64 (0.00)

Table 25 Disintegration time (min) of effervescent granules (n=3)

No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)
EF1	3.59 (0.25)	EF9	2.53 (0.30)	EF17	2.75 (0.24)	EF25	2.31 (0.18)
EF2	2.44 (0.08)	EF10	3.08 (0.26)	EF18	2.09 (0.08)	EF26	1.83 (0.10)
EF3	2.37 (0.08)	EF11	3.14 (0.29)	EF19	2.76(0.22)	EF27	2.17 (0.31)
EF4	2.85 (0.16)	EF12	2.30 (0.26)	EF20	2.71 (0.08)	EF28	3.19 (0.18)
EF5	2.48 (0.14)	EF13	3.66 (0.15)	EF21	2.34 (0.36)	EF29	2.92 (0.13)
EF6	2.88 (0.24)	EF14	3.76 (0.13)	EF22	2.48 (0.07)	EF30	2.48 (0.30)
EF7	3.02 (0.22)	EF15	2.28 (0.12)	EF23	2.80 (0.28)	EF31	3.70 (0.15)
EF8	1.96 (0.11)	EF16	2.54 (0.07)	EF24	2.34 (0.38)	EF32	2.10 (0.29)

Table 26 Total tannins in 100 g of effervescent granules (n=3)

No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)
EF1	0.87 (0.01)	EF9	4.74 (0.14)	EF17	2.11 (0.04)	EF25	4.77 (0.17)
EF2	2.38 (0.04)	EF10	4.97 (0.17)	EF18	2.33 (0.09)	EF26	4.61 (0.08)
EF3	4.52 (0.04)	EF11	3.99 (0.04)	EF19	6.54 (0.37)	EF27	4.19 (0.07)
EF4	6.67 (0.43)	EF12	4.72 (0.23)	EF20	6.50 (0.06)	EF28	4.44 (0.20)
EF5	8.96 (0.46)	EF13	4.07 (0.08)	EF21	2.10 (0.09)	EF29	4.62 (0.02)
EF6	3.82 (0.09)	EF14	2.36 (0.04)	EF22	4.86 (0.05)	EF30	4.87 (0.12)
EF7	4.43 (0.15)	EF15	6.76 (0.20)	EF23	4.26 (0.15)	EF31	3.73 (0.18)
EF8	4.10 (0.22)	EF16	6.25 (0.06)	EF24	4.36 (0.09)	EF32	4.18 (0.11)

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Table 27 Gallic acid in 100 g of effervescent granules (n=3)

No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)
EF1	0.05 (0.00)	EF9	0.24 (0.01)	EF17	0.12 (0.00)	EF25	0.24 (0.01)
EF2	0.11 (0.00)	EF10	0.24 (0.00)	EF18	0.12 (0.00)	EF26	0.24 (0.01)
EF3	0.24 (0.00)	EF11	0.23 (0.00)	EF19	0.35 (0.00)	EF27	0.26 (0.00)
EF4	0.35 (0.01)	EF12	0.25 (0.00)	EF20	0.37 (0.00)	EF28	0.24 (0.01)
EF5	0.48 (0.01)	EF13	0.24 (0.00)	EF21	0.12 (0.00)	EF29	0.23 (0.02)
EF6	0.23 (0.00)	EF14	0.12 (0.00)	EF22	0.24 (0.00)	EF30	0.23 (0.01)
EF7	0.24 (0.01)	EF15	0.37 (0.01)	EF23	0.22 (0.01)	EF31	0.23 (0.01)
EF8	0.23 (0.00)	EF16	0.35 (0.01)	EF24	0.22 (0.01)	EF32	0.24 (0.00)

2.3 Stability of effervescent granules containing *Phyllanthus emblica* extract

Effervescent granules formed agglomerates and changed color from yellowish to brownish as shown in figure 20. After storage at 40±2 ^{O}C 75% RH for 3 months, EF4, EF15, EF16, EF19, EF22, EF26 and EF30 did not change very much relative to other formulations as shown in table 48. After storage at 30±2 ^{O}C 75% RH for 3 months, change in apperance of EF4, EF10, EF16, EF22 and EF26 was not observed as shown in table 49. Quantity of *Phyllanthus emblica* extract was major effected to change of appearance because EF4, EF15, EF16 and EF19, containing 30% of *Phyllanthus emblica* extract in formulation, had low change of both color and aggomerate of granules. Colloidal silicon dioxide may be not increased stability of product because EF22, which did not contain colloidal silicon dioxide, was stable. Type of alkaline was not found effect in relation to stability. Level of citric acid did not affect to stability but it had effect on color change of solution after dissolved or dispersed in the water.

Levels of *Phyllanthus emblica* extract affected to % LOD. EF1 containing 5% of *Phyllanthus emblica* extract after stored at 40±2 ^{O}C 75% RH and 30±2 ^{O}C 75% RH for 3 months showed highest increase in % LOD as shown in figure 21-22 and table 50-51.

pH of all formulation were not changed at both 40±2 ^{O}C 75% RH and 30±2 ^{O}C 75% RH for 3 months as shown in table 52-53.

Results of % total tannins and % gallic acid after storage at 40±2 ^{O}C 75% RH and 30±2 ^{O}C 75% RH for 3 months were not clear changed by observed except for EF1 as shown in figure 23-26 and table 54-57. Increase of % total tannins and % gallic acid of EF1 may be related to increase in % LOD.

From figure 27-30, % total tannins and % gallic acid were likely related to % LOD; in contrast, they were not related to pH.

(a) EF1, room temperature for 3 months

(d) EF4, room temperature for 3 months

(b) EF1, 30±2 ^{O}C 75% RH for 3 months

(e) EF4, 30±2 ^{O}C 75% RH for 3 months

(c) EF1, 40±2 ^{O}C 75% RH for 3 months

(f) EF4, 40±2 ^{O}C 75% RH for 3 months

Figure 20 The apperance of EF1 and EF4 formulation.

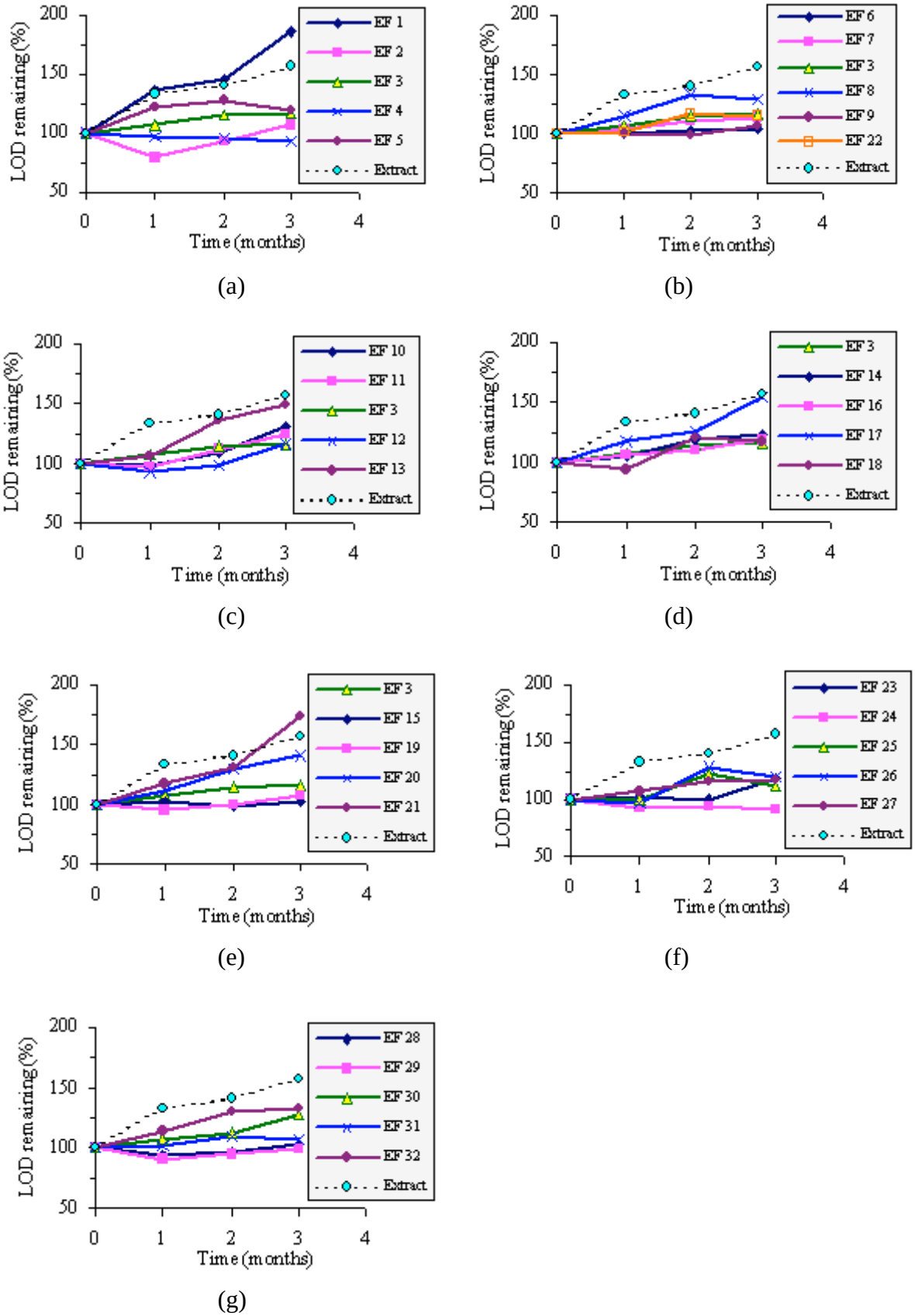


Figure 21 LOD remaining (%) of effervescent formulations and *Phyllanthus emblica* extract stored at 40±2 ^{O}C, 75% RH for 3 months.

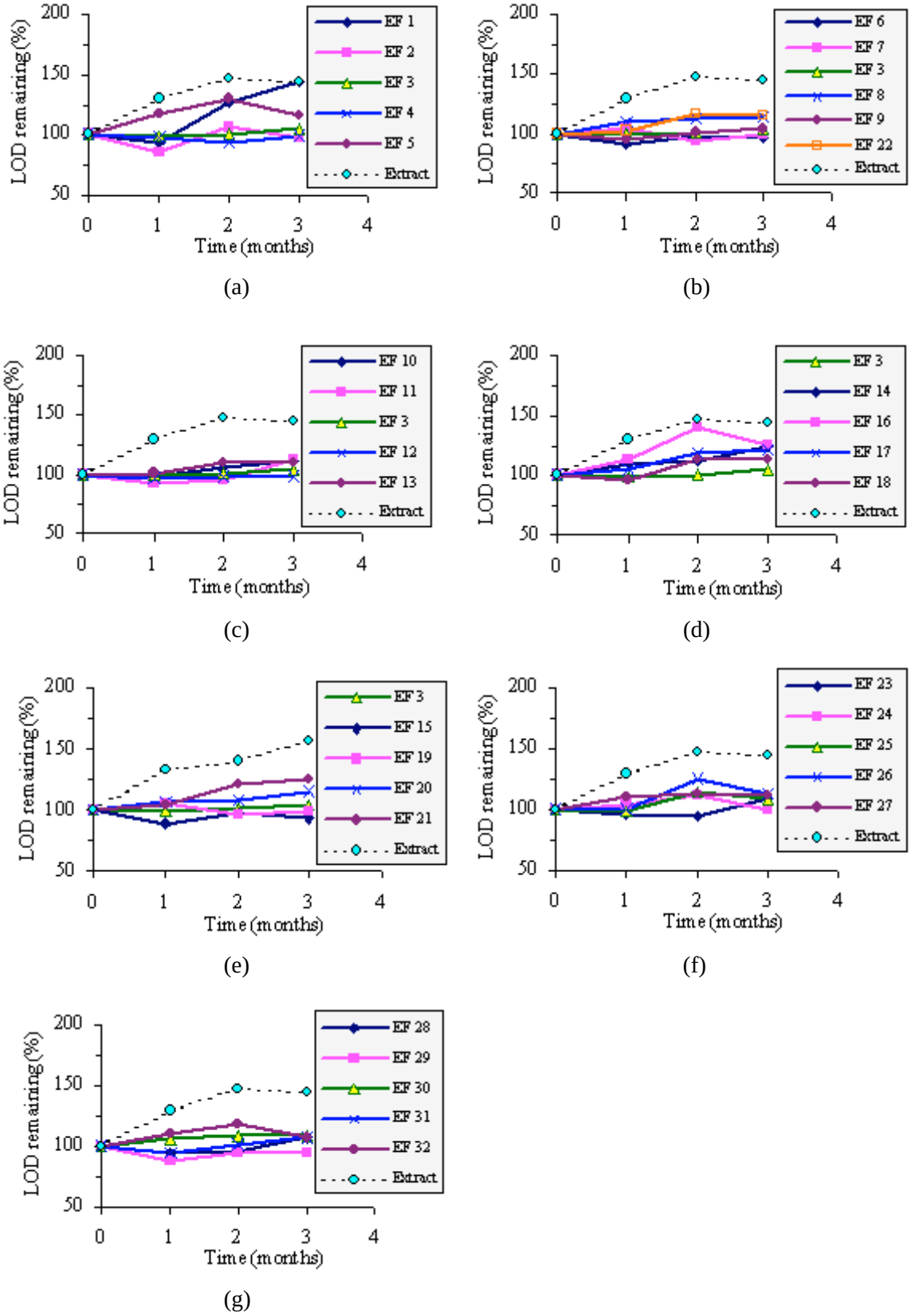


Figure 22 LOD remaining (%) of effervescent formulations and *Phyllanthus emblica* extract stored at 30±2 ^{O}C, 75% RH for 3 months.

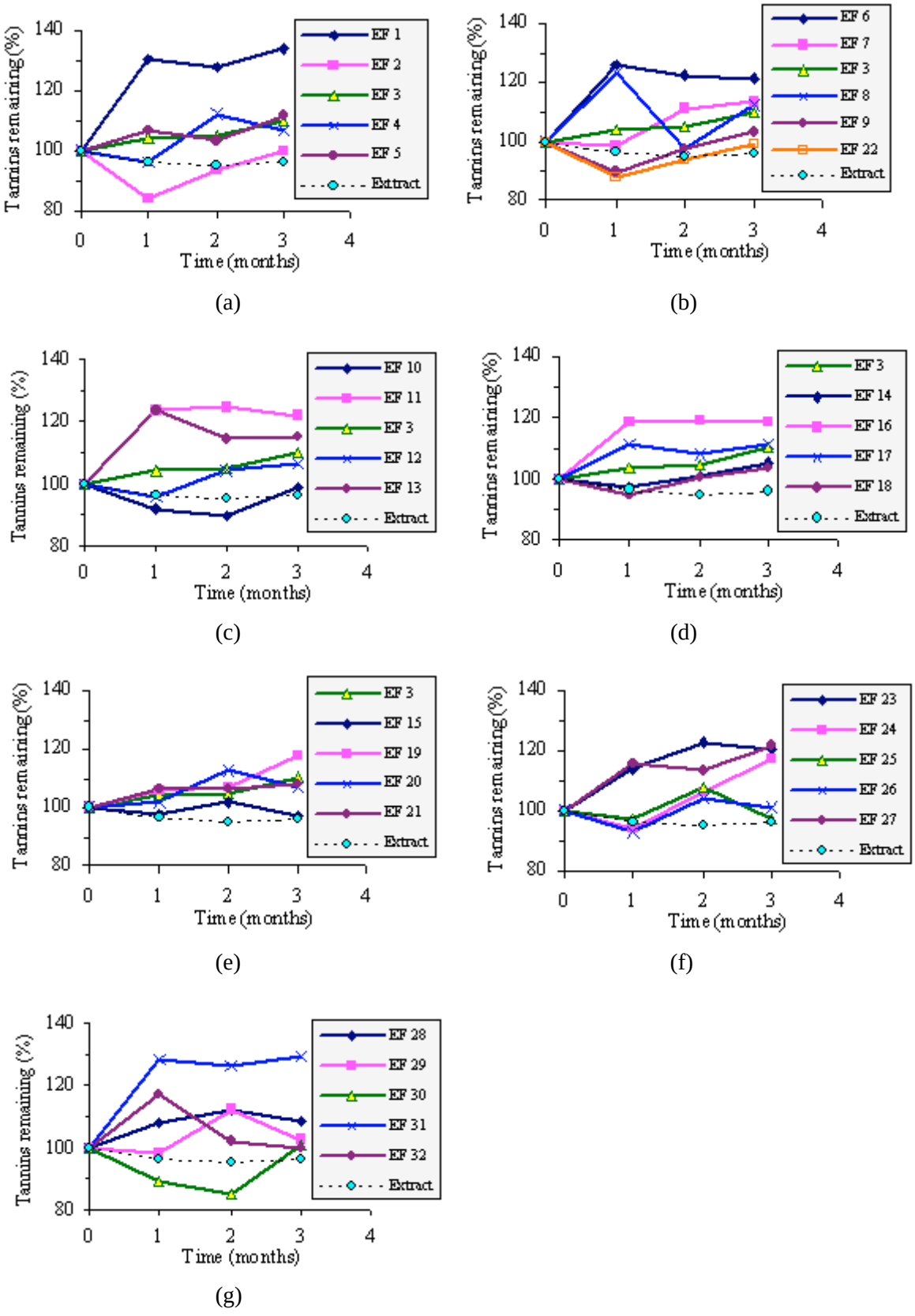


Figure 23 Tannin remaining (%) of effervescent formulations and *Phyllanthus emblica* extract stored at 40±2 ^{O}C, 75% RH for 3 months.

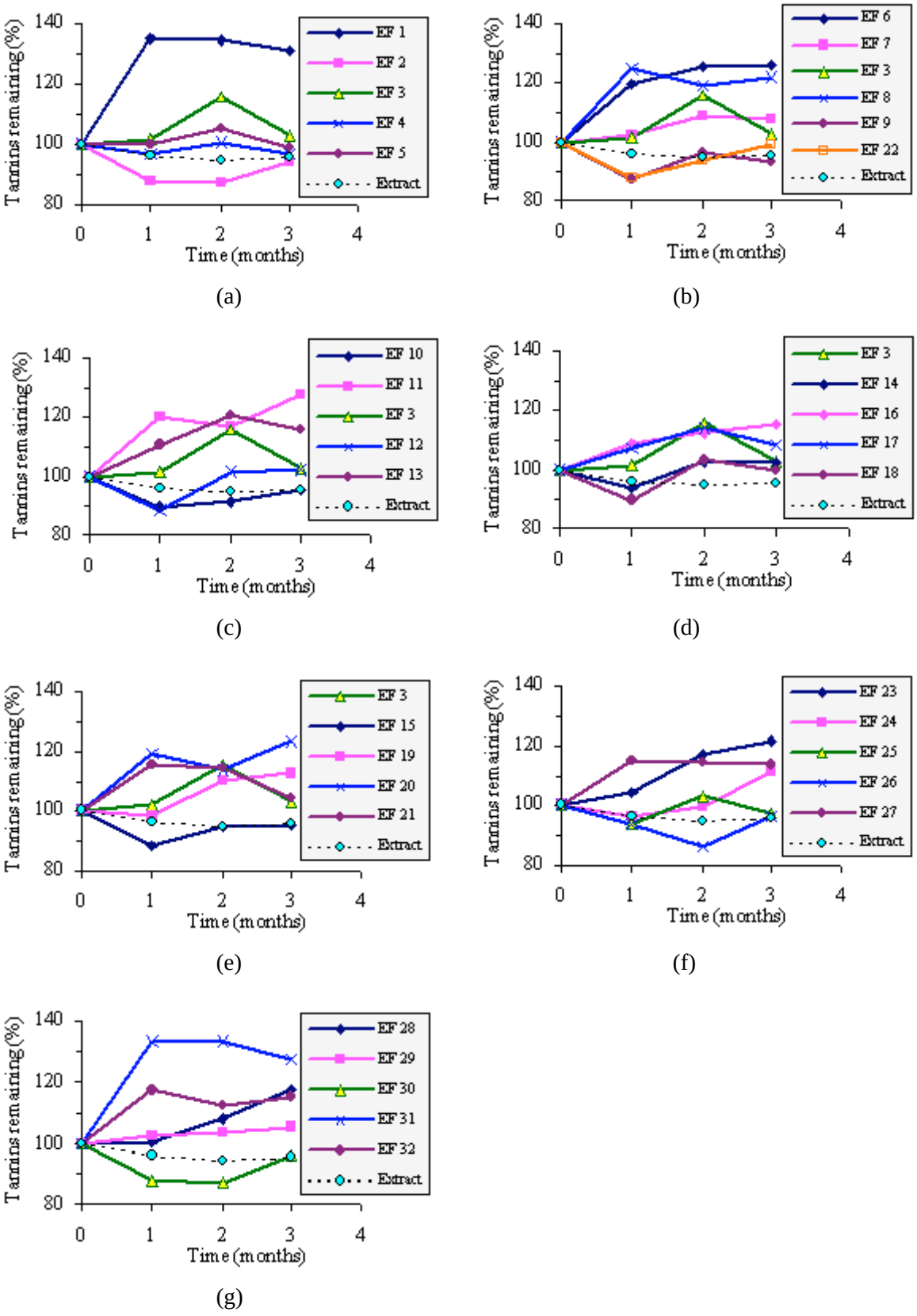


Figure 24 Tannin remaining (%) of effervescent formulations and *Phyllanthus emblica* extract stored at 30±2 ^{O}C, 75% RH for 3 months.

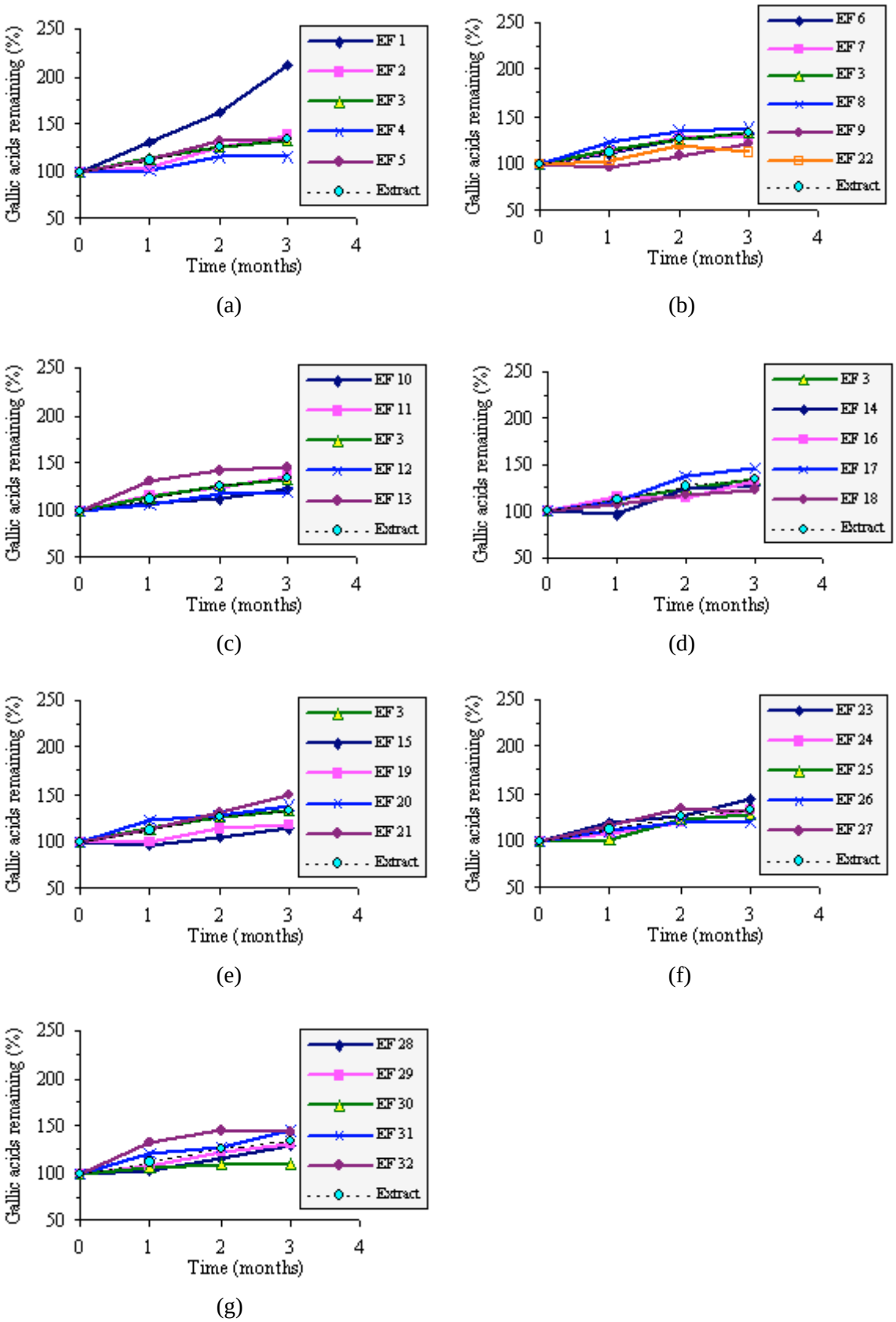


Figure 25 Gallic acid remaining (%) of effervescent formulations and *Phyllanthus emblica* extract stored at 40±2 ^{O}C, 75% RH for 3 months.

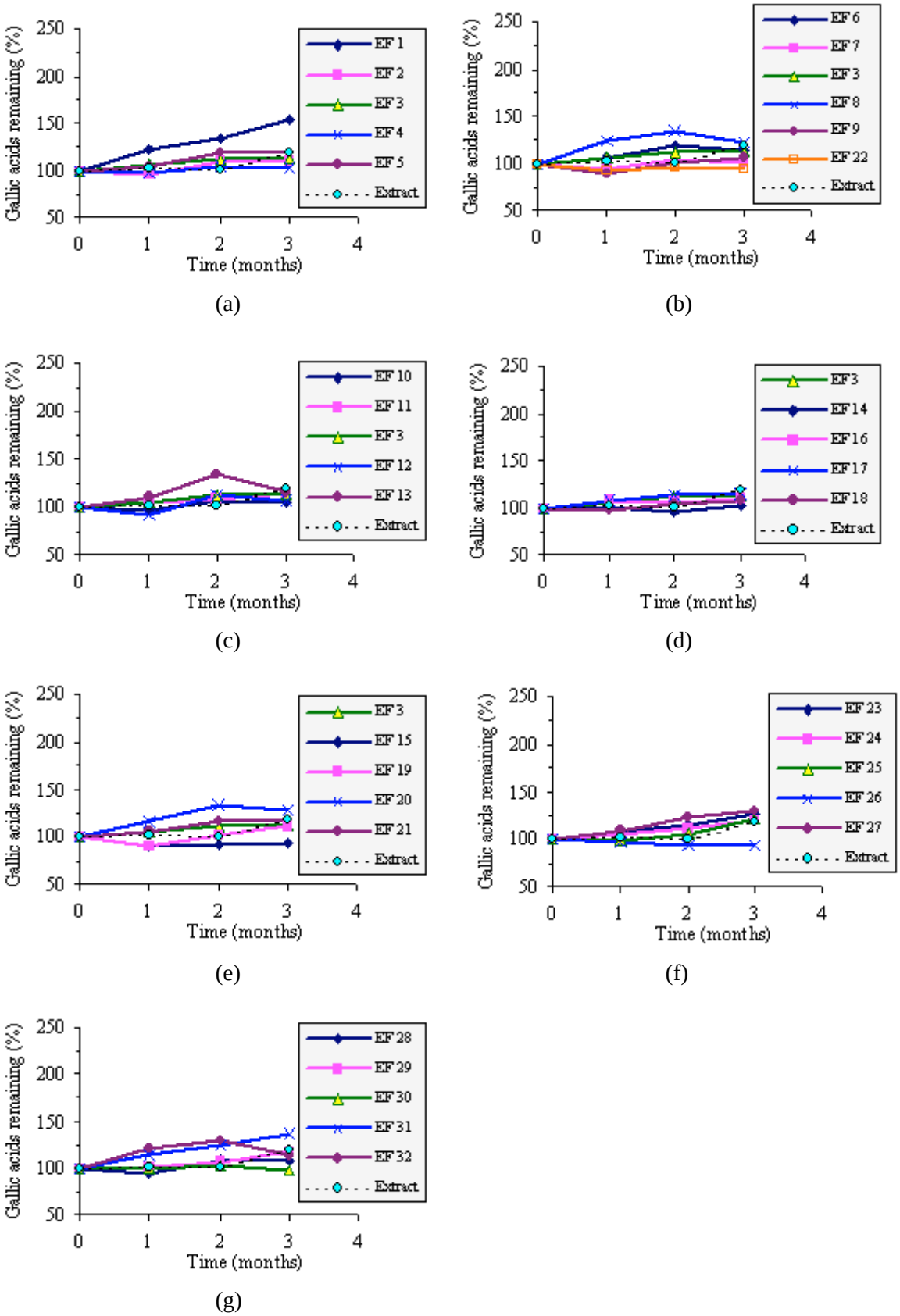
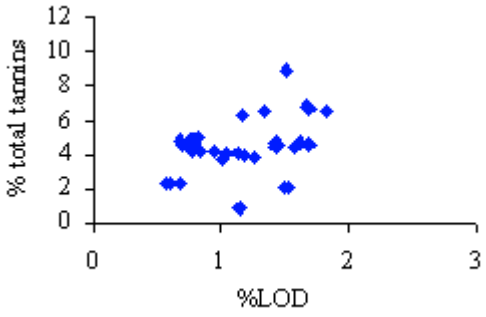
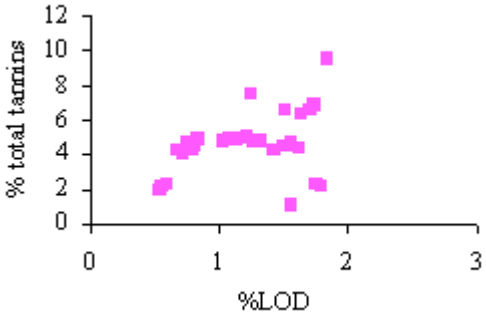


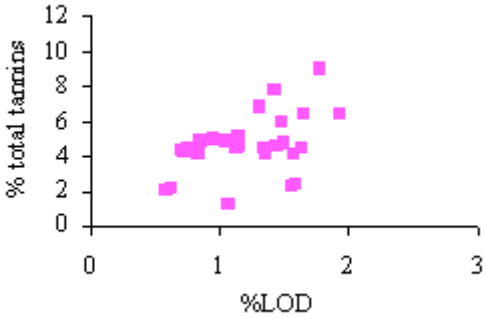
Figure 26 Gallic acid remaining (%) of effervescent formulations and *Phyllanthus emblica* extract stored at 30±2 ^{O}C, 75% RH for 3 months.



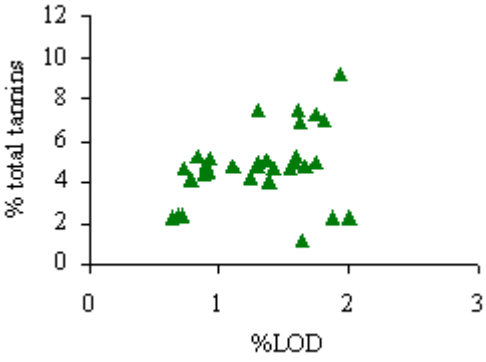
(a) Ambient, 0 month



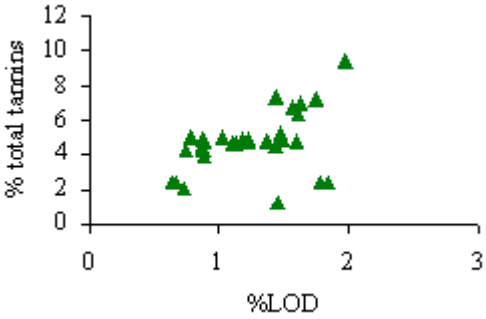
(b) 40±2 ^{O}C 75% RH, 1 month



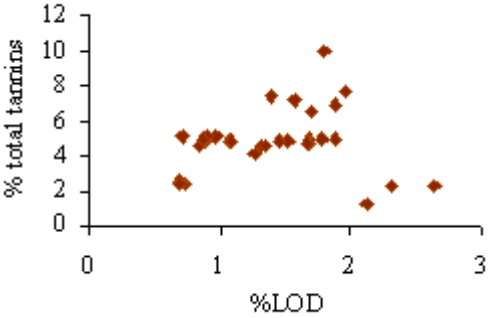
(e) 30±2 ^{O}C 75% RH, 1 month



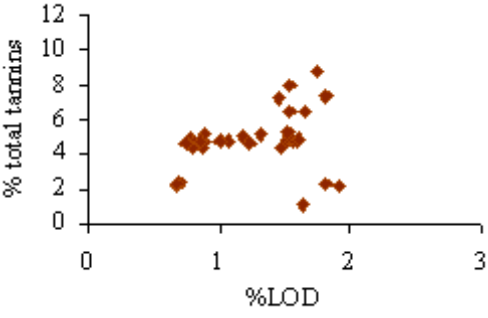
(c) 40±2 ^{O}C 75% RH, 2 months



(f) 30±2 ^{O}C 75% RH, 2 months

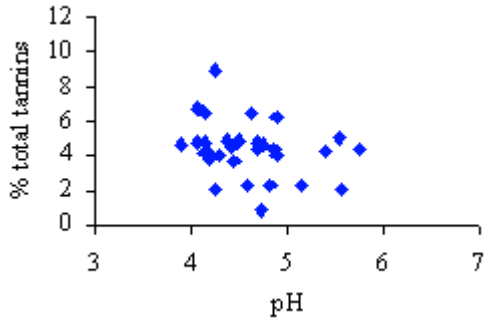


(d) 40±2 ^{O}C 75% RH, 3 months

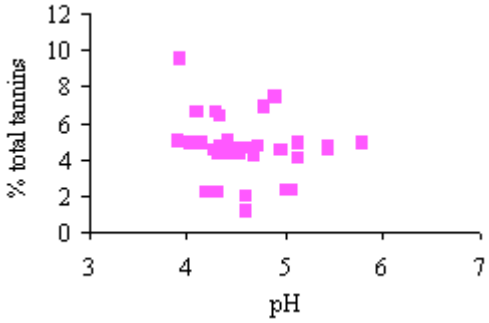


(g) 30±2 ^{O}C 75% RH, 3 months

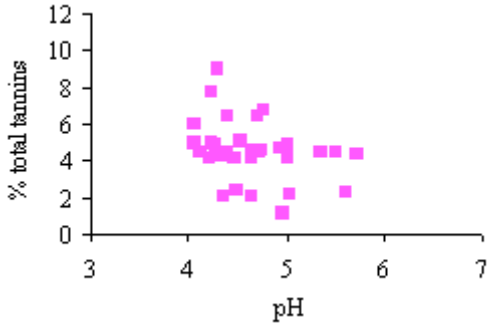
Figure 27 Total tannin versus loss on drying of effervescent formulations



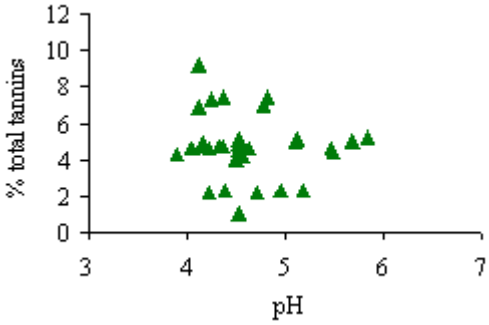
(a) Ambient, 0 month



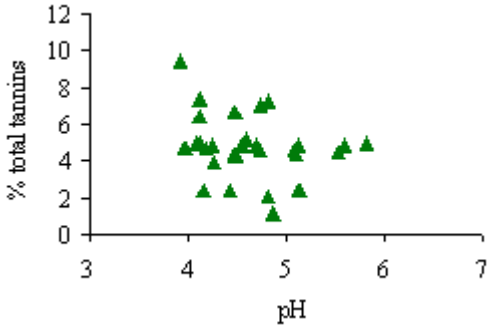
(b) 40±2 ^{O}C 75% RH, 1 month



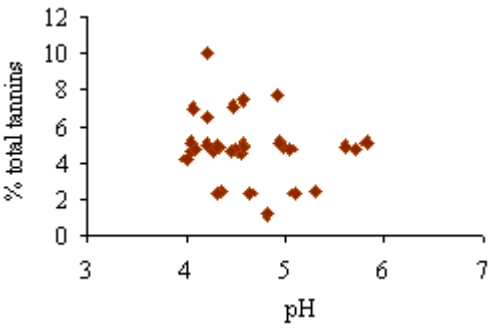
(e) 30±2 ^{O}C 75% RH, 1 month



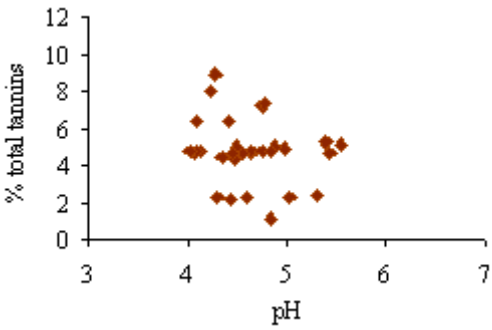
(c) 40±2 ^{O}C 75% RH, 2 months



(f) 30±2 ^{O}C 75% RH, 2 months

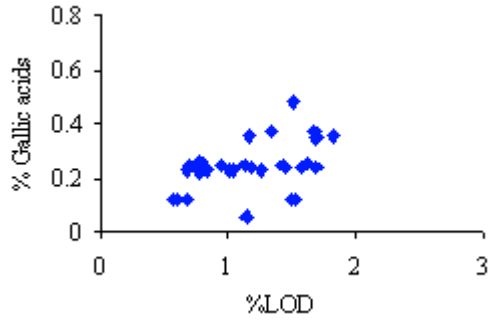


(d) 40±2 ^{O}C 75% RH, 3 months

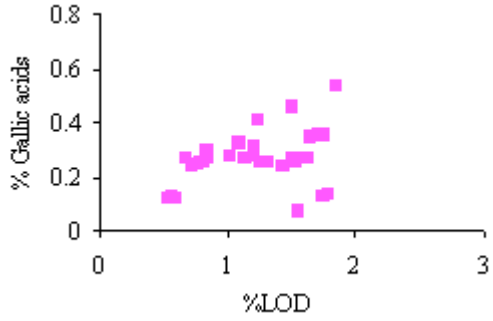


(g) 30±2 ^{O}C 75% RH, 3 months

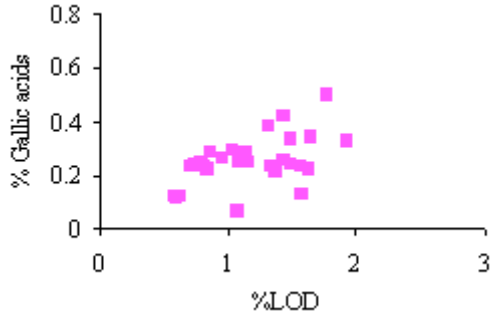
Figure 28 Total tannin versus pH of effervescent formulations.



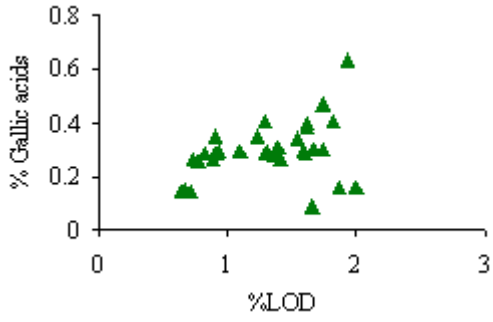
(a) Amblent, 0 month



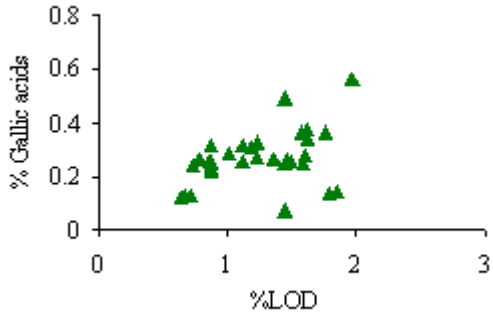
(b) 40±2 ^{O}C 75% RH, 1 month



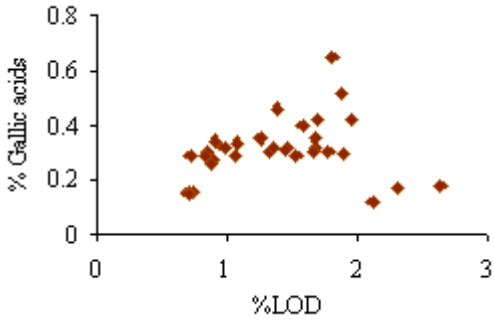
(e) 30±2 ^{O}C 75% RH, 1 month



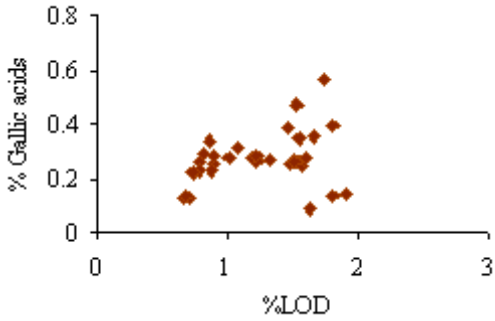
(c) 40±2 ^{O}C 75% RH, 2 months



(f) 30±2 ^{O}C 75% RH, 2 months

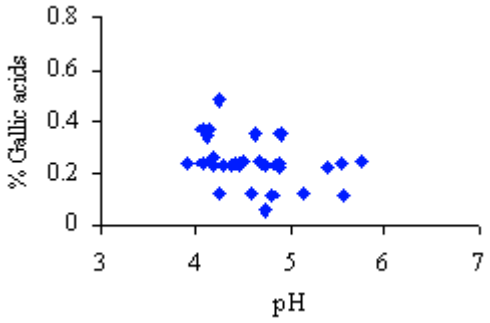


(d) 40±2 ^{O}C 75% RH, 3 months

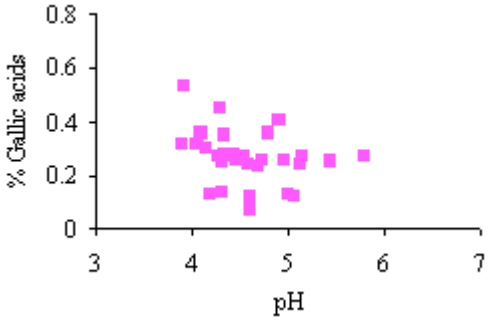


(g) 30±2 ^{O}C 75% RH, 3 months

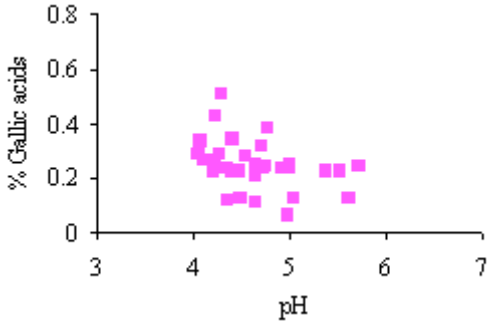
Figure 29 Gallic acid versus loss on drying of effervescent formulations.



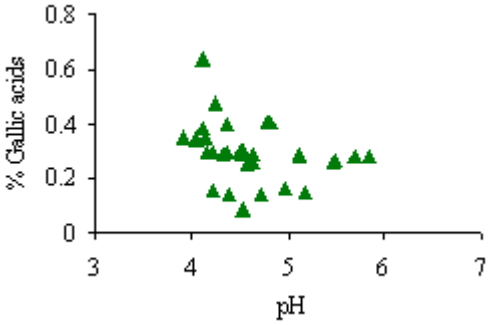
(a) Amblent, 0 month



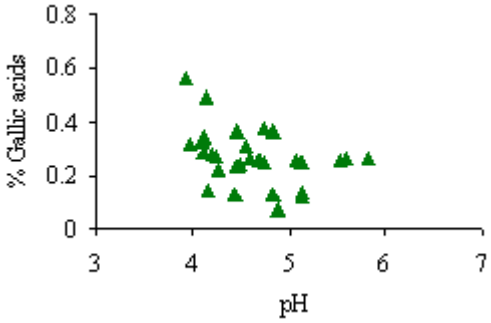
(b) 40±2 ^{O}C 75% RH, 1 month



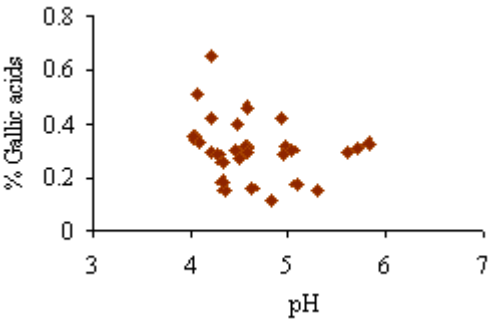
(e) 30±2 ^{O}C 75% RH, 1 month



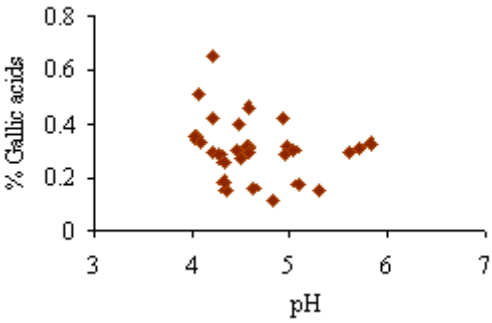
(c) 40±2 ^{O}C 75% RH, 2 months



(f) 30±2 ^{O}C 75% RH, 2 months



(d) 40±2 ^{O}C 75% RH, 3 months



(g) 30±2 ^{O}C 75% RH, 3 months

Figure 30 Gallic acid versus pH of effervescent formulations.

2.3 Statistical analysis

2.3.1 Before stability study

Effervescent granules formulations could classified to 5 groups for statistical analysis. The main effect of the quantity of *Phyllanthus emblica* extract (EF1-5) was analyzed by using 1-way ANOVA. The main effect of the quantity of colloidal silicon dioxide (EF3, 6-9, 22) was analyzed by using 1-way ANOVA. The main effect of the quantity of citric acid anhydrous (EF3, 10-13) was analyzed by using 1-way ANOVA. The interaction effect of the quantities of *Phyllanthus emblica* extract, colloidal silicon dioxide and citric acid anhydrous (EF 14-21) was analyzed by using 2-way ANOVA. Finally, the interaction effect of the quantities of citric acid anhydrous and type of alkaline (EF3, 10-13, 23-32) was analyzed by using 2-way ANOVA. The results from 1-way ANOVA were discussed for main effect because they were obtained from 5-levels of formulation variables. And the results from 2-way ANOVA were discussed for interaction effect.

The level of *Phyllanthus emblica* extract significantly affected on loss on drying, pH, flow rate, angle of repose, apparent density, disintegration time, total tannins and gallic acid contents ($p<0.05$). The level of colloidal silicon dioxide significantly affected on loss on drying, angle of repose, apparent density, disintegration time, total tannins and gallic acid contents ($p<0.05$). The level of citric acid anhydroud significantly affected on loss on drying, pH, angle of repose, apparent density, disintegration time, total tannins and gallic acid contents ($p<0.05$) as shown in table 28.

There was interaction effect of the level of extract and colloidal silicon dioxide on loss on drying, bulk density, flow rate, angle of repose, apparent density and total tannins content ($p<0.05$). The interaction effect of the level of extract and citric acid significantly affected on apparent density, disintegration time and gallic acid content ($p<0.05$). The interaction effect of the level of citric acid and colloidal silicon dioxide significantly affected on loss on drying, flow rate and angle of repose ($p<0.05$) as shown in table 29.

The interaction effect of the level of citric acid anhydrous and the type of alkaline significantly affected on loss on drying, tapped density, angle of repose, apparent density, disintegration time, total tannins and gallic acids ($p<0.05$) as shown in table 30.

Thus, the quantities of *Phyllanthus emblica* extract, colloidal silicon dioxide, citric acid anhydrous and type of alkaline affected on physicochemical properties of effervescent granule in different ways. In addition, the interaction effects between the levels of extract and colloidal silicon, the levels of extract and citric acid, and the level of citric acid and type of alkaline played a significant role in change of total tannins and gallic acid contents.

2.3.2 After stability study for 3 months

The results were analyzed by using repeated measures ANOVA. It was found that the quantity of *Phyllanthus emblica* extract significantly affected on loss on drying and gallic acid contents at 40±2 ^{O}C, 75% RH and 30±2 ^{O}C, 75% RH, and total tannins content at 40±2 ^{O}C, 75% RH ($p<0.05$). The results agreed with that of *Phyllanthus emblica* extract stored at the same stability test conditions as shown in table 46-47 (appendix C). The level of colloidal silicon dioxide significantly affected on total tannins and gallic acid contents at 40±2 ^{O}C, 75% RH and 30±2 ^{O}C, 75% RH ($p<0.05$). Citric acid anhydrous significantly affected on loss on drying at 40±2 ^{O}C, 75% RH and total tannins and gallic acid contents at 40±2 ^{O}C, 75% RH and 30±2 ^{O}C, 75% RH ($p<0.05$) as shown in table 31.

It was found that there was the interaction effect of the levels of extract and colloidal silicon dioxide on loss on drying, total tannins and gallic acid contents at 40±2 ^{O}C, 75% RH and 30±2 ^{O}C, 75% RH ($p<0.05$). The interaction effect of extract and citric acid significantly affected on total tannins at 40±2 ^{O}C, 75% RH ($p<0.05$). The interaction effect of citric acid and colloidal silicon dioxide significantly affected on total tannins and gallic acid at 30±2 ^{O}C, 75% RH ($p<0.05$) as shown in table 32.

Type of alkaline significantly affected on loss on drying, pH, total tannins content at 40±2 ^{O}C, 75% RH and on gallic acid contents at 30±2 ^{O}C, 75% RH ($p<0.05$). The interaction effect of the level of citric acid anhydrous and type of alkaline significantly affected on loss on drying at 40±2 ^{O}C, 75% RH, and on total tannins and gallic acid contents at 40±2 ^{O}C, 75% RH and 30±2 ^{O}C, 75% RH ($p<0.05$) as shown in table 33.

Thus, the excipients, i.e. colloidal silicon dioxide, citric acids and type of alkaline influenced on the stability of products because they significantly affected on loss on drying, total tannins and gallic acid.

Table 28 The results of the analysis of variance of main effect of effervescent granules

Response / Variables	Loss on drying (%)	pH	Bulk density (g/ml)	Tapped density (g/ml)	Compressibility (%)	Flow rate (g/sec.)	Angle of repose	Apparent density (g/cm^3)	DT (min)	Total tannins (%)	Gallic acid (%)
extract	**S**	**S**	NS	-	NS	**S**	**S**	**S**	**S**	**S**	**S**
aerosil	**S**	NS	NS	NS	NS	NS	**S**	**S**	**S**	**S**	**S**
citric	**S**	**S**	NS	-	NS	NS	**S**	**S**	**S**	**S**	**S**

Extract = Level of *Phyllanthus emblica* extract

Aerosil = Level of colloidal silicon dioxide

Citric = Level of citric acid anhydrous

S = Significant difference at a significant level ($P \leq 0.05$)

NS = No significant difference at a significant level ($P \leq 0.05$)

\- = Data could not be analyzed by 1-way ANONA

Table 29 The results of the analysis of variance of interaction between *Phyllanthus emblica* extract, colloidal silicon dioxide and citric acid anhydrous

Response / Variables	Loss on drying (%)	pH	Bulk density (g/ml)	Tapped density (g/ml)	Compressibility (%)	Flow rate (g/sec.)	Angle of repose	Apparent density (g/cm^3)	DT (min)	Total tannins (%)	Gallic acid (%)
extract	S	S	NS	NS	NS	NS	S	S	NS	S	S
aerosil	S	NS	NS	NS	NS	S	S	S	S	NS	NS
citric	NS	S	S	S	NS	NS	S	S	S	NS	S
extract * aerosil	**S**	NS	**S**	NS	NS	**S**	**S**	**S**	NS	**S**	NS
extract * citric	NS	NS	NS	NS	NS	NS	NS	**S**	**S**	NS	**S**
aerosil * citric	**S**	NS	NS	NS	NS	**S**	**S**	NS	NS	NS	NS
Adjusted R squared	0.984	0.756	0.574	0.420	0.134	0.700	0.739	0.994	0.863	0.994	0.999

Extract = Level of *Phyllanthus emblica* extract

Aerosil = Level of colloidal silicon dioxide

Citric = Level of citric acid anhydrous

S = Significant difference at a significant level ($P \leq 0.05$)

NS = No significant difference at a significant level ($P \leq 0.05$)

Table 30 The results of the analysis of variance of interaction between citric acid anhydrous and base type

Response / Variables	Loss on drying (%)	pH	Bulk density (g/ml)	Tapped density (g/ml)	Compressibility (%)	Flow rate (g/sec.)	Angle of repose	Apparent density (g/cm^3)	DT (min)	Total tannins (%)	Gallic acid (%)
citric	S	S	S	S	S	S	NS	S	S	S	S
base type	**S**	**S**	**S**	**S**	NS	NS	**S**	**S**	**S**	NS	NS
citric * base type	**S**	NS	NS	**S**	NS	NS	**S**	**S**	**S**	**S**	**S**
Adjusted R squared	0.967	0.885	0.529	0.623	0.022	0.322	0.473	0.995	0.837	0.844	0.586

Citric = Level of citric acid anhydrous

Base type = type of alkaline, i.e. sodium bicarbonate, effer-soda® or sodium bicarbonate mixed with sodium carbonate

S = Significant difference at a significant level ($P \leq 0.05$)

NS = No significant difference at a significant level ($P \leq 0.05$)

Table 31 The results of the analysis of variance of main effect of effervescent granules after stored at stability conditions

Response / Variables	Loss on drying		pH		Total tannins		Gallic acid	
	40±2 °C, 75% RH	30±2 °C, 75% RH	40±2 °C, 75% RH	30±2 °C, 75% RH	40±2 °C, 75% RH	30±2 °C, 75% RH	40±2 °C, 75% RH	30±2 °C, 75% RH
extract	**S**	**S**	NS	NS	**S**	NS	**S**	**S**
aerosil	NS	NS	NS	NS	**S**	**S**	**S**	**S**
citric	**S**	NS	NS	NS	**S**	**S**	**S**	**S**

Extract = Level of *Phyllanthus emblica* extract

Aerosil = Level of colloidal silicon dioxide

Citric = Level of citric acid anhydrous

S = Significant difference at a significant level ($P \leq 0.05$)

NS = No significant difference at a significant level ($P \leq 0.05$)

Table 32 The results of the analysis of variance of interaction between *Phyllanthus emblica* extract, colloidal silicon dioxide and citric acid after stored at stability conditions

Response / Variables	Loss on drying		pH		Total tannins		Gallic acid	
	40±2 °C, 75% RH	30±2 °C, 75% RH	40±2 °C, 75% RH	30±2 °C, 75% RH	40±2 °C, 75% RH	30±2 °C, 75% RH	40±2 °C, 75% RH	30±2 °C, 75% RH
extract	S	NS	NS	NS	S	S	S	S
aerosil	S	NS	NS	NS	NS	S	S	S
citric	S	NS	NS	NS	S	NS	NS	NS
extract * aerosil	**S**	**S**	NS	NS	**S**	**S**	**S**	**S**
extract * citric	NS	NS	NS	NS	**S**	NS	NS	NS
aerosil * citric	NS	NS	NS	NS	NS	**S**	NS	**S**

Extract = Level of *Phyllanthus emblica* extract

Aerosil = Level of colloidal silicon dioxide

Citric = Level of citric acid anhydrous

S = Significant difference at a significant level ($P \leq 0.05$)

NS = No significant difference at a significant level ($P \leq 0.05$)

Table 33 The results of the analysis of variance of interaction between citric acid anhydrous and base type after stored at stability conditions

Response / Variables	Loss on drying		pH		Total tannins		Gallic acid	
	40±2 ^{O}C, 75% RH	30±2 ^{O}C, 75% RH	40±2 ^{O}C, 75% RH	30±2 ^{O}C, 75% RH	40±2 ^{O}C, 75% RH	30±2 ^{O}C, 75% RH	40±2 ^{O}C, 75% RH	30±2 ^{O}C, 75% RH
citric	S	S	NS	NS	S	S	S	S
base type	**S**	NS	**S**	NS	**S**	NS	NS	**S**
citric * base type	**S**	NS	NS	NS	**S**	**S**	**S**	**S**

Citric = Level of citric acid anhydrous

Base type = type of alkaline, i.e. sodium bicarbonate, effer-soda® or sodium bicarbonate mixed with sodium carbonate

S = Significant difference at a significant level ($P \leq 0.05$)

NS = No significant difference at a significant level ($P \leq 0.05$)

3 Pellets containing *Phyllanthus emblica* extract

3.1 Preparation of pellets containing *Phyllanthus emblica* extract

Pellets were prepared by extrusion-spheronization. Solubility of *Phyllanthus emblica* extract and excipients were effected to quantity of binder liquid in wet mixing process. *Phyllanthus emblica* extract was good soluble in water. Increase in extract content in the formulations led to increase in wetted particle surface after adding binder liquid. If damp mass was too wet, it would induce partially large pellets and broad size distribution. In addition, increase in extract content brought about increase in sticky damp mass. In this case, formulation in containing microcrystalline cellulose less than 50% and high level of *Phyllanthus emblica* extract, the wet mass of which would very sticky. The sticky wet mass was extruded through extruder to form long cylindrical particle and spheronizer could not break this extrudate to form a pellet.

From results in table 34, formulation containing 20% of *Phyllanthus emblica* extract and 40-45% of binder liquid could prepare round shape. Whereas, formulation containing 40% of *Phyllanthus emblica* extract and 30% of binder liquid could prepare both dumbbell and round shape. So, high level of *Phyllanthus emblica* extract required lower quantity and narrow interval of binder liquid than low level of *Phyllanthus emblica* extract.

Formulation containing 30% of *Phyllanthus emblica* extract and 35-40% of binder liquid could prepare spherical particles. This formulations was central experimental (n=3). Its wet mass was not very sticky and could prepare to spherical particles.

Table 34 Appearance of pelletized products of varied binding liquid

No.	Quantity of liquid binder (% based on solid weight)					
	25%	30%	35%	40%	45%	50%
PE 1	-	-	-	Round shape	Round shape	Round shape
PE 2	Dumbbell shape	Dumbbell and round shape	Dumbbell and round shape	-	-	-
PE 3	-	-	-	Round shape	Round shape	Too wet
PE 4	Dumbbell shape	Dumbbell and round shape	Dumbbell and round shape	-	-	-
PE 5	-	-	-	Round shape	Round shape	Too wet
PE 6	Dumbbell shape	Dumbbell and round shape	Too wet	-	-	-
PE 7	-	-	-	Round shape	Round shape	Too wet
PE 8	Dumbbell shape	Dumbbell and round shape	Too wet	-	-	-
PE 9	-	Dumbbell and round shape	Round shape	Round shape	-	-

- = Formulation was not prepared

3.2 Characterization of pellets containing *Phyllanthus emblica* extract

3.2.1 Physical appearance

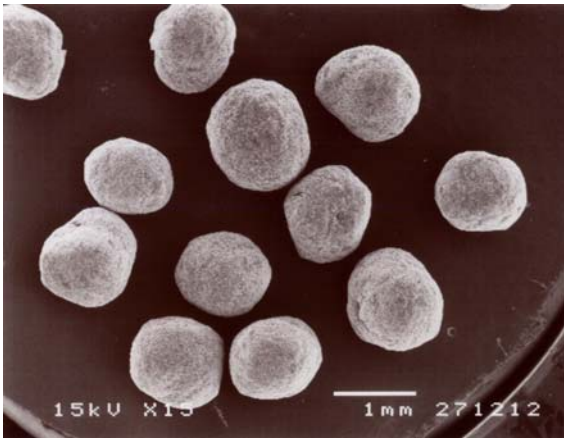
The apperance of pellets containing *Phyllanthus emblica* extract was observed. The pellets were yellowish particles. The yellowish color obtained from color of *Phyllanthus emblica* extract. The color of products were brownish, if the amounts of *Phyllanthus emblica* extract in the formulations were higher. Their shape were dumbbell or spherical with diameter of approximate 1-2 mm due to the quantity of the compositions and binder liquid as shown in table 34.

3.2.2 Morphology

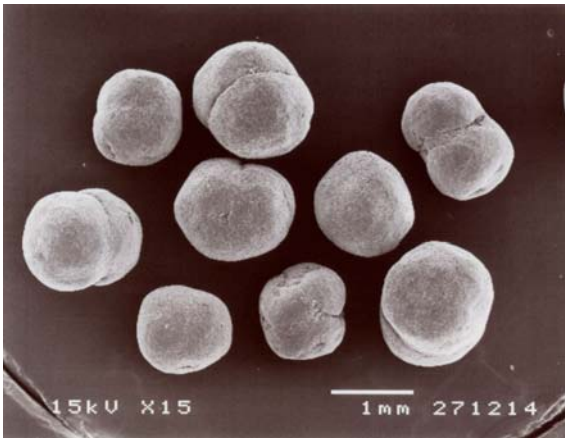
Shape, surface topography and cross-section of pellets was investigated by scanning electron microscopy using magnification of 15x, 15 kv. and 75x, 15 kv. as presented in figure 31-35. The pellets containing 20% of *Phyllanthus emblica* extract in formulation were small and spherical particle. The pellets containing 40% of *Phyllanthus emblica* extract in formulation were both dumbbell and spherical particle.

PE1-9 formulation had smooth surface. The cross-section of pellets showed that there were cavities in the pellets. Size of cavities in the pellets may result from the density of damp mass. The damp mass of low level of *Phyllanthus emblica* extract was less sticky than high level of *Phyllanthus emblica* extract.

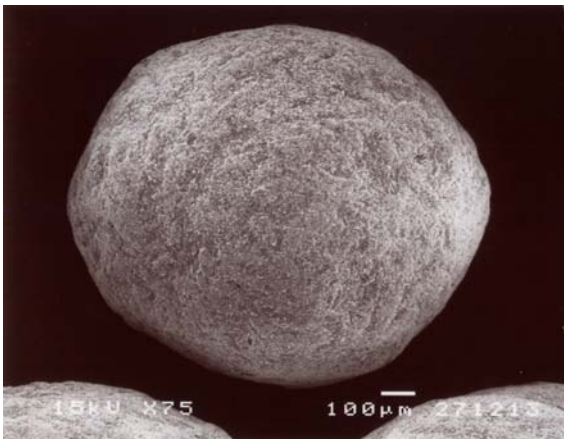
Difference of shape, surface topography and cross-section of pellets were resulted from the quantities and solubilities of compositions and the quantity of binder liquid.



(a) PE1, 15x

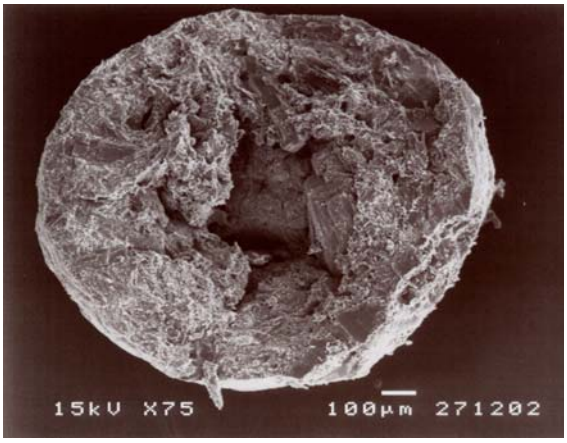


(d) PE2, 15x

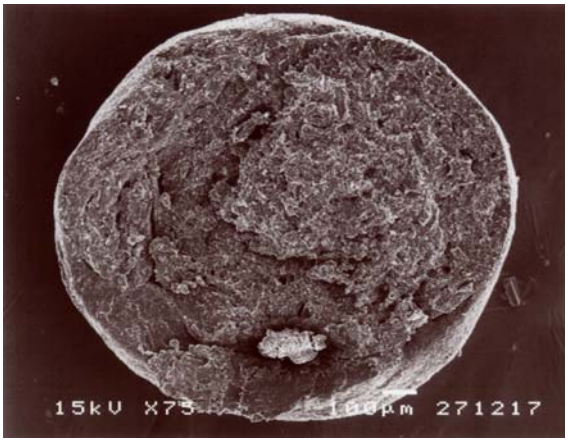


(b) PE1, 75x

(e) PE2, 75x

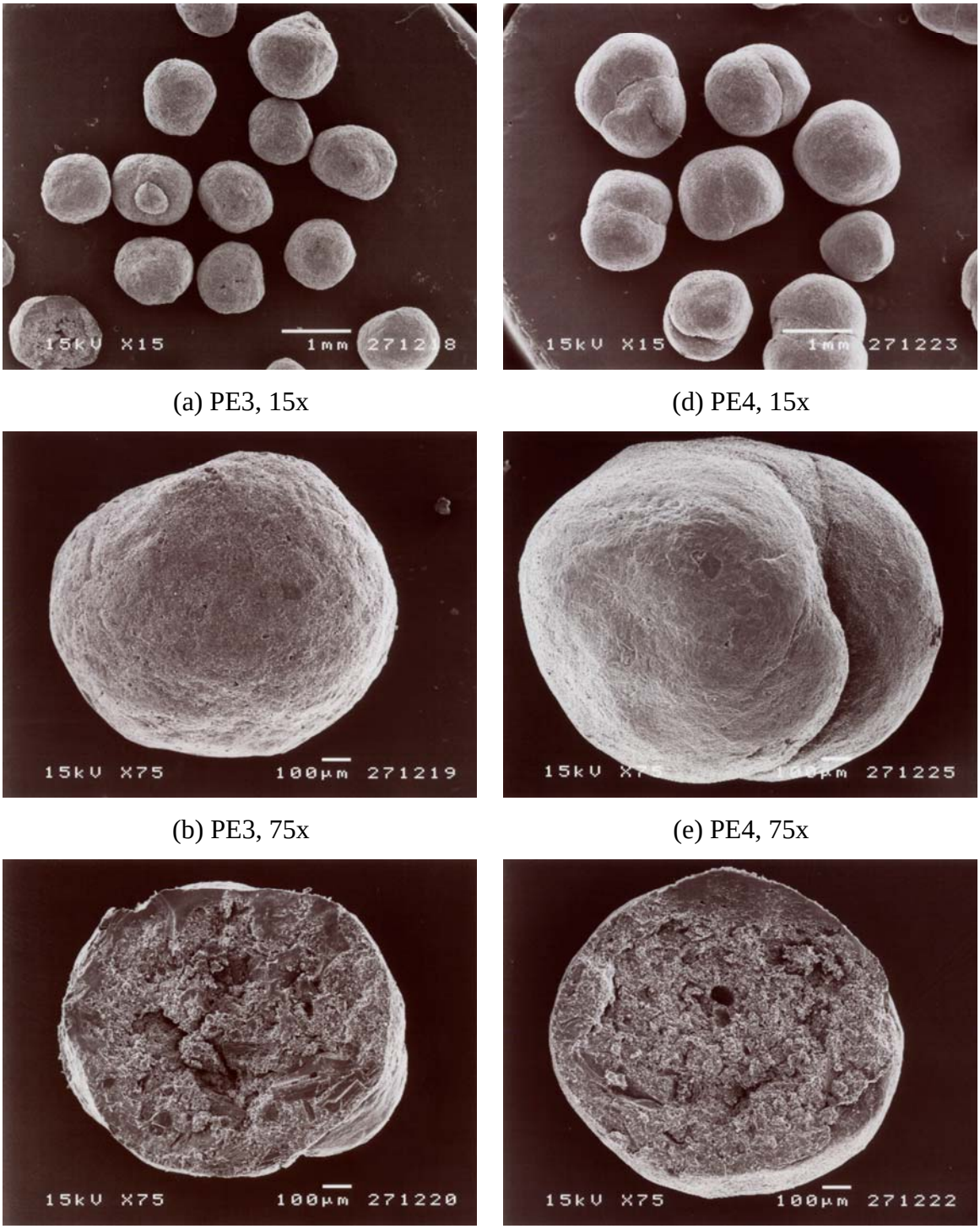


(c) PE1, 75x, cross section



(f) PE2, 75x, cross section

Figure 31 Scanning electron micrographs of PE1 and PE2 at magnification of 15x and 75x

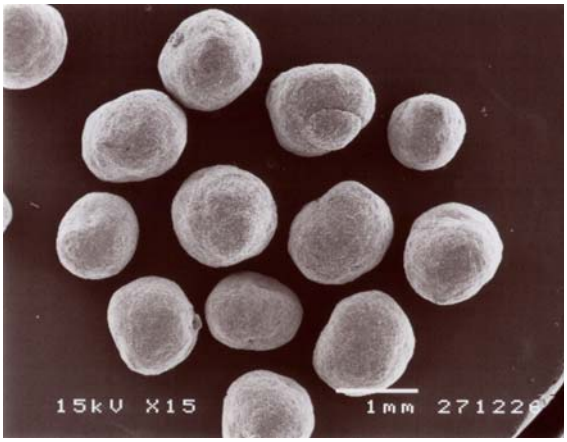


(a) PE3, 15x (d) PE4, 15x

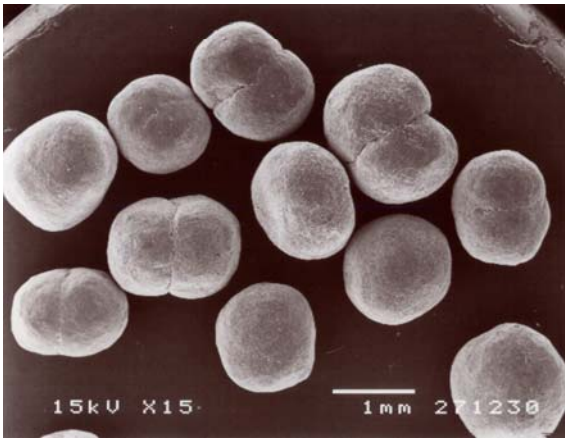
(b) PE3, 75x (e) PE4, 75x

(c) PE3, 75x, cross section (f) PE4, 75x, cross section

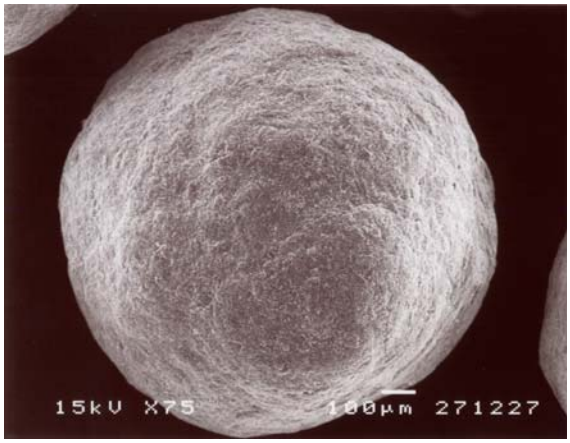
Figure 32 Scanning electron micrographs of PE3 and PE4 at magnification of 15x and 75x



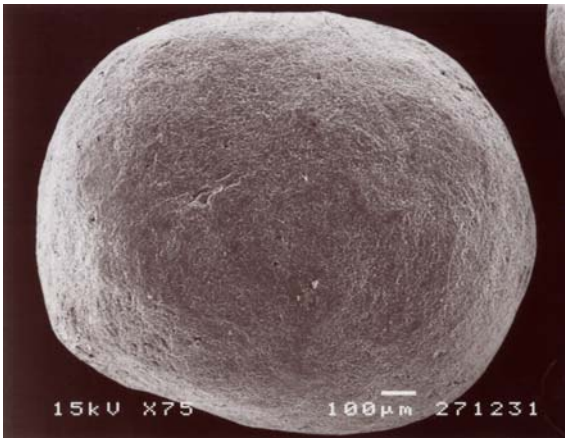
(a) PE5, 15x



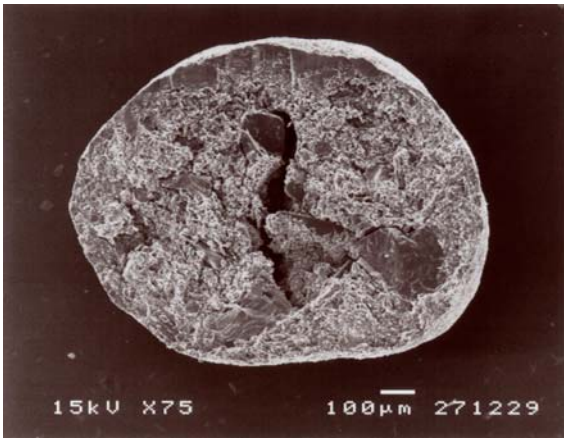
(d) PE6, 15x



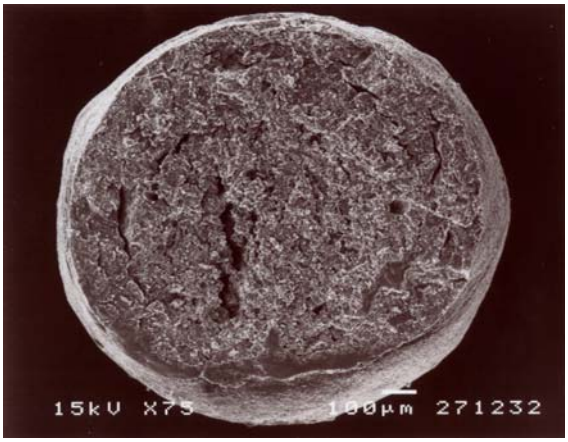
(b) PE5, 75x



(e) PE6, 75x

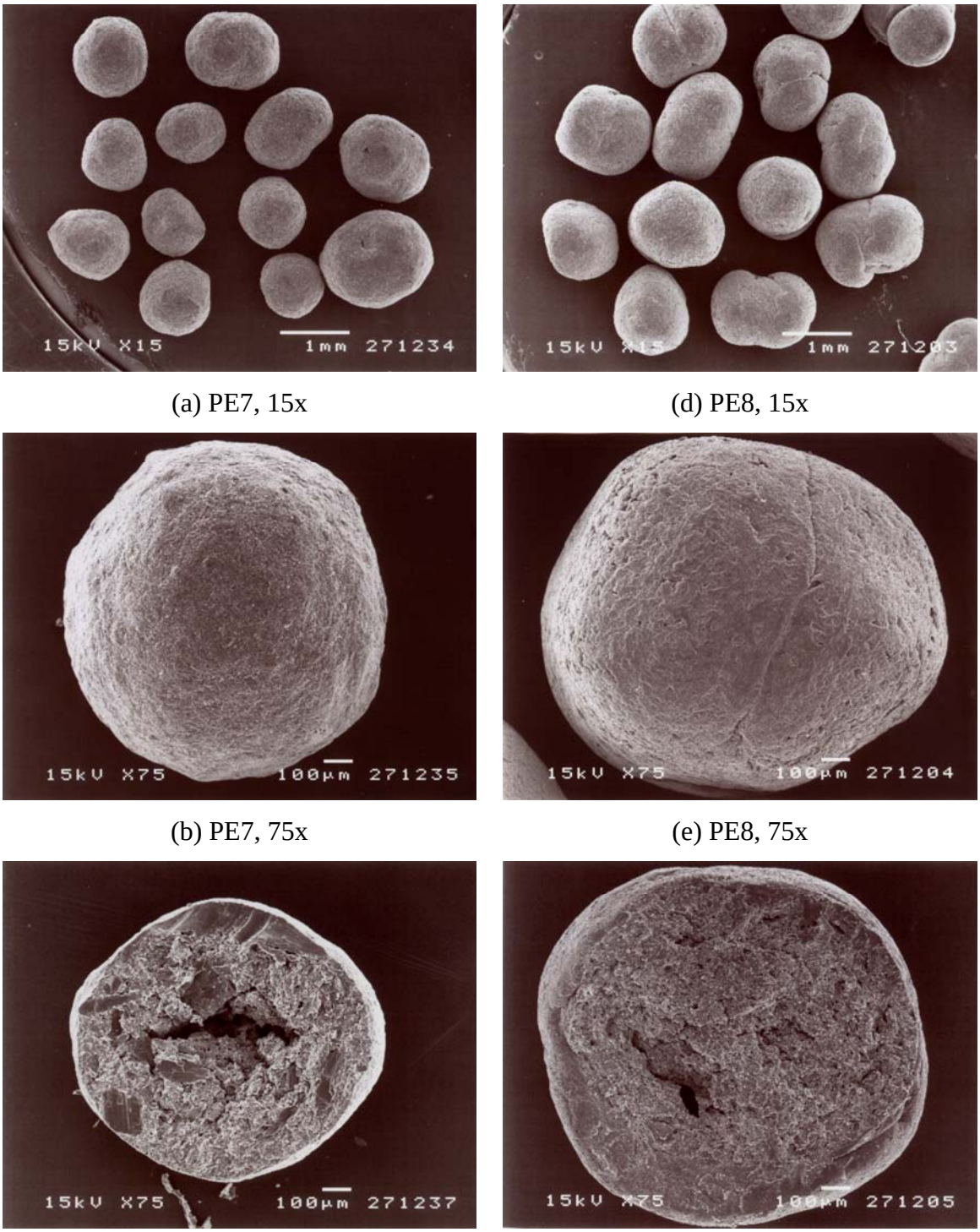


(c) PE5, 75x, cross section



(f) PE6, 75x, cross section

Figure 33 Scanning electron micrographs of PE5 and PE6 at magnification of 15x and 75x

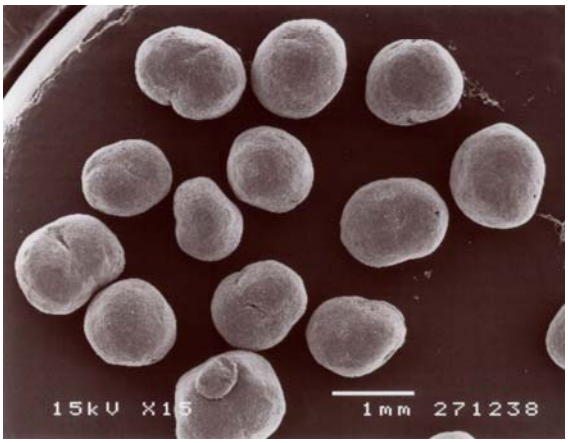


(a) PE7, 15x (d) PE8, 15x

(b) PE7, 75x (e) PE8, 75x

(c) PE7, 75x, cross section (f) PE8, 75x, cross section

Figure 34 Scanning electron micrographs of PE7 and PE8 at magnification of 15x and 75x



(a) PE9, 15x

(b) PE9, 75x

(c) PE9, 75x, cross section

Figure 35 Scanning electron micrographs of PE9 at magnification of 15x and 75x

3.2.3 Distribution

Size distributions of pellets were determined by sieve analysis. Characterization of particle size distribution could be divided into 2 patterns by the quantity of *Phyllanthus emblica* extract. Low level of *Phyllanthus emblica* extract gave small particle and narrow distribution than high level of *Phyllanthus emblica* extract.

The highest percent of size distribution of PE1 (34.68%), PE3 (35.59%), PE5 (39.13%), PE7 (40.30%) and PE9 (41.82%) were the particle size range between to 0.85-1.00 mm. The highest percent of size distribution of PE2 (51.03%), PE4 (46.80%), PE6 (49.47%) and PE8 (31.31%) were the particle size range between to 1.00-1.18 mm as displayed in figure 36 and table 35.

From this results, the particle size range selected of pellets each formulation for physiochemical analysis and stability test was 0.71-1.40 mm.

3.2.4 Loss on drying

Loss on drying of pellets was determined by a moisture analyzer. They had loss on drying between 0.26%-1.15% as shown in table 36. The difference of loss on drying should came from the quantity of remained binder liquid after dried at equal interval times.

3.2.5 pH

Solution of pellets had pH value between 2.78-3.03 as shown in table 36. pH value of solutions of the pellets varied slightly. The acidity of the solution of pellets were set from *Phyllanthus emblica* extract because the pH value of pellet solutions and extract solution similar.

Table 35 Size distribution of pellets

Size(mm) / No.	% Weight							
	< 0.50	**0.50**-0.71	**0.71**-0.85	**0.85**-1.00	**1.00**-1.18	**1.18**-1.40	**1.40**-1.70	> **1.7**
PE1	3.61	13.12	22.93	34.68	23.69	1.75	0.19	0
PE2	0.03	0.45	5.29	28.05	51.03	11.90	2.29	0.94
PE3	4.95	16.40	26.34	35.59	15.65	0.79	0.14	0.17
PE4	0.00	0.12	2.45	16.58	46.80	19.62	7.48	6.91
PE5	4.33	12.25	23.14	39.13	20.26	0.80	0.06	0.00
PE6	0.00	0.07	1.87	15.82	49.47	22.04	6.14	4.53
PE7	1.38	9.11	21.98	40.30	25.78	1.23	0.18	0.00
PE8	0.00	0.03	1.14	9.38	31.31	23.70	18.69	15.56
PE9, lot.1	0.57	2.41	12.14	41.82	38.81	3.21	0.67	0.34
PE9, lot.2	0.65	3.00	14.12	43.76	35.20	2.74	0.33	0.14
PE9, lot.3	0.47	3.04	14.00	43.66	35.06	2.62	0.56	0.59

* PE9 prepared and analysed in three replicates.

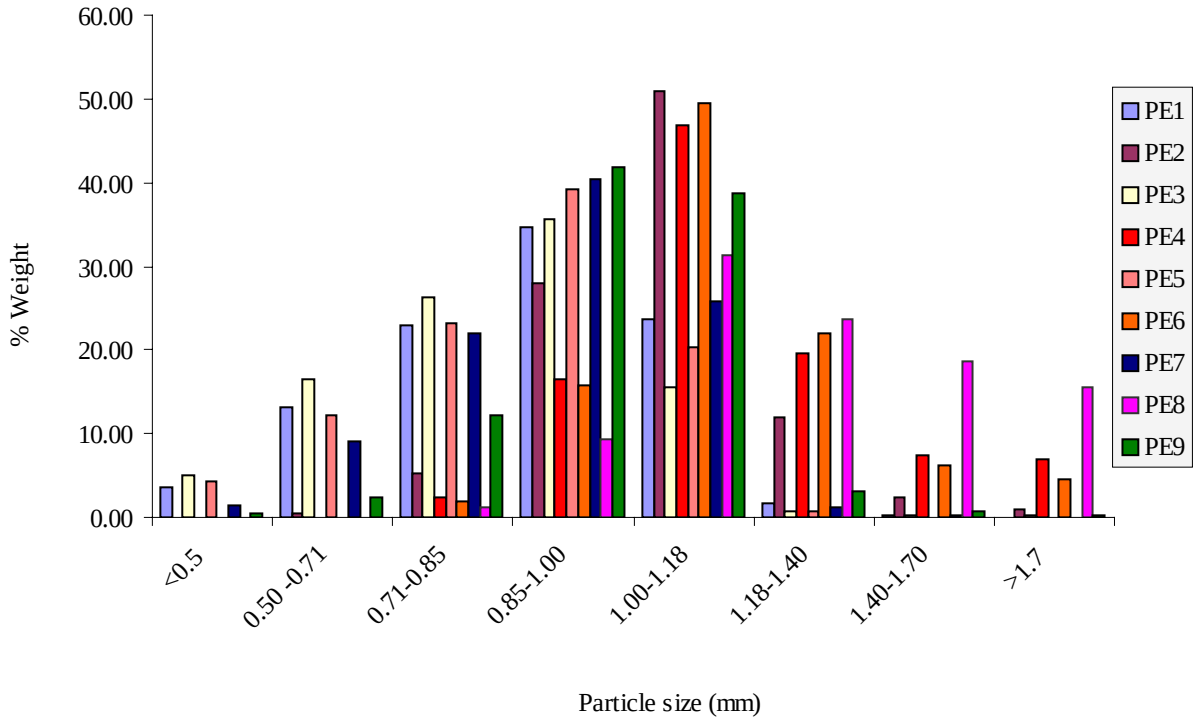


Figure 36 Size distribution of pellets

3.2.6 Bulk density, Tapped density and Percent compressibility

Pellets had bulk density, tapped density and % compressibility between 0.7 g/cm^3 -0.8 g/cm^3, 0.8 g/cm^3 - 0.9 g/cm^3 and 0.0%-12.5%, respectively as shown in table 36.

Bulk density and tapped density of pellets formulation varied slightly. Formulations containing 40% *Phyllanthus emblica* extract had higher value of bulk density and tapped density than formulations containing 20% *Phyllanthus emblica* extract.

From Carr's compressibility index in table 2, pellets were classified as free flowing characteristic level.

3.2.7 Flow rate

Flowability of pellets were determined by a glass funnel. All formulations could fast flowed through a glass funnel. They had flow rate between 19.7 g/sec - 36.0 g/sec as shown in table30.

3.2.8 Angle of repose

Pellets had angle of repose between 2.1^O-16.8^O as shown in table 36. They could classified to good flowability from table of relationship between the angle of repose and flowability in table 12. This results supported the flowing characteristics in term of compressibility in section 3.2.5.

3.2.9 Apparent density

Pellets had apparent density between 1.46 g/cm^3 -1.56 g/cm^3 as shown in table 36. Apparent density of pellets varied slightly. The apparent density of components related to apparent density of the products.

Table 36 Physicochemical properties of pellets (I)

No.	Loss on drying (%) (n=3)	pH (n=3)	Bulk density (g/ml) (n=3)	Tapped density (g/ml) (n=3)	Compressibility (%) (n=3)	Flow rate (g/sec.) (n=3)	Angle of repose (^{O}) (n=3)	Apparent density (g/cm^3) (n=5)
PE1	1.15 (0.31)	2.86 (0.01)	0.8 (0.0)	0.8 (0.0)	8.3 (7.2)	20.49 (1.04)	16.8 (0.1)	1.56 (0.00)
PE2	0.50 (0.09)	2.92 (0.01)	0.8 (0.0)	0.9 (0.0)	7.4 (6.4)	25.65 (1.45)	8.8 (0.1)	1.56 (0.00)
PE3	0.38 (0.07)	2.86 (0.01)	0.7 (0.0)	0.8 (0.0)	8.3 (7.2)	19.66 (1.79)	16.2 (0.1)	1.54 (0.00)
PE4	0.26 (0.02)	2.99 (0.01)	0.8 (0.1)	0.9 (0.0)	7.4 (6.4)	26.73 (4.44)	5.9 (0.5)	1.52 (0.00)
PE5	0.65 (0.12)	2.78 (0.01)	0.7 (0.0)	0.8 (0.0)	12.5 (0.0)	23.00 (1.41)	15.4 (0.1)	1.54 (0.00)
PE6	0.34 (0.11)	2.98 (0.01)	0.8 (0.0)	0.9 (0.0)	7.4 (6.4)	29.51 (4.06)	4.8 (0.0)	1.46 (0.01)
PE7	0.60 (0.07)	2.88 (0.02)	0.7 (0.0)	0.8 (0.0)	8.3 (7.2)	27.95 (0.38)	15.7 (0.9)	1.51 (0.00)
PE8	0.40 (0.16)	3.03 (0.02)	0.8 (0.0)	0.9 (0.0)	11.1 (0.0)	36.01 (3.21)	2.1 (0.0)	1.48 (0.00)
PE9, lot.1	0.38 (0.08)	2.97 (0.00)	0.8 (0.0)	0.8 (0.0)	0.0 (0.0)	28.94 (3.61)	9.7 (0.2)	1.54 (0.00)
PE9, lot.2	0.51 (0.14)	2.96 (0.00)	0.8 (0.0)	0.9 (0.0)	0.0 (0.0)	29.19 (1.16)	12.8 (0.3)	1.54 (0.00)
PE9, lot.3	0.39 (0.02)	2.97 (0.00)	0.8 (0.0)	0.9 (0.0)	11.1 (0.0)	25.50 (3.36)	13.1 (0.1)	1.53 (0.00)

* PE9 was prepared in three replicates.

3.2.10 Sphericity

One hundred pellets of each formulation were investigated by using image analyzer and analyzed by software program Image Pro Plus® of Image analyzer. Pellets had longest diameter between 1.06-1.43 mm., smallest diameter between 0.86-1.04 mm. and roundness between 1.23-1.60 as shown in table 37.

Sphericity of pellets are related with quantity of the water soluble components. *Phyllanthus emblica* extract was major factor affecting on sphericity. The pellets with high level of *Phyllanthus emblica* extract had higher roundness value or were of less spherical than the pellets with low level of *Phyllanthus emblica* extract. Level of acesulfame and citric acid should influence to sphericity but their influence was not clear. For example, PE8 containing high level of extract, acesulfame and citric acid had highest roundness value.

3.2.11 Hardness

Hardness of pellet was determined by texture analyser with 6-mm diameter cylinder stainless. Hardness of pellets were between 880.28-2486.55 g as shown in table 37. Characteristics of increased hardness was divided into 2 patterns by levels of *Phyllanthus emblica* extract. Hardness of formulations containing 20% of extract would increase, if the excipients were added at high levels. And, level of citric acid affect to increase hardness more than level of acesulfame. On the contrary, hardness of formulations containing 40% of extract would decrease, if the excipients were added at high level except for PE 8. PE8 had high level of extract, acesulfame and citric acid but its hardness was not decreased.

3.2.12 Friability

All formulations of pellets had low friability. Friability of pellets were between 0.00% - 0.30% as shown in table 37.

3.2.13 Amount of Total tannins

Total tannins were determined by Folin-Ciocalteau method. The percentage of total tannins depended on the quantity of *Phyllanthus emblica* extract.

From data as shown in table 37, formulation containing 20% *Phyllanthus emblica* extract had total tannins between 4.81%-5.08%. Formulation of 40% *Phyllanthus emblica* extract had total tannins between 9.66%-9.83%. And formulation containing 30% *Phyllanthus emblica* extract had total tannins between 7.29%-7.46%.

3.2.14 Amount of Gallic acid

The percentage of gallic acid was measured by high performance liquid chromatographic method. The percentage of total tannins also depended on the quantity of *Phyllanthus emblica* extract.

From data as shown in table 37, formulation containing 20% *Phyllanthus emblica* extract had gallic acid between 0.15% - 0.17%. Formulation of 40% *Phyllanthus emblica* extract had gallic acid as 0.30%. And formulation containing 30% *Phyllanthus emblica* extract had gallic acid between 0.21% - 0.22%.

Table 37 Physiochemical properties of pellets (II)

No.	Longest diameter (mm) (n=100)	Smallest diameter (mm) (n=100)	Roundness (n=100)	Hardness (g) (n=50)	Friability (%) (n=3)	Total tannins (%) (n=3)	Gallic acid (%) (n=3)
PE1	1.06 (0.16)	0.87 (0.13)	1.28 (0.28)	880.28 (198.86)	0.30 (0.39)	4.81 (0.04)	0.16 (0.00)
PE2	1.27 (0.21)	0.95 (0.12)	1.35 (0.20)	2486.55 (504.42)	0.03 (0.05)	9.80 (0.09)	0.30 (0.00)
PE3	1.09 (0.23)	0.86 (0.11)	1.32 (0.61)	955.76 (207.62)	0.05 (0.00)	5.08 (0.07)	0.15 (0.00)
PE4	1.41 (0.22)	0.99 (0.12)	1.47 (0.38)	2281.58 (483.47)	0.25 (0.20)	9.83 (0.10)	0.30 (0.00)
PE5	1.10 (0.14)	0.89 (0.10)	1.23 (0.12)	1065.76 (228.75)	0.08 (0.05)	5.08 (0.03)	0.15 (0.00)
PE6	1.42 (0.20)	1.02 (0.11)	1.51 (0.27)	1891.20 (510.25)	0.00 (0.00)	9.66 (0.12)	0.30 (0.00)
PE7	1.07 (0.15)	0.90 (0.12)	1.24 (0.13)	1177.49 (194.87)	0.11 (0.07)	5.01 (0.06)	0.17 (0.00)
PE8	1.43 (0.24)	1.04 (0.15)	1.60 (0.54)	2427.47 (711.81)	0.04 (0.04)	9.76 (0.11)	0.30 (0.01)
PE9, lot.1	1.26 (0.15)	0.98 (0.10)	1.28 (0.13)	1547.08 (352.01)	0.10 (0.05)	7.30 (0.17)	0.22 (0.01)
PE9, lot.2	1.23 (0.17)	0.94 (0.10)	1.30 (0.15)	1515.54 (264.65)	0.02 (0.03)	7.29 (0.09)	0.21 (0.00)
PE9, lot.3	1.14 (0.17)	0.90 (0.10)	1.25 (0.11)	1476.32 (268.77)	0.08 (0.03)	7.46 (0.08)	0.21 (0.00)

* PE9 was prepared from three replicates.

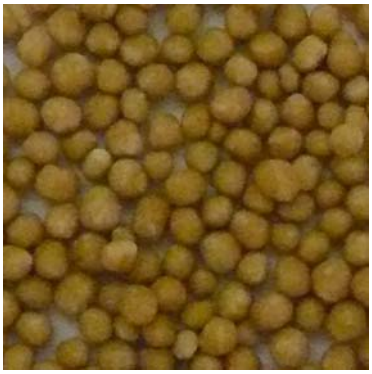
3.3 Stability of pellets containing *Phyllanthus emblica* extract

The color of all pellets at 40±2 ^{O}C 75% RH for 3 months were darkened. The color of pellets at 30±2 ^{O}C 75% RH for 3 months did not change except for formulation containing high level of *Phyllanthus emblica* extract as shown in figure 37. The color of pellets were influenced by storing temperature and the extract content because the pellets color changed at 40 ^{O}C rather than at 30 ^{O}C. At 30 ^{O}C, only the color of the pellets containing high level of *Phyllanthus emblica* extract rather changed.

At 40±2 ^{O}C 75% RH for 3 months, % LOD of PE1, PE2, and PE7 had little change and % LOD of PE3, PE4, PE5, PE6, PE8 and PE9 increased as shown in figure 38 and table 58. At 30±2 ^{O}C 75% RH for 3 months, % LOD of PE1, PE2, PE5 and PE7 had little change and % LOD of PE3, PE4, PE6, PE8 and PE9 was likely to increase as shown in figure 39 and table 59 (appendix C). This results, % LOD of pellets at 30±2 ^{O}C 75% RH and 40±2 ^{O}C 75% RH for 3 months were similar. LOD may be influenced by the quantities of acesulfame and *Phyllanthus emblica* extract because PE4 containing high level of acesulfame and extract had highest LOD.

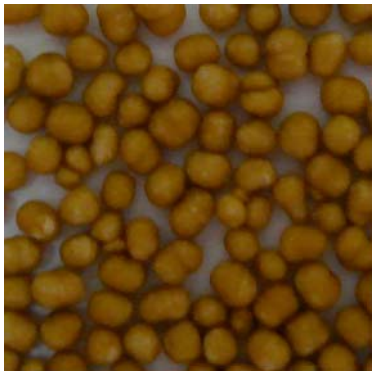
The amount of total tannins of all formulations at 30±2 ^{O}C 75% RH and 40±2 ^{O}C 75% RH for 3 months veried slightly as shown in figure 40-41 and table 60-61 (appendix C). But, the excipients may lead to decrease in tannins because the *Phyllanthus emblica* extract, which was stored at 30±2 ^{O}C 75% RH and 40±2 ^{O}C 75% RH for 3 months, had lower change in tannins content than the prepared pellets.

Gallic acid contents of all formulation increased and PE5 containing 20% of *Phyllanthus emblica* extract, 2% of acesulfame potassium and 6% of citric acid had highest change in gallic acid as shown in figure 42-43 and table 62-63 (appendix C). Gallic acid of all formulations increased at 40 ^{O}C more than at 30 ^{O}C. Gallic acid content of PE2 containing 40% of *Phyllanthus emblica* extract, 2% of acesulfame and 3% of citric acid had lowest value. In addition, the excipients may lead to increase in gallic acid because the *Phyllanthus emblica* extract, which was stored at 30±2 ^{O}C 75% RH and 40±2 ^{O}C 75% RH for 3 months, had lower change in gallic acid content than the prepared pellets.

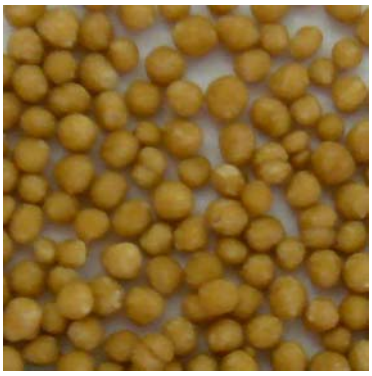


(a) PE1, room temperature for 3 months

(d) PE8, room temperature for 3 months



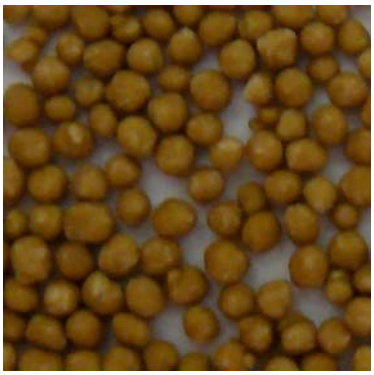
(g) PE9, room temperature for 3 months



(b) PE1, after 30±2 ^{O}C 75% RH for 3 months

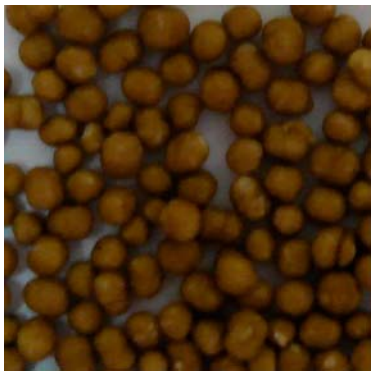
(e) PE8, after 30±2 ^{O}C 75% RH for 3 months

(h) PE9, after 30±2 ^{O}C 75% RH for 3 months



(c) PE1, after 40±2 ^{O}C 75% RH for 3 months

(f) PE8, after 40±2 ^{O}C 75% RH for 3 months



(i) PE9, after 40±2 ^{O}C 75% RH for 3 months

Figure 37 Apperance of PE1, PE8 and PE9 formulations

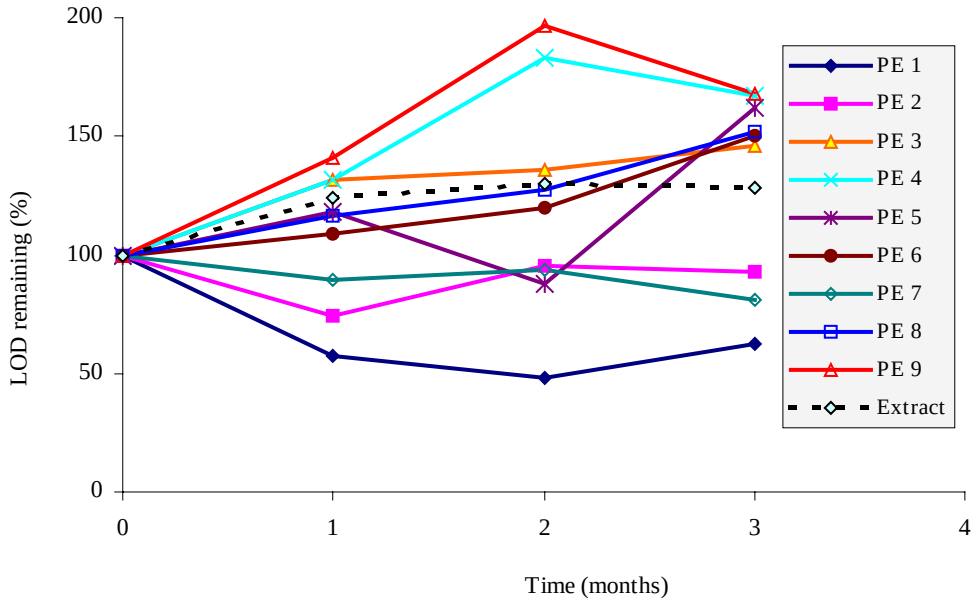


Figure 38 LOD remaining (%) of pellets and *Phyllanthus emblica* extract stored at 40±2 ^{O}C, 75% RH.

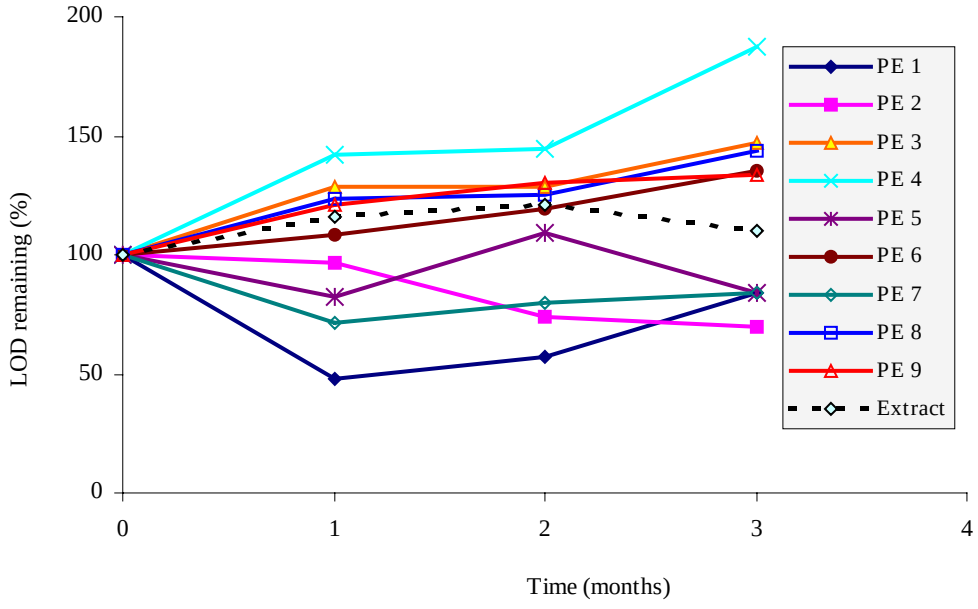


Figure 39 LOD remaining (%) of pellets and *Phyllanthus emblica* extract stored at 30±2 ^{O}C, 75% RH.

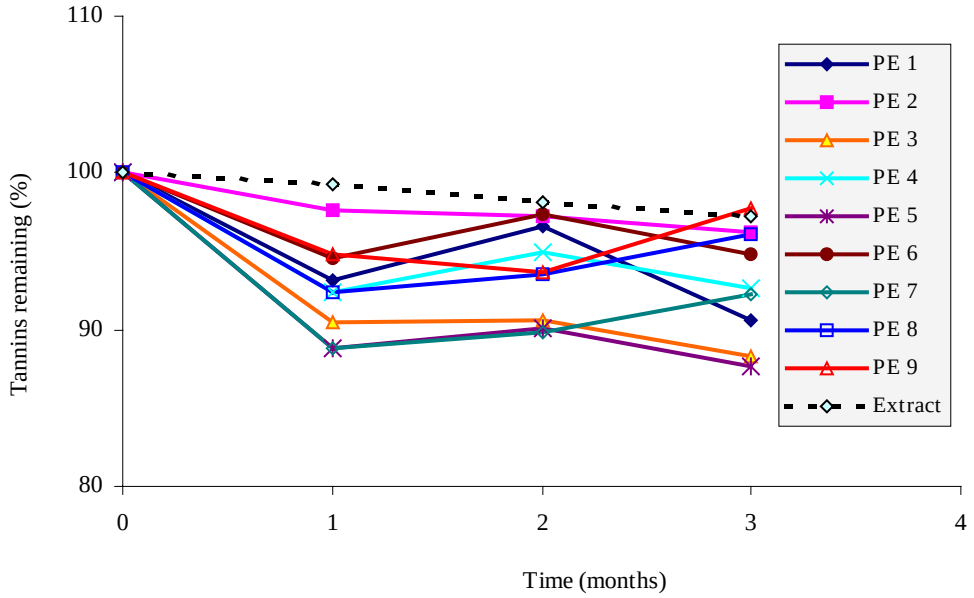


Figure 40 Total tannins remaining (%) of pellets and *Phyllanthus emblica* extract stored at 40±2 ^{O}C, 75% RH.

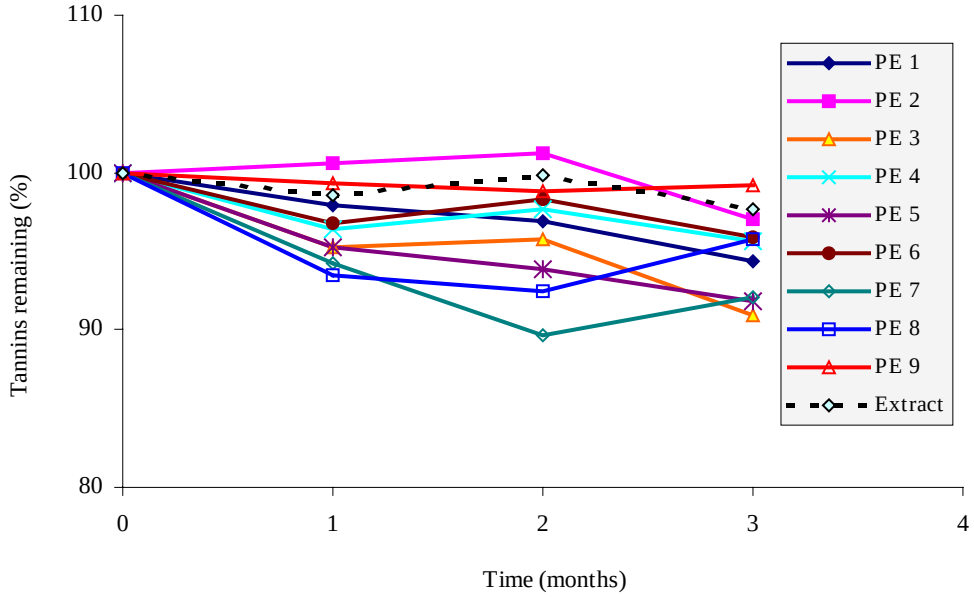


Figure 41 Total tannins remaining (%) of pellets and *Phyllanthus emblica* extract stored at 30±2 ^{O}C, 75% RH.

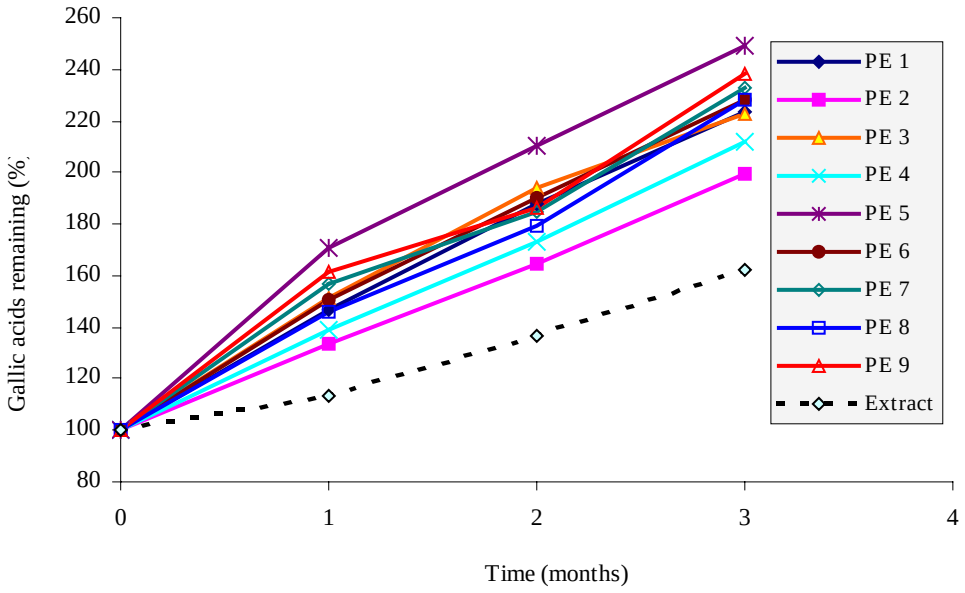


Figure 42 Gallic acid remaining (%) of pellets and *Phyllanthus emblica* extract stored at 40±2 ^{O}C, 75% RH.

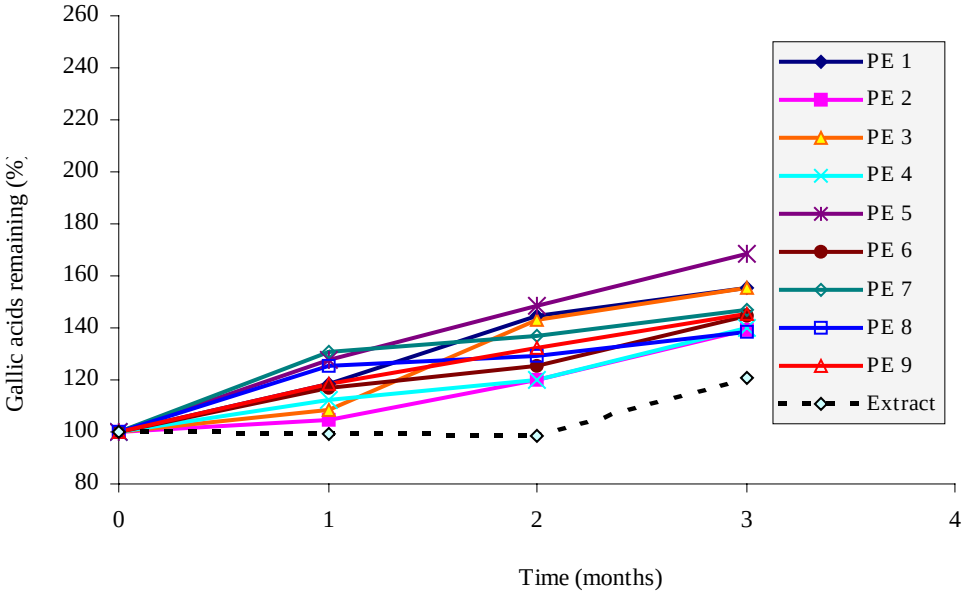


Figure 43 Gallic acid remaining (%) of pellets and *Phyllanthus emblica* extract stored at 30±2 ^{O}C, 75% RH

3.3 Statistical analysis

3.3.1 Before stability study

The effect of formulation variables on physicochemical properties of the pellets produced was statistically analyzed by using 2-way ANOVA. It was found that the formulation variables studied, i.e. quantities of *Phyllanthus emblica* extract, acesulfame potassium and citric acid anhydrous influenced on physicochemical properties of the pellets except for %compressibility as shown in table 38.

The level of *Phyllanthus emblica* extract was the important variable because it significantly affected on many properties of the pellets, i.e. loss on drying, pH, bulk density, tapped density, flow rate, angle of repose, apparent density, roundness, hardness, total tannins and gallic acid contents ($p<0.05$). The level of acesulfame potassium significantly affected on loss on drying, pH, flow rate, angle of repose, apparent density, roundness and hardness ($p<0.05$). The level of citric acid anhydrous significantly affected on pH, flow rate, angle of repose, apparent density and gallic acid content ($p<0.05$).

There was also interaction effect of the levels of the extract and acesulfame potassium on loss on drying, angle of repose and apparent density. The interaction effect of the levels of extract and citric acid significantly affected on pH, angle of repose, apparent density, roundness, hardness and total tannins content ($p<0.05$). The interaction effect of the levels of citric acid and acesulfame potassium significantly affected on loss on drying, pH, flow rate, apparent density, hardness and gallic acid content ($p<0.05$).

Thus, the level of *Phyllanthus emblica* extract was major variable affecting on physicochemical properties of the pellets. Total tannins and gallic acid were expected to be the main compounds indicating the product stability. In this study, the quantity of citric acid anhydrous affected to active ingredient of *Phyllanthus emblica* extract because it significantly affected on gallic acids content ($p<0.05$). The interaction effect of the levels of the extract and citric acid, or the levels of citric acid and acesulfame potassium should be of interest because it also significantly affected on total tannins and gallic acids ($p <0.05$).

3.3.2 After stability study for 3 months

The results were analyzed by using repeated measures ANOVA. It was found that the level of *Phyllanthus emblica* extract significantly affected on loss on drying and gallic acid content at 40±2 ^{O}C, 75% RH and 30±2 ^{O}C, 75% RH ($p<0.05$). The level of acesulfame potassium significantly affected on loss on drying, total tannins and gallic acid content at 40±2 ^{O}C, 75% RH and 30±2 ^{O}C, 75% RH ($p<0.05$). The level of citric acid anhydrous significantly affected on loss on drying at 40±2 ^{O}C, 75% RH, and total tannins and gallic acid content at 40^{O}±2 ^{O}C, 75% RH and 30±2 ^{O}C, 75% RH ($p<0.05$) as shown in table 39.

Moreover, it was shown the interaction effect of the levels of the extract and acesulfame potassium on total tannins content at 30±2 ^{O}C, 75% RH and gallic acid content at 40±2 ^{O}C, 75% RH and 30±2 ^{O}C, 75% RH. The interaction effect of the levels of the extract and citric acid significantly affected on gallic acid content at 40±2 ^{O}C, 75% RH and 30±2 ^{O}C, 75% RH ($p<0.05$). The interaction effect of the levels of citric acid and acesulfame potassium significantly affected on loss on drying, total tannins and gallic acid content at 40±2 ^{O}C, 75% RH and 30±2 ^{O}C, 75% RH ($p<0.05$).

Phyllanthus emblica extract was also stored at 40±2 ^{O}C, 75% RH and 30±2 ^{O}C, 75% RH. From table 46-47 (appendix C), it was found that there was an increase in loss on drying and gallic acid in *Phyllanthus emblica* extract. This results agreed with the effect of *Phyllanthus emblica* extract in pellets as resulted from statistical analysis; the level of *Phyllanthus emblica* extract significantly affected on loss on drying and gallic acid ($p<0.05$).

The levels of acesulfame potassium and citric acid anhydrous major influenced to stability of pellets because they significantly affected on loss on drying, total tannins and gallic acid content ($p<0.05$).

Table 38 The results of the analysis of variance of pellets before stability study

Response / Variables	Loss on drying (%)	pH	Bulk density (g/ml)	Tapped density (g/ml)	Compressibility (%)	Flow rate (g/sec)	Angle of repose	Apparent density (g/cm^3)	Round-ness	Hardness (g)	Total tannins (%)	Gallic acid (%)
Extract	**S**	**S**	**S**	**S**	NS	**S**	**S**	**S**	**S**	**S**	**S**	**S**
Acesulfame	**S**	**S**	NS	NS	NS	**S**	**S**	**S**	**S**	**S**	NS	NS
Citric	NS	**S**	NS	NS	NS	**S**	**S**	**S**	NS	NS	NS	**S**
Extract * Acesulfame	**S**	NS	NS	NS	NS	NS	**S**	**S**	NS	NS	NS	NS
Extract * Citric	NS	**S**	NS	NS	NS	NS	**S**	**S**	**S**	**S**	**S**	NS
Acesulfame * Citric	**S**	**S**	NS	NS	NS	**S**	NS	**S**	NS	**S**	NS	**S**
Adjusted R squared	0.740	0.981	0.614	0.674	-0.017	0.686	0.960	0.997	0.990	0.974	0.118	0.669

Extract = Level of *Phyllanthus emblica* extract

Acesulfame = Level of acesulfame potassium

Citric = Level of citric acid anhydrous

S = Significant difference at a significant level ($P \leq 0.05$)

NS = No significant difference at a significant level ($P \leq 0.05$)

Table 39 The results of the analysis of variance after stored at stability study for 3 months

Response / Variables	Loss on drying		Total tannins		Gallic acid	
	40±2 ^{O}C, 75% RH	30±2 ^{O}C, 75% RH	40±2 ^{O}C, 75% RH	30±2 ^{O}C, 75% RH	40±2 ^{O}C, 75% RH	30±2 ^{O}C, 75% RH
extract	**S**	**S**	NS	NS	**S**	**S**
acesulfame	**S**	**S**	**S**	**S**	**S**	**S**
citric	**S**	NS	**S**	**S**	**S**	**S**
extract * acesulfame	NS	NS	NS	**S**	**S**	**S**
extract * citric	NS	NS	NS	NS	**S**	**S**
acesulfame * citric	**S**	**S**	**S**	**S**	**S**	**S**

Extract = Level of *Phyllanthus emblica* extract

Acesulfame = Level of acesulfame potassium

Citric = Level of citric acid anhydrous

S = Significant difference at a significant level ($P \leq 0.05$)

NS = No significant difference at a significant level ($P \leq 0.05$)

CHAPTER V

CONCLUSIONS

Phyllanthus Emblica L. extracts were hygroscopic and yellowish powder. Their particles were mostly spherical and possessed smooth surface. The powder showed poor flowability due to the static charge. The extracts complied to limit of microbial contamination according to Thai pharmacopoeia 1997. Its solution had pH value of 2.97 (lot 171106E) and 3.02 (lot 081206G). Total tannins content was 26.92% (lot 171106E) and 24.54% (lot 081206G). Gallic acid content was 0.69% (lot 171106E) and 1.24% (lot 081206G).

Effervescent granules containing *Phyllanthus emblica* fruit extract were yellowish. They showed good flowability and could dissolve or disperse in water within 5 minutes. pH of the solution had an effect on the solution color after dissolving or dispersing granules in the water.

After storage at $40\pm2\ ^{O}C$, 75% relative humidity or $30\pm2\ ^{O}C$, 75% relative humidity for 3 months, the appearance of the effervescent granules changed, in many cases, caking and formation of large agglomerates occurred. Change in appearance could not be related to change in the contents of total tannins and gallic acid, which were the major compounds determined in the present study. In addition, the appearance of granules could not be directly related to formulation variables, moisture content in terms of loss on drying and pH. However, there were significant main and interaction effects of formulation variables, i.e. the levels of *Phyllanthus emblica* fruit extract, colloidal silicon dioxide, citric acid and types of alkaline, on many properties of effervescent granules ($p < 0.05$). Types of alkaline and the quantity of citric acid were not found to stabilize the products. The total tannins and gallic acid contents may be related to moisture content in the formulations.

For pelletized product, the quantities of *Phyllanthus emblica* fruit extract and the excipients, the solubility of compositions and the quantity of binder liquid affected on

characteristics of pellets. Pellets containing 20% of the extract in formulation were round, while those containing 40% of the extract in formulation remained dumbbell-shape. The damp mass containing the low level of the extract required higher quantity of binder liquid and was less sticky than that of the high level of the extract due to microcrystalline cellulose content in the formulation.

After storage at 40$\pm$2 ^{O}C, 75% relative humidity or 30$\pm$2 ^{O}C, 75% relative humidity for 3 months, change in color of pellets was observed depending on storage temperature and the extract content. The color of the pellets containing the high level of the extract stored at the high temperature was darker. The moisture content of stored pellets may be influened by formulation variables, i.e. the levels of *Phyllanthus emblica* extract, acesulfame potassium and citric acid, as well as the quantity of binder liquid employed in the formulation. There were significant main effects of the levels of acesulfame potassium and citric acid, also their interaction effects on total tannins content ($p<0.05$). The main and interaction effects of the levels of *Phyllanthus emblica* extract, acesulfame potassium and citric acid significantly influenced on gallic acid content ($p <0.05$).

The results indicate that there is possibility of developing effervescent granules and pellets containing *Phyllanthus emblica* fruit extract; and the properties of the extract may play a significant role in successful development. The impact of the extract properties and the effect of formulation variables on the physical and chemical stability of the products still need to be further investigated.

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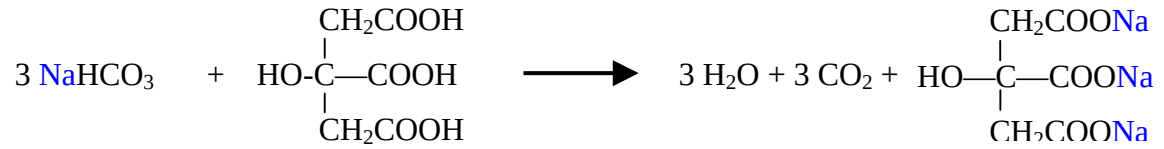
Appendices

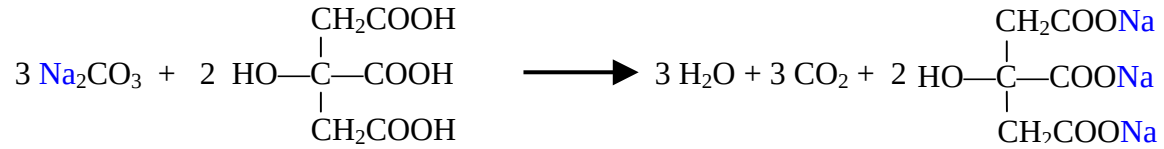
Appendix A

Calculation of acid-base reaction

Acid-base reactions between citric acid and sodium bicarbonate or sodium carbonate will start after added into water. One mole of citric acid anhydrous are completely neutralized with three mole of sodium bicarbonate, while two moles of citric acid anhydrous are completely neutralized with three moles of sodium carbonate. The reaction equation is shown as follows :



Sodium bicarbonate (MW = 84.01) Citric acid anhydrous (MW = 192.12)



Sodium carbonate (MW = 106) Citric acid anhydrous (MW = 192.12)

To neutralize 100 g alkalines with citric acid anhydrous, the amounts of acid required can be calculated as the followings:

1. Sodium bicarbonate 100 g

From the reaction equation, 3 moles of sodium bicarbonate (MW = 84.01 g) reacts with 1 mole of citric acid anhydrous (MW = 192.12 g)

Thus, 100 g sodium bicarbonate can be neutralized with citric acid anhydrous

$$= \frac{100 \times 192.12}{(3 \times 84.01)} = 76.229 \text{ grams}$$

2. Sodium bicarbonate 86 g and sodium carbonate 14 g

Sodium bicarbonate 86 g together with sodium carbonate 14 g, which is equivalent to sodium bicarbonate and sodium carbonate contents in Effer-soda® (Certificate of analysis for Effer-soda®, SPI Pharma) indicating that Effer-soda® is sodium bicarbonate which is partly, converted to sodium carbonate. For Effer-soda™ 12 lot no. 05H066 for used to preparation of effervescent granules, it has sodium carbonate approximate 14%.

From the reaction equation, 3 moles of sodium bicarbonate (MW = 84.01 g) reacts with 1 mole of citric acid anhydrous (MW = 192.12 g) and 3 moles of sodium carbonate (MW = 106 g) reacts with 2 mole of citric acid anhydrous.

Thus, 86 g sodium bicarbonate can be neutralized with citric acid anhydrous

$$= \frac{86 \times 192.12}{(3 \times 84.01)} = 65.557 \text{ g}$$

and 14 g sodium carbonate can be neutralized with citric acid anhydrous

$$= \frac{14 \times (2 \times 192.12)}{(3 \times 106)} = 16.916 \text{ g}$$

Therefore, 86 g sodium bicarbonate together with 14 g sodium carbonate, or Effer-soda® can be neutralized with citric acid anhydrous

$$= (65.557 + 16.916) = 82.473 \text{ g}$$

To neutralize a constant level of citric acid anhydrous of 15% in the formulation, the calculation is as the followings:

As above calculation, 100 g sodium bicarbonate can be neutralized with citric acid anhydrous 76.229 g and 86 g sodium bicarbonate together with 14 g sodium carbonate, or Effer-soda® can be neutralized with citric acid anhydrous 82.473 g

Thus, 15% or 15 g citric acid anhydrouscan be neutralized with sodium bicarbonate $= \frac{15 \times 100}{76.229} = 19.7\%$

or neutralized with Effer-soda®

$= \frac{15 \times 100}{82.473} = 18.2\%$

100 g Effer-soda® is equivalent to sodium bicarbonate 86 g together with sodium carbonate 14 g

Thus, 18.2% Effer-soda will equivalent with sodium bicarbonate

$= 18.2\% \times 0.86 = 15.7\%$

mixing with sodium carbonate

$= 18.2\% \times 0.14 = 2.5\%$

So, the amounts of acid and alkalines in the formulation were designed as follow:

- Citric acid anhydrous 15% react with sodium bicarbonate 19.7%
- Citric acid anhydrous 15% react with Effer-soda 18.2%
- Citric acid anhydrous 15% react with sodium bicarbonate 15.7% and sodium carbonate 2.5%

Appendix B

Table 40 Calibration data of gallic acid at 270 nm

Concentration (μg/ml)	Peak area at 270 nm
0.25	14351.33
1.00	59818.33
2.99	175433.67
4.98	293346.00
7.47	441660.67

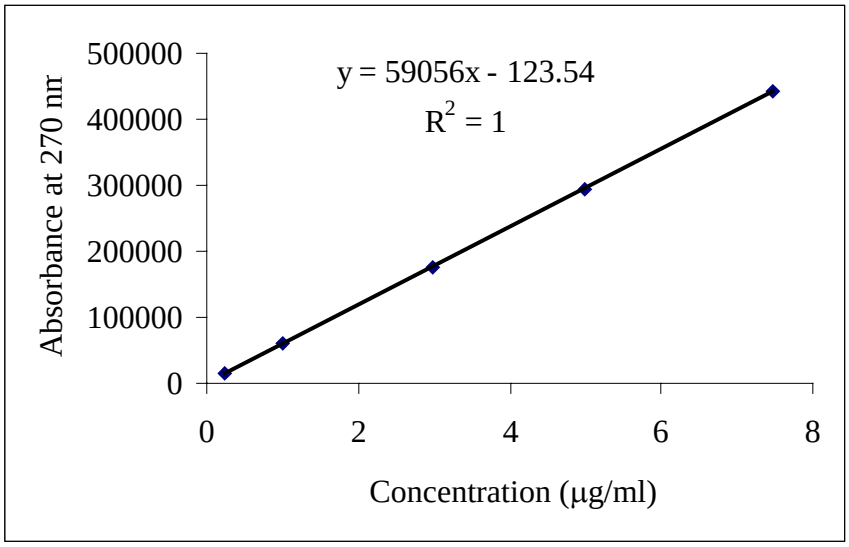


Figure 44 Calibration curve of gallic acid at 270 nm

Table 41 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	%RSD
0.4980	0.5018	0.5020	0.5072	100.77	100.80	101.85	101.14	0.61
0.9960	1.0206	1.0232	1.0247	102.47	102.73	102.88	102.69	0.20
2.4900	2.4829	2.4810	2.4905	99.72	99.64	100.02	99.79	0.20
3.9840	4.0735	3.9977	4.0280	102.25	100.34	101.10	101.23	0.95
4.9800	5.0364	5.0246	5.0214	101.13	100.89	100.83	100.95	0.16

Table 42 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	%RSD
0.4960	0.5062	0.4986	0.5097	102.06	100.52	102.76	101.78	1.13
0.9920	1.0123	1.0197	1.0094	102.05	102.79	101.75	102.20	0.52
2.4800	2.5469	2.5568	2.5140	102.69	103.10	101.37	102.39	0.88
3.9681	4.0474	4.0032	4.0932	102.00	100.89	103.15	102.01	1.11
4.9601	5.0651	5.0924	5.0690	102.12	102.67	102.20	102.33	0.29

Table 43 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	%RSD
0.4960	0.5033	0.5015	0.4935	101.48	101.12	99.50	100.70	1.04
0.9920	1.0047	1.0072	1.0042	101.28	101.53	101.23	101.35	0.16
2.4800	2.5209	2.5058	2.5324	101.65	101.04	102.11	101.60	0.53
3.9681	4.0611	4.1445	4.0574	102.34	104.45	102.25	103.01	1.21
4.9601	5.0774	4.9890	5.0000	102.37	100.58	100.81	101.25	0.96

Table 44 Precision of HPLC method

Number	Peak area at 270 nm		
	1st day	2nd day	3rd day
1	63816	62482	62833
2	63969	62928	62987
3	62708	62306	63424
4	63787	63265	63172
5	63763	62836	63072
6	63790	64012	63756
Mean	63639	62972	63207
%RSD	0.73	0.97	0.53

Table 45 System suitability of HPLC method

	Mean	%RSD
Peak area	60131	1.16
Tailing factor	1.25	1.10

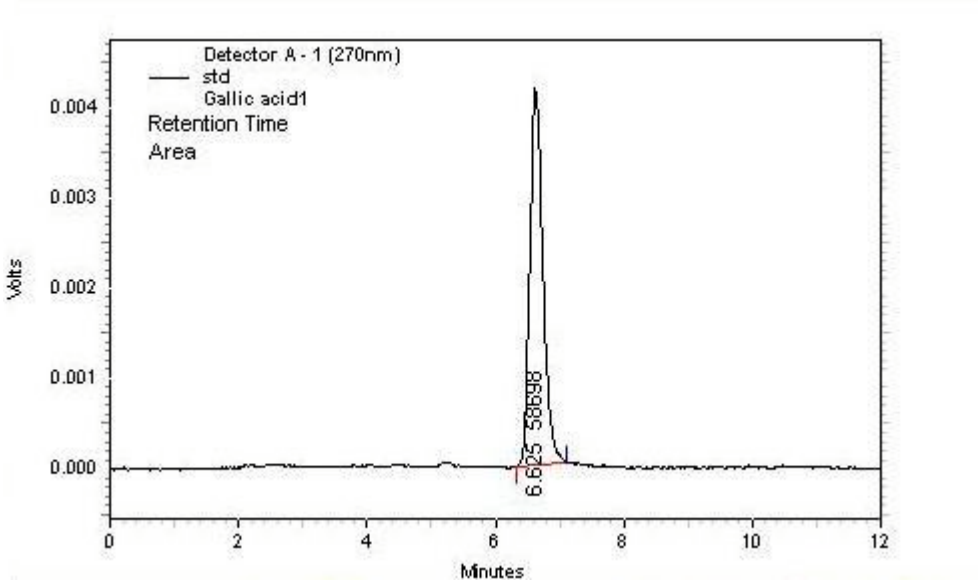


Figure 45 Chromatogram of gallic acid solution

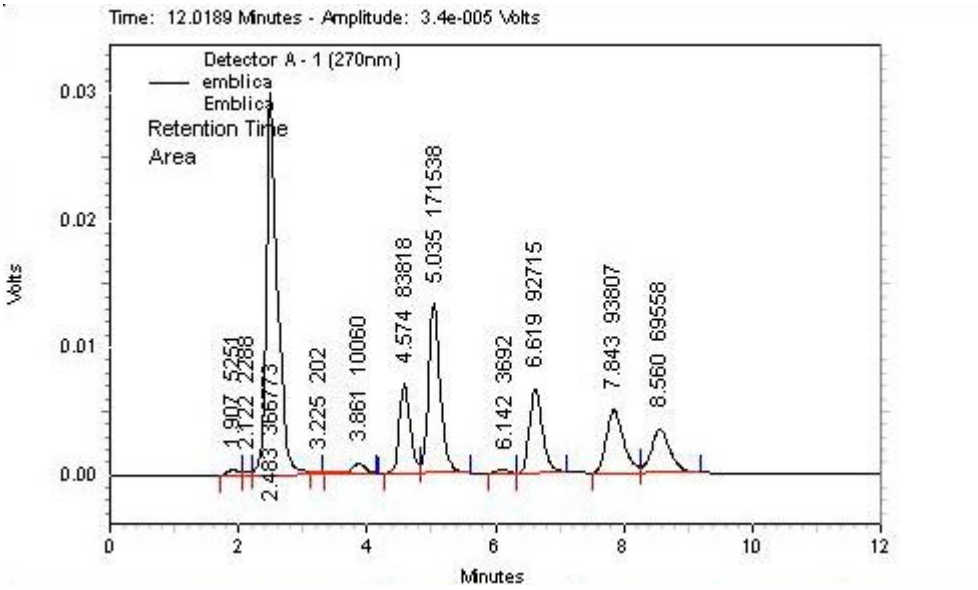


Figure 46 Chromatogram of *Phyllanthus Emblica* extract solution

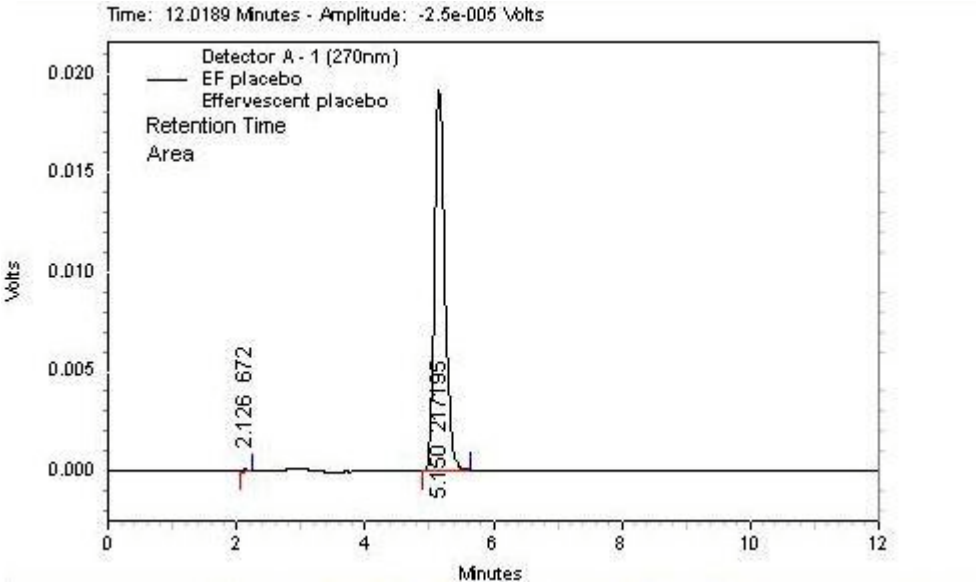


Figure 47 Chromatogram of EF3 composition

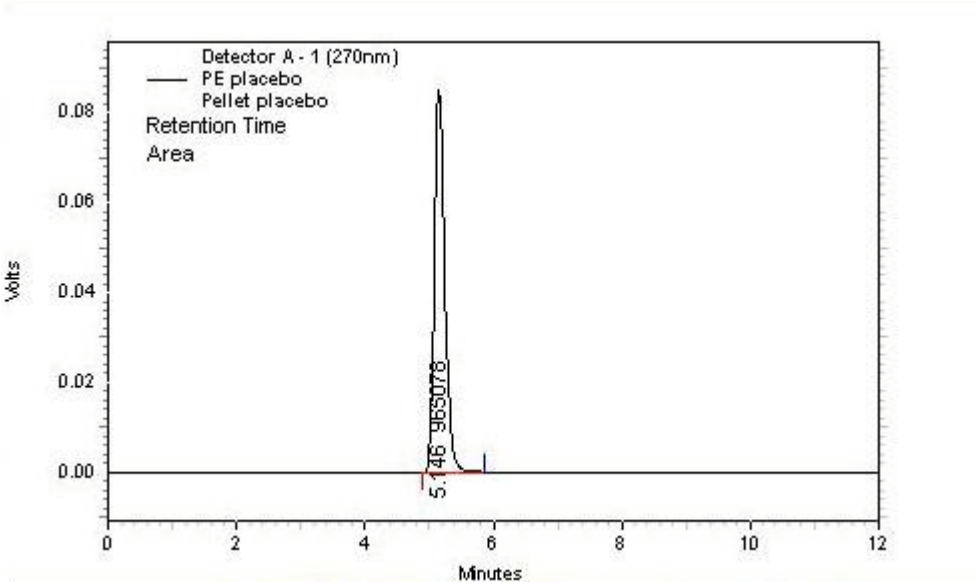


Figure 48 Chromatogram of PE7 composition

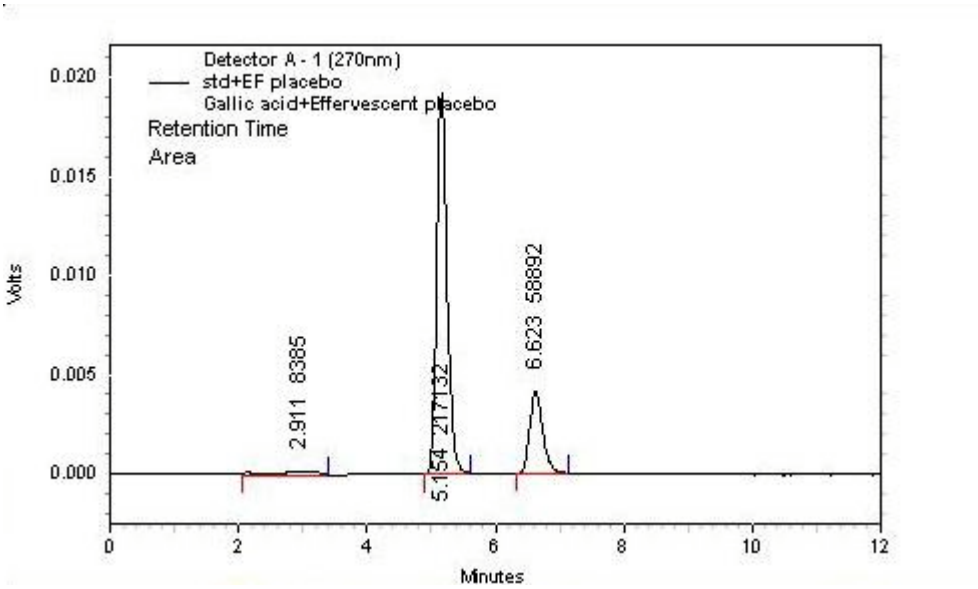


Figure 49 Chromatogram of solution of gallic acid and EF3 composition

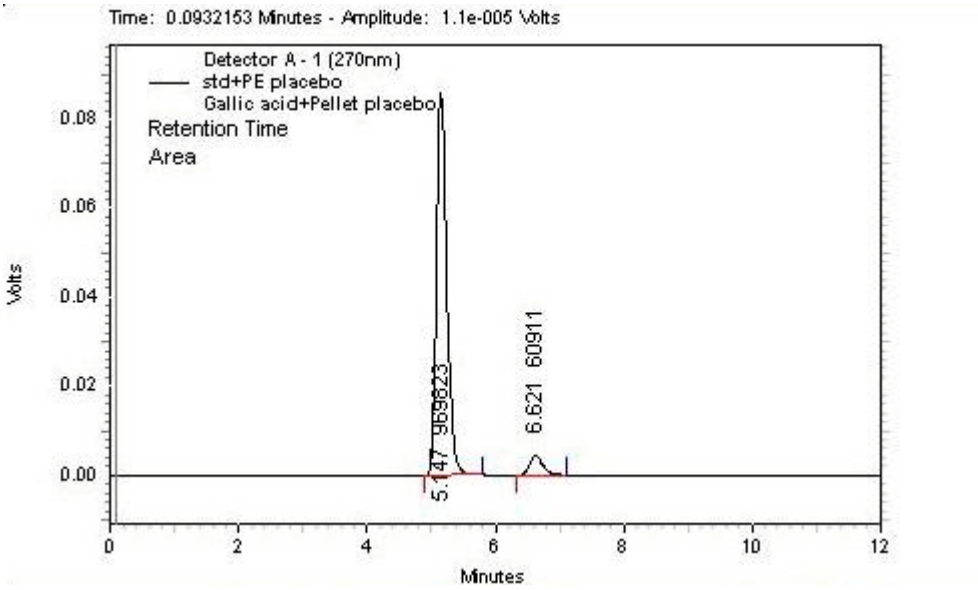


Figure 50 Chromatogram of solution of gallic acid and PE7 composition

Appendix C

Table 46 Stability data of *Phyllanthus Emblica* extract stored at 40±2 ^{O}C, 75% RH.

Lot no.	171106 E			081206G		
	1 month	2 months	3 months	1 month	2 months	3 months
%Loss on drying	3.66 (0.12)	3.84 (0.03)	3.79 (0.06)	3.14 (0.15)	3.31 (0.11)	3.69 (0.16)
pH	3.12 (0.01)	3.13 (0.01)	3.13 (0.01)	3.05 (0.01)	3.20 (0.01)	2.98 (0.01)
%Total tannins	26.73 (0.29)	26.40 (0.39)	26.17 (0.30)	23.64 (0.11)	23.29 (0.42)	23.58 (0.14)
%Gallic acid	0.78 (0.01)	0.94 (0.01)	1.12 (0.01)	1.39 (0.02)	1.56 (0.01)	1.65 (0.02)

Table 47 Stability data of *Phyllanthus Emblica* extract stored at 30±2 ^{O}C, 75% RH.

Lot no.	171106 E			081206G		
	1 month	2 months	3 months	1 month	2 months	3 months
Loss on drying (%)	3.42 (0.05)	3.58 (0.02)	3.23 (0.10)	3.04 (0.01)	3.46 (0.40)	3.39 (0.42)
pH	3.10 (0.01)	3.11 (0.01)	3.10 (0.01)	3.04 (0.01)	2.99 (0.03)	2.98 (0.03)
Total tannins (%)	26.54 (0.43)	26.88 (0.41)	26.29 (0.18)	23.56 (0.27)	23.18 (0.35)	23.42 (0.07)
Gallic acid (%)	0.68 (0.00)	0.68 (0.00)	0.83 (0.01)	1.26 (0.01)	1.25 (0.01)	1.47 (0.03)

Table 48 Appearance of effervescent granules stored at 40±2 ^{O}C, 75% RH.

No.	1 month	2 months	3 months	No.	1 month	2 months	3 months
EF1	+++	++++	++++	EF17	++	+++	+++
EF2	0	+	++	EF18	0	+	+++
EF3	+	+++	+++	EF19	0	+	+
EF4	0	+	+	EF20	+++	+++	+++
EF5	++	+++	+++	EF21	++	+++	++++
EF6	++	++	++	EF22	0	+	+
EF7	+	+++	+++	EF23	+	+	++
EF8	+++	+++	+++	EF24	0	++	++
EF9	0	++	+++	EF25	+	+++	+++
EF10	0	0	++	EF26	0	+	+
EF11	+	++	+++	EF27	+++	+++	+++
EF12	+	+++	++++	EF28	+	++	++
EF13	+++	+++	+++	EF29	+	+++	+++
EF14	0	0	++	EF30	0	+	+
EF15	0	0	+	EF31	+	++	++
EF16	0	0	+	EF32	++	+++	+++

+ + + + ⟶ + ; High degree of agglomeration ⟶ Low degree of agglomeration

0 ; No change of product was observed

Table 49 Appearance of effervescent granules stored at 30±2 ^{O}C, 75% RH.

No.	1 month	2 months	3 months	No.	1 month	2 months	3 months
EF1	+++	+++	+++	EF17	++	+++	+++
EF2	0	0	+	EF18	0	+	+
EF3	0	++	+++	EF19	0	0	+
EF4	0	0	0	EF20	++	+	+
EF5	+	+	+	EF21	++	+++	+++
EF6	+	+	+	EF22	0	0	0
EF7	+	++	+++	EF23	0	+	+
EF8	+	++	++	EF24	0	+	+
EF9	0	+	+	EF25	0	++	+++
EF10	0	0	0	EF26	0	0	0
EF11	0	+	+	EF27	++	++	+
EF12	0	+	++	EF28	0	+	+
EF13	++	++	++	EF29	+	++	+++
EF14	0	0	+	EF30	0	0	+
EF15	0	0	0	EF31	+	+	++
EF16	0	0	0	EF32	++	++	+

+ + + + ⟶ + ; High degree of agglomeration ⟶ Low degree of agglomeration

0 ; No change of product was observed

Table 50 %Loss on drying of effervescent granules stored at 40±2 ^{O}C, 75% RH.

No.	Mean (SD)			No.	Mean (SD)		
	1 month	2 months	3 months		1 month	2 months	3 months
EF1	1.55 (0.22)	1.65 (0.26)	2.12 (0.51)	EF17	1.75 (0.07)	1.88 (0.07)	2.31 (0.16)
EF2	0.54 (0.06)	0.64 (0.05)	0.73 (0.11)	EF18	0.56 (0.03)	0.72 (0.07)	0.70 (0.09)
EF3	1.56 (0.05)	1.67 (0.12)	1.69 (0.08)	EF19	1.74 (0.06)	1.81 (0.11)	1.96 (0.13)
EF4	1.65 (0.08)	1.62 (0.04)	1.58 (0.06)	EF20	1.50 (0.02)	1.74 (0.08)	1.87 (0.18)
EF5	1.84 (0.03)	1.94 (0.10)	1.80 (0.15)	EF21	1.78 (0.07)	2.00 (0.04)	2.65 (0.03)
EF6	1.26 (0.11)	1.30 (0.16)	1.31 (0.14)	EF22	0.79 (0.03)	0.91 (0.14)	0.90 (0.03)
EF7	1.63 (0.05)	1.74 (0.17)	1.78 (0.17)	EF23	0.84 (0.07)	0.83 (0.04)	0.98 (0.03)
EF8	1.21 (0.08)	1.39 (0.08)	1.35 (0.09)	EF24	0.73 (0.05)	0.74 (0.01)	0.72 (0.10)
EF9	1.43 (0.12)	1.42 (0.06)	1.53 (0.13)	EF25	0.76 (0.06)	0.93 (0.09)	0.84 (0.08)
EF10	0.80 (0.03)	0.88 (0.09)	1.06 (0.08)	EF26	0.68 (0.03)	0.90 (0.10)	0.84 (0.06)
EF11	1.13 (0.13)	1.31 (0.07)	1.46 (0.20)	EF27	0.84 (0.03)	0.90 (0.09)	0.91 (0.05)
EF12	1.50 (0.08)	1.58 (0.04)	1.88 (0.11)	EF28	1.32 (0.08)	1.36 (0.09)	1.46 (0.07)
EF13	1.21 (0.04)	1.55 (0.05)	1.69 (0.07)	EF29	1.53 (0.05)	1.60 (0.07)	1.67 (0.10)
EF14	0.59 (0.05)	0.68 (0.03)	0.69 (0.04)	EF30	0.74 (0.05)	0.78 (0.02)	0.87 (0.01)
EF15	1.71 (0.02)	1.63 (0.08)	1.70 (0.04)	EF31	1.03 (0.07)	1.10 (0.12)	1.08 (0.07)
EF16	1.24 (0.05)	1.30 (0.06)	1.39 (0.06)	EF32	1.09 (0.12)	1.23 (0.19)	1.26 (0.03)

Table 51 %Loss on drying of effervescent granules stored at 30±2 ^{O}C, 75% RH.

No.	Mean (SD)			No.	Mean (SD)		
	1 month	2 months	3 months		1 month	2 months	3 months
EF1	1.07 (0.03)	1.45 (0.09)	1.63 (0.06)	EF17	1.56 (0.06)	1.78 (0.07)	1.81 (0.08)
EF2	0.59 (0.06)	0.73 (0.06)	0.67 (0.09)	EF18	0.57 (0.05)	0.67 (0.06)	0.68 (0.03)
EF3	1.44 (0.03)	1.47 (0.13)	1.51 (0.08)	EF19	1.94 (0.15)	1.75 (0.08)	1.81 (0.10)
EF4	1.65 (0.07)	1.57 (0.03)	1.66 (0.07)	EF20	1.43 (0.03)	1.44 (0.18)	1.54 (0.07)
EF5	1.77 (0.09)	1.97 (0.07)	1.74 (0.11)	EF21	1.58 (0.05)	1.85 (0.06)	1.91 (0.04)
EF6	1.15 (0.21)	1.22 (0.12)	1.22 (0.09)	EF22	0.84 (0.05)	0.87 (0.02)	0.88 (0.08)
EF7	1.64 (0.04)	1.49 (0.09)	1.56 (0.13)	EF23	0.78 (0.19)	0.78 (0.00)	0.89 (0.05)
EF8	1.14 (0.08)	1.18 (0.01)	1.18 (0.03)	EF24	0.81 (0.02)	0.86 (0.13)	0.78 (0.02)
EF9	1.37 (0.07)	1.44 (0.08)	1.48 (0.05)	EF25	0.75 (0.04)	0.86 (0.16)	0.82 (0.02)
EF10	0.80 (0.05)	0.86 (0.05)	0.90 (0.09)	EF26	0.70 (0.04)	0.87 (0.10)	0.79 (0.04)
EF11	1.08 (0.13)	1.11 (0.02)	1.32 (0.03)	EF27	0.86 (0.08)	0.88 (0.02)	0.87 (0.05)
EF12	1.57 (0.08)	1.60 (0.10)	1.58 (0.06)	EF28	1.34 (0.08)	1.36 (0.09)	1.52 (0.06)
EF13	1.14 (0.02)	1.23 (0.04)	1.23 (0.04)	EF29	1.49 (0.12)	1.59 (0.08)	1.60 (0.10)
EF14	0.62 (0.02)	0.63 (0.03)	0.70 (0.06)	EF30	0.72 (0.05)	0.74 (0.05)	0.74 (0.04)
EF15	1.48 (0.07)	1.62 (0.03)	1.55 (0.21)	EF31	0.95 (0.01)	1.02 (0.02)	1.07 (0.04)
EF16	1.32 (0.14)	1.63 (0.33)	1.46 (0.05)	EF32	1.04 (0.08)	1.12 (0.05)	1.01 (0.08)

Table 52 pH of dispersion of effervescent granules stored at 40±2 ^{O}C, 75% RH.

No.	Mean (SD)			No.	Mean (SD)		
	1 month	2 months	3 months		1 month	2 months	3 months
EF1	4.60 (0.27)	4.53 (0.05)	4.82 (0.17)	EF17	5.00 (0.34)	4.96 (0.12)	5.09 (0.38)
EF2	4.61 (0.06)	4.72 (0.12)	4.64 (0.10)	EF18	4.19 (0.08)	4.39 (0.13)	4.36 (0.14)
EF3	4.48 (0.38)	4.37 (0.48)	4.59 (0.20)	EF19	4.80 (0.03)	4.79 (0.41)	4.93 (0.32)
EF4	4.35 (0.17)	4.37 (0.25)	4.47 (0.34)	EF20	4.29 (0.22)	4.26 (0.14)	4.07 (0.05)
EF5	3.92 (0.18)	4.11 (0.14)	4.23 (0.35)	EF21	4.33 (0.20)	4.22 (0.18)	4.32 (0.05)
EF6	4.73 (0.09)	4.63 (0.23)	4.53 (0.31)	EF22	4.47 (0.04)	4.53 (0.14)	4.51 (0.12)
EF7	4.53 (0.44)	4.53 (0.34)	4.58 (0.08)	EF23	5.80 (0.27)	5.84 (0.46)	5.83 (0.19)
EF8	4.43 (0.29)	4.50 (0.32)	4.57 (0.18)	EF24	5.13 (0.37)	5.48 (0.03)	4.95 (0.17)
EF9	4.69 (0.45)	4.64 (0.60)	4.58 (0.09)	EF25	4.59 (0.45)	4.53 (0.28)	4.46 (0.37)
EF10	5.44 (0.20)	5.49 (0.30)	5.60 (0.26)	EF26	4.37 (0.20)	4.32 (0.11)	4.28 (0.26)
EF11	5.14 (0.21)	5.12 (0.10)	4.99 (0.47)	EF27	4.15 (0.15)	4.15 (0.06)	4.06 (0.18)
EF12	4.28 (0.23)	4.17 (0.12)	4.22 (0.21)	EF28	5.45 (0.27)	5.69 (0.23)	5.72 (0.07)
EF13	3.90 (0.12)	4.06 (0.16)	4.05 (0.05)	EF29	4.96 (0.75)	5.12 (0.35)	5.05 (0.41)
EF14	5.07 (0.38)	5.20 (0.14)	5.30 (0.28)	EF30	4.31 (0.06)	4.58 (0.25)	4.34 (0.16)
EF15	4.10 (0.35)	4.12 (0.10)	4.22 (0.39)	EF31	4.33 (0.18)	4.22 (0.10)	4.09 (0.08)
EF16	4.91 (0.22)	4.82 (0.17)	4.59 (0.23)	EF32	4.05 (0.08)	3.91 (0.19)	4.01 (0.03)

Table 53 pH of dispersion of effervescent granules stored at 30±2 ^{O}C, 75% RH.

No.	Mean (SD)			No.	Mean (SD)		
	1 month	2 months	3 months		1 month	2 months	3 months
EF1	4.97 (0.36)	4.88 (0.25)	4.83 (0.22)	EF17	5.61 (0.62)	5.14 (0.63)	5.03 (0.23)
EF2	4.65 (0.13)	4.83 (0.19)	4.60 (0.27)	EF18	4.37 (0.08)	4.43 (0.12)	4.31 (0.10)
EF3	4.65 (0.10)	4.59 (0.58)	4.65 (0.26)	EF19	4.70 (0.06)	4.84 (0.34)	4.77 (0.10)
EF4	4.41 (0.29)	4.47 (0.09)	4.42 (0.28)	EF20	4.24 (0.26)	4.13 (0.04)	4.25 (0.26)
EF5	4.30 (0.13)	3.93 (0.17)	4.29 (0.34)	EF21	4.49 (0.05)	4.15 (0.18)	4.44 (0.23)
EF6	4.76 (0.29)	4.24 (0.24)	4.64 (0.09)	EF22	4.47 (0.04)	4.47 (0.02)	4.48 (0.02)
EF7	4.41 (0.46)	4.70 (0.20)	4.75 (0.40)	EF23	5.72 (0.18)	5.81 (0.31)	5.55 (0.12)
EF8	4.54 (0.22)	4.55 (0.49)	4.49 (0.16)	EF24	5.00 (0.47)	5.12 (0.26)	4.85 (0.07)
EF9	4.65 (0.09)	4.74 (0.11)	4.49 (0.18)	EF25	4.73 (0.34)	4.69 (0.61)	4.57 (0.30)
EF10	5.36 (0.24)	5.54 (0.29)	5.44 (0.18)	EF26	4.37 (0.76)	4.28 (0.29)	4.34 (0.33)
EF11	5.00 (0.12)	5.08 (0.25)	4.88 (0.36)	EF27	4.28 (0.19)	3.97 (0.28)	4.09 (0.25)
EF12	4.21 (0.20)	4.19 (0.37)	4.13 (0.30)	EF28	5.52 (0.28)	5.60 (0.57)	5.40 (0.54)
EF13	4.12 (0.24)	4.10 (0.12)	4.08 (0.21)	EF29	4.94 (0.18)	5.14 (0.62)	4.99 (0.42)
EF14	5.04 (0.19)	5.15 (0.18)	5.32 (0.45)	EF30	4.30 (0.20)	4.49 (0.11)	4.46 (0.08)
EF15	4.07 (0.26)	4.11 (0.07)	4.09 (0.03)	EF31	4.23 (0.22)	4.12 (0.05)	4.04 (0.19)
EF16	4.77 (0.09)	4.74 (0.20)	4.74 (0.08)	EF32	4.06 (0.13)	3.98 (0.18)	4.03 (0.07)

Table 54 Total tannins in 100 g effervescent granules stored at 40±2 ^{O}C, 75% RH.

No.	Mean (SD)			No.	Mean (SD)		
	1 month	2 months	3 months		1 month	2 months	3 months
EF1	1.13 (0.03)	1.11 (0.01)	1.16 (0.03)	EF17	2.35 (0.10)	2.28 (0.05)	2.35 (0.02)
EF2	1.99 (0.04)	2.22 (0.10)	2.38 (0.12)	EF18	2.21 (0.18)	2.33 (0.19)	2.42 (0.16)
EF3	4.70 (0.20)	4.74 (0.28)	4.98 (0.28)	EF19	6.92 (0.49)	6.99 (0.33)	7.69 (0.57)
EF4	6.40 (0.29)	7.49 (0.36)	7.12 (0.23)	EF20	6.62 (0.18)	7.30 (0.37)	6.96 (0.38)
EF5	9.54 (0.80)	9.25 (0.48)	10.00 (0.66)	EF21	2.24 (0.10)	2.24 (0.03)	2.28 (0.03)
EF6	4.80 (0.10)	4.68 (0.37)	4.64 (0.40)	EF22	4.28 (0.15)	4.54 (0.14)	4.82 (0.16)
EF7	4.37 (0.21)	4.92 (0.32)	5.02 (0.26)	EF23	4.84 (0.10)	5.22 (0.34)	5.13 (0.68)
EF8	5.05 (0.46)	3.99 (0.24)	4.60 (0.37)	EF24	4.08 (0.12)	4.63 (0.13)	5.10 (0.12)
EF9	4.24 (0.08)	4.61 (0.09)	4.89 (0.18)	EF25	4.64 (0.09)	5.14 (0.15)	4.63 (0.07)
EF10	4.57 (0.22)	4.45 (0.11)	4.91 (0.33)	EF26	4.27 (0.24)	4.79 (0.37)	4.65 (0.27)
EF11	4.91 (0.16)	4.97 (0.15)	4.85 (0.05)	EF27	4.85 (0.34)	4.76 (0.07)	5.09 (0.32)
EF12	4.51 (0.23)	4.92 (0.30)	5.02 (0.16)	EF28	4.79 (0.10)	4.98 (0.23)	4.81 (0.21)
EF13	5.03 (0.44)	4.64 (0.36)	4.68 (0.11)	EF29	4.53 (0.08)	5.18 (0.11)	4.75 (0.09)
EF14	2.30 (0.07)	2.38 (0.13)	2.49 (0.20)	EF30	4.34 (0.14)	4.14 (0.13)	4.92 (0.17)
EF15	6.61 (0.29)	6.86 (0.16)	6.58 (0.26)	EF31	4.79 (0.02)	4.70 (0.17)	4.82 (0.16)
EF16	7.42 (0.21)	7.44 (0.19)	7.41 (0.28)	EF32	4.89 (0.50)	4.25 (0.15)	4.17 (0.12)

Table 55 Total tannins in 100 g effervescent granules stored at 30±2 ^{O}C, 75% RH.

No.	Mean (SD)			No.	Mean (SD)		
	1 month	2 months	3 months		1 month	2 months	3 months
EF1	1.17 (0.08)	1.17 (0.03)	1.14 (0.02)	EF17	2.25 (0.13)	2.40 (0.04)	2.29 (0.05)
EF2	2.10 (0.11)	2.07 (0.09)	2.24 (0.07)	EF18	2.08 (0.04)	2.40 (0.20)	2.32 (0.13)
EF3	4.60 (0.17)	5.22 (0.22)	4.65 (0.15)	EF19	6.46 (0.12)	7.22 (0.09)	7.36 (0.16)
EF4	6.47 (0.34)	6.73 (0.59)	6.45 (0.40)	EF20	7.75 (0.52)	7.40 (0.28)	8.00 (0.61)
EF5	8.97 (0.59)	9.43 (0.55)	8.87 (0.26)	EF21	2.42 (0.08)	2.40 (0.06)	2.19 (0.09)
EF6	4.57 (0.23)	4.79 (0.05)	4.80 (0.10)	EF22	4.12 (0.04)	4.34 (0.05)	4.39 (0.07)
EF7	4.52 (0.19)	4.84 (0.31)	4.77 (0.40)	EF23	4.44 (0.15)	4.98 (0.22)	5.17 (0.20)
EF8	5.11 (0.11)	4.89 (0.19)	5.00 (0.12)	EF24	4.19 (0.25)	4.33 (0.19)	4.85 (0.30)
EF9	4.13 (0.04)	4.56 (0.12)	4.40 (0.20)	EF25	4.45 (0.21)	4.90 (0.29)	4.64 (0.36)
EF10	4.45 (0.17)	4.54 (0.21)	4.74 (0.24)	EF26	4.31 (0.10)	3.97 (0.23)	4.45 (0.09)
EF11	4.80 (0.29)	4.66 (0.23)	5.08 (0.16)	EF27	4.80 (0.44)	4.78 (0.22)	4.77 (0.35)
EF12	4.17 (0.19)	4.78 (0.03)	4.83 (0.04)	EF28	4.46 (0.09)	4.80 (0.48)	5.22 (0.35)
EF13	4.51 (0.23)	4.93 (0.12)	4.71 (0.16)	EF29	4.75 (0.16)	4.79 (0.05)	4.87 (0.19)
EF14	2.21 (0.08)	2.43 (0.06)	2.41 (0.12)	EF30	4.29 (0.09)	4.24 (0.11)	4.67 (0.06)
EF15	5.97 (0.38)	6.40 (0.14)	6.44 (0.38)	EF31	4.97 (0.17)	4.98 (0.24)	4.75 (0.18)
EF16	6.79 (0.24)	7.01 (0.18)	7.20 (0.13)	EF32	4.90 (0.32)	4.71 (0.08)	4.82 (0.27)

Table 56 Gallic acid in 100 g effervescent granules stored at 40±2 ^{O}C, 75% RH.

No.	Mean (SD)			No.	Mean (SD)		
	1 month	2 months	3 months		1 month	2 months	3 months
EF1	0.07 (0.00)	0.09 (0.00)	0.11 (0.00)	EF17	0.13 (0.00)	0.16 (0.00)	0.17 (0.00)
EF2	0.12 (0.00)	0.14 (0.01)	0.16 (0.01)	EF18	0.13 (0.01)	0.14 (0.01)	0.15 (0.00)
EF3	0.27 (0.00)	0.30 (0.00)	0.32 (0.01)	EF19	0.35 (0.01)	0.40 (0.02)	0.42 (0.02)
EF4	0.35 (0.01)	0.40 (0.02)	0.40 (0.02)	EF20	0.45 (0.01)	0.47 (0.05)	0.51 (0.03)
EF5	0.53 (0.02)	0.64 (0.02)	0.65 (0.03)	EF21	0.13 (0.00)	0.16 (0.00)	0.18 (0.00)
EF6	0.25 (0.01)	0.29 (0.01)	0.30 (0.01)	EF22	0.25 (0.01)	0.29 (0.02)	0.27 (0.01)
EF7	0.27 (0.01)	0.30 (0.01)	0.31 (0.02)	EF23	0.27 (0.00)	0.28 (0.02)	0.32 (0.01)
EF8	0.28 (0.02)	0.31 (0.01)	0.32 (0.01)	EF24	0.23 (0.01)	0.26 (0.00)	0.29 (0.01)
EF9	0.23 (0.00)	0.26 (0.01)	0.29 (0.00)	EF25	0.24 (0.01)	0.29 (0.01)	0.30 (0.01)
EF10	0.25 (0.010	0.26 (0.00)	0.29 (0.00)	EF26	0.27 (0.01)	0.28 (0.01)	0.29 (0.00)
EF11	0.27 (0.01)	0.29 (0.01)	0.32 (0.01)	EF27	0.30 (0.02)	0.35 (0.02)	0.34 (0.01)
EF12	0.27 (0.01)	0.29 (0.02)	0.30 (0.00)	EF28	0.25 (0.01)	0.28 (0.01)	0.31 (0.01)
EF13	0.31 (0.02)	0.34 (0.02)	0.35 (0.01)	EF29	0.25 (0.01)	0.28 (0.01)	0.30 (0.01)
EF14	0.12 (0.00)	0.15 (0.01)	0.15 (0.01)	EF30	0.24 (0.01)	0.25 (0.02)	0.25 (0.01)
EF15	0.35 (0.01)	0.38 (0.00)	0.42 (0.02)	EF31	0.28 (0.01)	0.29 (0.02)	0.33 (0.01)
EF16	0.41 (0.01)	0.40 (0.02)	0.46 (0.03)	EF32	0.32 (0.02)	0.35 (0.01)	0.35 (0.00)

Table 57 Gallic acid in 100 g effervescent granules stored at 30±2 ^{O}C, 75% RH.

No.	Mean (SD)			No.	Mean (SD)		
	1 month	2 months	3 months		1 month	2 months	3 months
EF1	0.06 (0.00)	0.07 (0.00)	0.08 (0.00)	EF17	0.12 (0.00)	0.13 (0.00)	0.13 (0.00)
EF2	0.11 (0.00)	0.13 (0.01)	0.13 (0.01)	EF18	0.12 (0.00)	0.13 (0.00)	0.13 (0.00)
EF3	0.25 (0.00)	0.26 (0.02)	0.27 (0.01)	EF19	0.32 (0.01)	0.36 (0.02)	0.39 (0.01)
EF4	0.34 (0.00)	0.36 (0.02)	0.36 (0.01)	EF20	0.42 (0.04)	0.49 (0.07)	0.47 (0.04)
EF5	0.50 (0.03)	0.57 (0.02)	0.57 (0.00)	EF21	0.13 (0.00)	0.14 (0.00)	0.14 (0.00)
EF6	0.24 (0.01)	0.27 (0.01)	0.26 (0.02)	EF22	0.22 (0.01)	0.23 (0.01)	0.23 (0.01)
EF7	0.22 (0.00)	0.25 (0.00)	0.24 (0.01)	EF23	0.24 (0.01)	0.26 (0.01)	0.28 (0.01)
EF8	0.28 (0.01)	0.31 (0.00)	0.28 (0.00)	EF24	0.23 (0.01)	0.24 (0.02)	0.26 (0.01)
EF9	0.21 (0.01)	0.24 (0.01)	0.25 (0.00)	EF25	0.23 (0.01)	0.25 (0.01)	0.29 (0.03)
EF10	0.23 (0.01)	0.25 (0.01)	0.25 (0.01)	EF26	0.23 (0.01)	0.22 (0.02)	0.22 (0.02)
EF11	0.25 (0.01)	0.25 (0.02)	0.26 (0.01)	EF27	0.28 (0.02)	0.32 (0.01)	0.34 (0.01)
EF12	0.23 (0.01)	0.28 (0.00)	0.27 (0.01)	EF28	0.23 (0.01)	0.26 (0.01)	0.26 (0.00)
EF13	0.27 (0.01)	0.32 (0.01)	0.28 (0.01)	EF29	0.24 (0.01)	0.25 (0.00)	0.27 (0.01)
EF14	0.12 (0.01)	0.11 (0.01)	0.12 (0.00)	EF30	0.23 (0.00)	0.23 (0.02)	0.22 (0.00)
EF15	0.33 (0.01)	0.34 (0.01)	0.34 (0.00)	EF31	0.26 (0.01)	0.29 (0.01)	0.32 (0.01)
EF16	0.38 (0.02)	0.37 (0.03)	0.38 (0.02)	EF32	0.29 (0.01)	0.31 (0.02)	0.27 (0.03)

Table 58 %Loss on drying of pellets stored at 40±2 ^{O}C, 75% RH

No.	Mean (SD)		
	1 month	2 months	3 months
PE1	0.66 (0.16)	0.55 (0.06)	0.71 (0.16)
PE2	0.37 (0.04)	0.48 (0.05)	0.47 (0.08)
PE3	0.50 (0.01)	0.51 (0.06)	0.55 (0.03)
PE4	0.35 (0.05)	0.48 (0.05)	0.44 (0.01)
PE5	0.77 (0.07)	0.57 (0.06)	1.06 (0.09)
PE6	0.37 (0.02)	0.41 (0.08)	0.52 (0.07)
PE7	0.54 (0.13)	0.56 (0.08)	0.49 (0.07)
PE8	0.47 (0.03)	0.51 (0.05)	0.61 (0.06)
PE9, lot.1	0.54 (0.09)	0.75 (0.31)	0.64 (0.02)
PE9, lot.2	0.51 (0.10)	0.52 (0.04)	0.61 (0.04)
PE9, lot.3	0.46 (0.10)	0.50 (0.03)	0.55 (0.08)

* PE9 was prepared from three replicates.

Table 59 %Loss on drying of pellets stored at 30±2 ^{O}C, 75% RH.

No.	Mean (SD)		
	1 month	2 months	3 months
PE1	0.55 (0.04)	0.65 (0.22)	0.96 (0.20)
PE2	0.49 (0.09)	0.37 (0.02)	0.35 (0.02)
PE3	0.48 (0.04)	0.48 (0.07)	0.55 (0.07)
PE4	0.37 (0.03)	0.38 (0.07)	0.49 (0.08)
PE5	0.54 (0.03)	0.71 (0.06)	0.55 (0.11)
PE6	0.37 (0.01)	0.41 (0.06)	0.46 (0.10)
PE7	0.43 (0.04)	0.48 (0.04)	0.51 (0.11)
PE8	0.50 (0.07)	0.50 (0.11)	0.58 (0.05)

PE9, lot.1	0.62 (0.15)	0.69 (0.25)	0.59 (0.07)
PE9, lot.2	0.51 (0.08)	0.56 (0.070	0.53 (0.08)
PE9, lot.3	0.48 (0.08)	0.51 (0.06)	0.53 (0.05)

* PE9 was prepared from three replicates.

Table 60 Total tannins in 100 g pellets stored at 40±2 ^{O}C, 75% RH.

No.	Mean (SD)		
	1 month	2 months	3 months
PE1	4.48 (0.04)	4.65 (0.05)	4.36 (0.12)
PE2	9.56 (0.05)	9.52 (0.17)	9.43 (0.09)
PE3	4.60 (0.05)	4.61 (0.04)	4.49 (0.10)
PE4	9.09 (0.12)	9.33 (0.09)	9.11 (0.16)
PE5	4.51 (0.05)	4.58 (0.07)	4.46 (0.09)
PE6	9.13 (0.06)	9.40 (0.04)	9.15 (0.11)
PE7	4.45 (0.04)	4.50 (0.04)	4.62 (0.04)
PE8	9.02 (0.11)	9.13 (0.21)	9.38 (0.06)
PE9, lot.1	6.92 (0.08)	6.84 (0.15)	7.14 (0.08)
PE9, lot.2	6.98 (0.06)	6.96 (0.14)	7.15 (0.04)
PE9, lot.3	6.99 (0.19)	7.02 (0.06)	7.18 (0.05)

* PE9 was prepared from three replicates.

Table 61 Total tannins in 100 g pellets stored at 30±2 ^{O}C, 75% RH.

No.	Mean (SD)		
	1 month	2 months	3 months
PE1	4.71 (0.02)	4.66 (0.06)	4.54 (0.04)
PE2	9.86 (0.11)	9.91 (0.07)	9.51 (0.13)
PE3	4.84 (0.05)	4.87 (0.04)	4.62 (0.03)

PE4	9.47 (0.10)	9.61 (0.15)	9.41 (0.14)
PE5	4.84 (0.09)	4.77 (0.03)	4.67 (0.04)
PE6	9.34 (0.13)	9.49 (0.20)	9.26 (0.14)
PE7	4.72 (0.05)	4.49 (0.04)	4.61 (0.09)
PE8	9.12 (0.15)	9.03 (0.11)	9.35 (0.31)
PE9, lot.1	7.25 (0.07)	7.22 (0.07)	7.24 (0.05)
PE9, lot.2	7.28 (0.08)	7.30 (0.14)	7.16 (0.08)
PE9, lot.3	7.34 (0.09)	7.40 (0.07)	7.22 (0.06)

* PE9 was prepared from three replicates.

Table 62 Gallic acid in 100 g pellets stored at 40±2 ^{O}C, 75% RH.

No.	Mean (SD)		
	1 month	2 months	3 months
PE1	0.23 (0.00)	0.29 (0.00)	0.35 (0.00)
PE2	0.40 (0.01)	0.49 (0.00)	0.60 (0.01)
PE3	0.22 (0.00)	0.28 (0.00)	0.33 (0.00)
PE4	0.41 (0.00)	0.51 (0.00)	0.63 (0.00)
PE5	0.26 (0.00)	0.32 (0.01)	0.38 (0.00)
PE6	0.45 (0.02)	0.57 (0.00)	0.69 (0.01)
PE7	0.26 (0.00)	0.31 (0.01)	0.39 (0.00)
PE8	0.45 (0.00)	0.55 (0.01)	0.69 (0.01)
PE9, lot.1	0.36 (0.00)	0.41 (0.00)	0.53 (0.00)
PE9, lot.2	0.35 (0.00)	0.40 (0.00)	0.51 (0.00)
PE9, lot.3	0.36 (0.00)	0.41 (0.00)	0.51 (0.01)

* PE9 was prepared from three replicates.

Table 63 Gallic acid in 100 g pellets stored at 30±2 ^{O}C, 75% RH.

No.	Mean (SD)		
	1 month	2 months	3 months
PE1	0.18 (0.00)	0.23 (0.00)	0.24 (0.00)
PE2	0.31 (0.00)	0.36 (0.03)	0.42 (0.00)
PE3	0.16 (0.00)	0.21 (0.00)	0.23 (0.00)
PE4	0.33 (0.00)	0.35 (0.00)	0.42 (0.01)
PE5	0.20 (0.00)	0.23 (0.01)	0.26 (0.00)
PE6	0.35 (0.01)	0.38 (0.00)	0.44 (0.02)
PE7	0.22 (0.00)	0.23 (0.00)	0.24 (0.00)
PE8	0.38 (0.01)	0.39 (0.00)	0.42 (0.00)
PE9, lot.1	0.26 (0.01)	0.29 (0.00)	0.32 (0.00)
PE9, lot.2	0.27 (0.00)	0.29 (0.00)	0.31 (0.00)
PE9, lot.3	0.28 (0.00)	0.29 (0.00)	0.32 (0.00)

* PE9 was prepared and analysed from three replicates.

Data presentation

The results from stability studies of effervescent granules and pellets were plotted between percentage remaining of loss on drying (LOD), total tannins or gallic acid content versus time. The percentage remaining of loss on drying (LOD), total tannins or gallic acid content were calculated as the following equations:

$$\text{LOD remaining (\%)} = \frac{\text{LOD}_{\text{t month}}}{\text{LOD}_{\text{0 month}}} \times 100$$

$\text{LOD}_{\text{t month}}$ = Loss on drying after storage for 1, 2 or 3 months

$\text{LOD}_{\text{0 month}}$ = Loss on drying after product preparation

$$\text{Total tannins remaining (\%)} = \frac{\text{Total tannins}_{\text{t month}}}{\text{Total tannins}_{\text{0 month}}} \times 100$$

$\text{Total tannins}_{\text{t month}}$ = Total tannins content after storage for 1, 2 or 3 months

$\text{Total tannins}_{\text{0 month}}$ = Total tannins content after product preparation

$$\text{Gallic acid remaining (\%)} = \frac{\text{Gallic acid}_{\text{t month}}}{\text{Gallic acid}_{\text{0 month}}} \times 100$$

$\text{Gallic acid}_{\text{t month}}$ = Gallic acid content after storage for 1, 2 or 3 months

$\text{Gallic acid}_{\text{0 month}}$ = Gallic acid content after product preparation

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