

Research article

Preparation of Beijing Grass Tablets from Spray-dried Powder of Juice of *Murdannia loriformis* (Hassk.) Rolla Rao *et* Kammathy

Kaewnoi A., Duanyai S., Tongthong K., Manok S., Rodpo P., Jateleela S.*

Program in Thai Traditional Medicine, Faculty of Science and Technology, Bansomdejchaopraya Rajabhat University, Bangkok 10600, Thailand.

Key words: *Murdannia loriformis*, Beijing grass tablets, spray dried powder, formulations.

***Corresponding Author: Jateleela S.,**
Program in Thai Traditional Medicine,
Faculty of Science and Technology,
Bansomdejchaopraya Rajabhat University,
Bangkok 10600, Thailand.

Abstract

Murdannia loriformis (Hassk.) Rolla Rao *et* Kammathy, namely Beijing grass (BJG) is a favourite herb utilized to treat various cancers and improve the life quality for patients. The purpose of this study is aimed to develop BJG tablet formulations (Rxs) from 4 formulas of spray dried BJG powder. For the juices extracted from BJG, formula I to IV contained 4.0% of 4 ratios of maltodextrin (MDX):lactose, i.e., 3:1, 2:2, 1:3, and 4:0 that provided spray dried BJG powder formula I to IV, respectively. The 12 batch Rxs of BJG tablets were prepared by a wet granulation method. Microcrystalline cellulose (MCC) was used as a binder and disintegrant at the content of BJG powder formula I to IV as follows: 6.0% for Rx 1- 4, 10.0% for Rx 5- 8, and 16.0% for Rx 9 -12, respectively. Four concentrations of 100 ml ethanol were used as granulants at 90%, 80%, 70%, and 60% v/v for the Rxs of BJG powder formula I, II, III, and IV, respectively. Croscarmellose sodium, magnesium stearate, and fumed silica were used as an external disintegrant, a lubricant and a glidant, respectively. All Rxs met the weight variation criteria of Dietary Supplements, USP 40. Rx 5-12 significantly provided tablets with higher hardness, and thinner thickness than Rx 1- 4 did. For tablet friability, the decreased content of MDX in BJG powder formula III or IV would significantly cause more friable tablets than that of powder formula I or II. For tablet disintegration time (DT), both increases in lactose content of BJG powder, and in MCC content could significantly reduce DT. For chemical ID test, same ID was obtained from BJG tablet Rx 12 compared with BJG juice.

Introduction

The medicinal herbal, nutraceutical and pharmaceutical industries are focusing their interest on different natural compounds as a strategy to enhance the health benefits of their products. Beijing grass (BJG) is a good example of an herbal source of bioactive compounds with proven benefits for human health. The botanical name of BJG is *Murdannia loriformis* (Hassk.) Rolla Rao *et* Kammathy (*M. loriformis*), family: Commelinaceae, has long been used by Chinese practitioners as a folk herb in cancer related therapy and as a remedy for cancer in an early stage, and for treating other diseases including colds, throat infections, pneumonia, diabetes mellitus, flu and inflammation such as inflamed wound [1]. In 1984, *M. loriformis* has become very famous in Thailand when some cancer patients have recovered after drinking its fresh juice [2]. It has been claimed to have a potential for treating, healing or protecting against cancer by strengthening the immunomodulatory mechanism. Glycoside and aglycone of the plant have been reported to be cytotoxic against breast cancer and colon cancer cell lines [2]. BJG extracts exert an inhibitory effect on the cell growth of breast and colon carcinomas through the apoptosis mediated pathway [3]. Many studies have revealed its effectiveness as an anticancer, anticarcinogen

[1-3] and also as an antioxidant [4, 5]. The ethanol extract of BJG has been shown to possess the anti-inflammatory, analgesic and antipyretic activities, without any abnormality in acute toxicity test in rats. Moreover, the study using *in vivo* experiments has revealed that BJG exhibits such effects in rat models without adverse effect on gastric mucosa [6]. These results have supported the use of BJG in traditional medicine for various ailments. The phytochemical studies have revealed that BJG contains phytosteryl glycoside (G1a), glycosphingolipid (G1b), namely, 2, β -O-D-glucopyranosyl-2-(2'-hydroxy-Z-6'-enecosamide) sphingosine, amino acid, flavonoids, plant membrane lipid [2-3], syringic acid, and isovitexin [1]. The study has showed that (G1a) and (G1b), in BJG elicit immunomodulatory properties and a moderate cytotoxicity with an ED₅₀ of 16 μ g/ml against human breast ATCC HTB20 (BT474) and colon (SW620) cancer cell lines [3]. Several studies have supported that BJG possesses anti-mutagenic, anti-proliferative, antitumour, and immunomodulator activity [1-3, 7, 8] and stimulated T-lymphocyte proliferation [1, 2]. The ethanol extract of BJG has been shown to possess the anti-inflammatory, analgesic and antipyretic activities, without any abnormality in acute toxicity test in rats [6]. From investigating the antioxidant activity of BJG, BJG tea extract contains a phenolic content of around 47 mg gallic

acid equivalent (GAE) /g dry weight with antioxidant activity of 4.14 mmol Fe (II) /g dry weight evaluated by ferric reducing antioxidant power (FRAP) assay [5]. Furthermore, the antioxidant activity of BJG extract by DPPH (2, 2'-diphenyl-1-picrylhydrazyl) assay has been found that BJG at 500 µg/ml exhibits a scavenging effect of 91.50 [4].

Spray-drying is defined as the transformation of liquid state feed into a dried particulate form. This is achieved by an atomizing the fluid into a drying chamber of a spray dryer, where the liquid droplets are passed through a hot-air stream [9, 10]. The heat and mass transfer during the drying occurs rapidly between the air and vapor films surrounding the droplets at the saturation temperature. This technique, consequently, is suitable for the heat-sensitive products [11]. Characterization of physicochemical properties of plant extracts, processing and formulation are widely significant in the product development to stabilize the products with rapid degradation of active compounds and to increase their low bioavailability [12]. Plant extracts can be transformed from liquid to a spray dried powder that includes the carriers [13]. The spray drying technique is a powerful tool for delivering cost-effective and high quality ingredients. Spray dried extracts powders are easily transported, handled and reduced in bulk size since they possess a good flowability and high stability [14]. Maltodextrin (MDX) is a water soluble modified starch derivative widely used without synthetic fillers for improving the flowability of powders [15]. Recently, the 3 various ratios of lactose-MDX mixture were used as the carrier in the spray drying of the passion fruit juice, under various operation conditions, e.g., the inlet air temperatures of 180- 190°C, the air pressures of 0.10-0.20 MPa in order to obtain appropriate powdered products [14]. Various formulas of BJG juice, the industrial production of powders using spray drying as a processing method for various BJG powder formulas, and the formulations (Rxs) of BJG tablets from the various BJG powder formulas by a wet granulation technique were developed. The studies were aimed to obtain the best formula of spray dried BJG powder and best BJG tablet formulation with best acceptable physicochemical properties. Furthermore, the appropriate chemical identification tests of various components in BJG were set for BJG tablet compared with a sample of BJG juice.

Materials and methods

Authenticity of beijing grass

The BJG with flowers has already been first authenticated by Miss Petnumpung Rodpo, the instructor at the Program in Thai Traditional Medicine, Faculty of Science and Technology, Bansomdejchaopraya Rajabhat University, Bangkok 10600, Thailand. The herbarium

number of identification was BSRUTTMCM0003, as shown in Figure 1 (a) and (b).

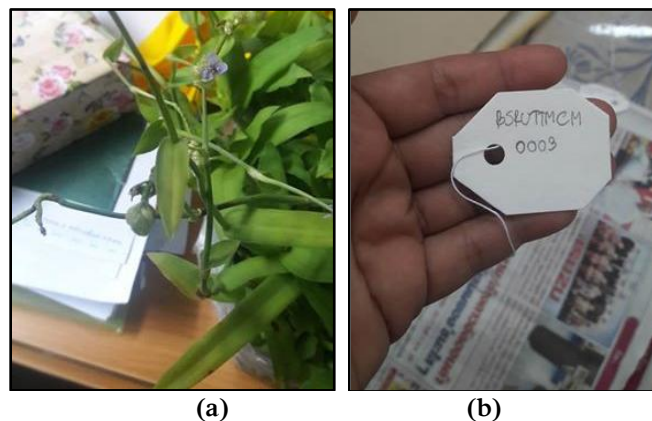


Figure 1. (a) Beijing grass with flowers, (b) herbarium number of identification of BJG.

Materials

Fresh Beijing grass with 3-4 months growing period was purchased from medicinal plant cultivation group in Bangpakong district, Chachoengsao, Thailand. MDX was purchased from Chemipan Corporation, Bangkok, Thailand. Lactose was purchased from Krittiya Royal, Nakhon-Pathom, Thailand. PVP K90 was Povidone K90 purchased from GAP Corp, New Jersey, USA. Croscarmellose sodium (Ac-di-sol®) and microcrystalline cellulose (Avicel®PH 102), both were purchased from FMC Health & Nutrition, Newark, Delaware, USA. Magnesium stearate was purchased from Peter Greven, Netherlands. Ethanol, 95% v/v was purchased from SSCV Corporation, Samut-Prakan, Thailand. Fumed silica (Aerosil®) was purchased from Evonik Industries AG, Leverk, Germany. And purified water was prepared at Faculty of Pharmacy, Mahidol University, Thailand. All chemicals for the chemical identification tests were analytical grade purchased from Merck, Thailand.

Preparation of various BJG juices

The 430 kg fresh BJG delivered from farm was cleaned with purified water. The cleaned root of BJG was cut off 63.50 kg by a cleaned sharp knife. The cleaned bunches without root, were weighed about 366.5 kg and were compressed with a cold press juicer (at Perfect Natural Food Powder and Favor 2002 Plant, Samut-Sakhon, Thailand) to receive the herbal juice of around 270 kg and its separated pomade was eliminated. The herbal juice was filtrated through a muslin cloth to gain around 255 kg. The filtrate was divided into 8 portions as follows: (1) the juice sample portion of 30.00 g for chemical identification test (2) the 3 portions of around 4 kg BJG juice without filler, and (3) the 4 portions of about 60 kg (4 x 60 kg) for developing the spray dried powder formulas I- IV, respectively, as depicted in table 1. The 4 portions of about 60 kg BJG juices were incorporated

with total soluble carriers of 4.0% by weight to make easier handling the spray dried BJJ powder in order to develop various BJJ tablet Rx's. MDX and lactose were added in BJJ juice formulas I- IV by the ratio of 3:1, 2:2, 3:1, and 4:0 to provide spray dried BJJ powder formulas I- IV, respectively, by the spray drying process.

Preparation of spray dried BJJ powder

Each portion of BJJ juice, was delivered to a spray dryer (at Perfect Natural Food Powder and Favor 2002 Plant, Samut-Sakhon, Thailand) with a delivery rate of 18 L/h. The process conditions were constantly set as follows: an inlet and outlet air temperatures of 180°C and 85°C, respectively, a pump velocity of 12 Hz, a rotary atomizer velocity of 14,000 rpm with a pressure of 0.36 MPa. The spray dried BJJ powder collected from chamber- and cyclone-product collectors were well mixed after spray drying process in a V-blender (Hand made lab type of 5.0 L, Department of Manufacturing Pharmacy, Mahidol University, Bangkok, Thailand) for 5 min. The spray dried BJJ powder of all formulas I – IV were well kept in the pouches of aluminum foil laminated with a low density polyethylene (LDPE, for sealing purpose) to protect spray dried BJJ powder from the moisture transmission from ambient and the photolysis from light.

Formulations of BJJ tablets

We prepared 12 tablet Rx's from spray dried BJJ powder formulas I-IV. Each tablet contains 500 mg spray dried BJJ powder and various excipients used in suitable percentage to prepare 600 mg tablets as depicted in tables 2 and 3. For intragranular excipients, microcrystalline cellulose (MCC, Avicel® PH 102) was incorporated as a self-disintegrating filler at the % contents by weight of spray dried BJJ powder formulas I-IV as follows: (1) 6% for Rx1-4, (2) 10% for Rx 5-8, and (3) 16% for Rx 9-12, respectively. Lactose was added in all tablet Rx's in order to make the same tablet weight of 600 mg. For extra granular excipients added, croscarmellose, magnesium

stearate and fumed silica were used as a disintegrant, a lubricant, and a glidant at the tablet concentration of 2.5, 0.50, and 0.25% by weight, respectively. All 12 batch Rx's for 400 tablets were prepared to develop the tablet Rx from spray dried BJJ powder formulas I-IV. The normal oral dose for adult will be discussed on the determination of oral dosage unit which is 2-3 tablets of 500 mg BJJ, bid.

Wet granulation technique and tablet preparation

Each batch formulation of 400 tablets as depicted, 200.0 g BJJ powder formula I, II, III, or IV, and intragranular excipients, namely MCC, and lactose were weighed with triple beam balance (700/800 Series 2610g model, Ohaus, USA) and an electric precision balance (Entris323-1S model, Sartorius, Germany), respectively. The spray dried BJJ powder, MCC, and lactose were well thoroughly mixed with the V-blender for 5 min. Each mixture of Rx's 1- 12 was well kneaded with the 100 ml ethanol solution with the specified concentration as depicted in table 4, in a planetary mixer (5KPM5EGR Standard Mixer 4.8 L, KitchenAid®, USA) for 15min to obtain a good equilibrium damp mass. The damp mass was passed through 14-mesh sieve equipped in an oscillating granulator (Lab type, KSL Engineering, Bangkok, Thailand) to gain wet granules which were latterly dried in a tray dryer (50 kg type, KSL Engineering, Bangkok, Thailand). The dried BJJ granules were passed through 18-mesh sieve of the oscillating granulator, and were well mixed with extragranular excipients, namely, croscarmellose sodium, magnesium stearate and fumed silica in the V-blender for 5 min. The well mixed granules were compressed with a single tablet machine (Korsch EK III-G model, Charatchai Machinery, Bangkok, Thailand) equipped with 13-mm standard concaved tools to obtain average tablet weight of 600 mg for all Rx's with the hardness about 5 kg for Rx 1-4 and about 7 kg for Rx 5-12.

Table 1. Various formulas of BJJ juice and the results of spray drying process.

Beijing Grass Juice (BJG Juice)	Carriers		BJG Juice (kg)	Spray-dried BJJ powder (g)	% Yield (w/w)	Collection Efficacy*
	Maltodextrin (w/w)	Lactose (w/w)				
Sample without carrier	-	-	3.84	11.95 g	0.312	100%
	-	-	4.02	12.46 g	0.310	100%
	-	-	4.08	12.57 g	<u>0.308</u>	100%
					Mean: 0.31	
BJG Juice with 4.0% filler	4%		100.00	4.31	4.31	100%
Formula I	3 %	1%	60.78	2,160	3.55	82.37%
Formula II	2%	2%	60.05	2,049	3.41	79.11%
Formula III	1%	3%	61.50	2,171	3.53	81.90%
Formula IV	0	4%	58.80	1,983	3.37	78.19%

Table 2. The 12 tablet formulations containing 500 mg spray dried BJJ powder and various excipients used at % by weight in order to prepare for 600 mg tablet.

Rx	BJG juice formula	Spray dried BJJ powder	Intragranular Excipients % w/w of BJJ powder		Extragranular Excipients % w/w of 600 mg tablet weight		
			Avicel® (MCC)	Lactose qs to 600 mg	Croscar-mellose	Fumed silica	Magnesium stearate
1	I	500 mg	6.0%	53 mg	2.5%	0.25%	0.5%
2	II	500 mg	6.0%	53 mg	2.5%	0.25%	0.5%
3	III	500 mg	6.0%	53 mg	2.5%	0.25%	0.5%
4	IV	500 mg	6.0%	53 mg	2.5%	0.25%	0.5%
5	I	500 mg	10.0%	33 mg	2.5%	0.25%	0.5%
6	II	500 mg	10.0%	33 mg	2.5%	0.25%	0.5%
7	III	500 mg	10.0%	33 mg	2.5%	0.25%	0.5%
8	IV	500 mg	10.0%	33 mg	2.5%	0.25%	0.5%
9	I	500 mg	16.0%	3 mg	2.5%	0.25%	0.5%
10	II	500 mg	16.0%	3 mg	2.5%	0.25%	0.5%
11	III	500 mg	16.0%	3 mg	2.5%	0.25%	0.5%
12	IV	500 mg	16.0%	3 mg	2.5%	0.25%	0.5%

*0.25 ml ethanol solution was used as granulating liquid to make a good wet granulation.

Table 3. The 12 master tablet formulations containing 500 mg spray dried BJJ powder and various excipients used in mg in order to prepare for 600 mg tablet.

Rx	BJG juice formula	Spray dried BJJ powder	Intragranular Excipients		Extragranular Excipients		
			Avicel® (MCC)	Lactose qs to 600 mg	Croscar-mellose	Fumed silica	Magnesium stearate
1	I	500 mg	30 mg	50.5 mg	15 mg	1.5 mg	3 mg
2	II	500 mg	30 mg	50.5 mg	15 mg	1.5 mg	3 mg
3	III	500 mg	30 mg	50.5 mg	15 mg	1.5 mg	3 mg
4	IV	500 mg	30 mg	50.5 mg	15 mg	1.5 mg	3 mg
5	I	500 mg	50 mg	30.5 mg	15 mg	1.5 mg	3 mg
6	II	500 mg	50 mg	30.5 mg	15 mg	1.5 mg	3 mg
7	III	500 mg	50 mg	30.5 mg	15 mg	1.5 mg	3 mg
8	IV	500 mg	50 mg	30.5 mg	15 mg	1.5 mg	3 mg
9	I	500 mg	80 mg	0.5 mg	15 mg	1.5 mg	3 mg
10	II	500 mg	80 mg	0.5 mg	15 mg	1.5 mg	3 mg
11	III	500 mg	80 mg	0.5 mg	15 mg	1.5 mg	3 mg
12	IV	500 mg	80 mg	0.5 mg	15 mg	1.5 mg	3 mg

*0.25 ml ethanol solution was used as granulating liquid to make a good wet granulation.

Table 4. The 12 batch formulations containing 200 g spray dried BJJ powder and various excipients used in g (ml) in order to prepare for 400 tablets.

Rx	BJG juice formula	Spray dried BJJ powder	Intragranular Excipients			Extragranular Excipients		
			Avicel® (MCC)	Lactose	100 ml Ethanol, Conc. of *	Croscar-mellose	Fumed silica	Magnesium stearate
1	I	200.0 g	12.0 g	20.2 g	90% v/v	6.0 g	0.6 g	1.2 g
2	II	200.0 g	12.0 g	20.2 g	80% v/v	6.0 g	0.6 g	1.2 g
3	III	200.0 g	12.0 g	20.2 g	70% v/v	6.0 g	0.6 g	1.2 g
4	IV	200.0 g	12.0 g	20.2 g	60% v/v	6.0 g	0.6 g	1.2 g
5	I	200.0 g	20.0 g	12.2 g	90% v/v	6.0 g	0.6 g	1.2 g
6	II	200.0 g	20.0 g	12.2 g	80% v/v	6.0 g	0.6 g	1.2 g
7	III	200.0 g	20.0 g	12.2 g	70% v/v	6.0 g	0.6 g	1.2 g
8	IV	200.0 g	20.0 g	12.2 g	60% v/v	6.0 g	0.6 g	1.2 g
9	I	200.0 g	32.0 g	0.2 g	90% v/v	6.0 g	0.6 g	1.2 g
10	II	200.0 g	32.0 g	0.2 g	80% v/v	6.0 g	0.6 g	1.2 g
11	III	200.0 g	32.0 g	0.2 g	70% v/v	6.0 g	0.6 g	1.2 g
12	IV	200.0 g	32.0 g	0.2 g	60% v/v	6.0 g	0.6 g	1.2 g

*100 ml ethanol solution was used as granulating liquid to make a good wet granulation.

Loss on drying (LOD) of spray dried BJJ powder formulas I - IV

The LOD of 2.5 g spray dried BJJ powder formulas I - IV were detected as % with a moisture analyzer (HB43-S model, Mettler Toledo, Fisher Scientific UK, England). The LODs were done in triplicate, and the mean was calculated.

Microbiological examination of spray dried BJJ powder

From Supplement to Thai Herbal Pharmacopoeia [15], the herb which used as a starting raw material for finished product for oral use must be required an examination for the absence of these pathogenic microbial contamination [14] as follows: (1) *Clostridium Spp.* per 1 g (2) *Salmonella Spp.* per 10 g, and (3) *E. Coli* per 1 g. The sample of spray dried BJJ powder was sent to a certified laboratory unit of Asia Medical and Agricultural Laboratory and Research Center, Bangkok, Thailand in order to be whether met such requirements or not.

Flowability of excipients, spray dried BJJ powder and dry BJJ granules with and without extragranular excipients

The Carr's Compressibility Index was used to evaluate a flowability of a material with a jolting volumeter (Type EU 42 E2/89 S WE, J. Engelsmann AG, Ludwigshafen am Rheine, Germany) by the following equation [17, 18]:

$$\text{Carr's Compressibility Index} = 100 \times [\rho_{\text{tapped}} - \rho_{\text{bulk}}] / \rho_{\text{tapped}} \quad \text{Equation (1)}$$

where ρ_{tapped} is the tapped density of powder or granules at 1,000 taps of stamping the 100 ml measuring glass cylinder containing 40.00 g or less in order to detect a constant volume of a material, and ρ_{bulk} is the bulk density of the material. For an evaluating the flowability of various materials, the compressibility index were done in triplicate and calculated for their mean and standard deviation. Compare the calculated values with ranges of % compressibility in the scale of flowability table of Carr's Compressibility Index [17, 18] for predicting the flowability of 6 tablet excipients used, 4 different formulas of spray dried BJJ powder, as well as dry BJJ granules with and without extragranular excipients.

Physicochemical evaluation of BJJ tablets of all formulations

Tablet weight variation

From the weight variation criteria in dietary supplements of USP 40 [19], the 20 whole tablets were weighed individually, and calculated the mean weight. For mean tablet weight more than 324 mg, the requirements was met if the weights of not more than 2 of the tablets differed from the mean weight by more than 5.0% and no tablet differed in weight by more than 10.0%. Another

appropriate weight parameter is a percentage coefficient of variation, % CV which is a standard deviation per mean tablet weight of 100 mg as follow:-

$$\% \text{ CV} = (\text{Standard Deviation} / \text{Mean}) \times 100 \quad \text{Equation (2)}$$

Tablet hardness and thickness

During in-process, the tablets were detected individually for their hardness by a hardness tester (type: 1-10 kg, Stokes-Monsanto, USA). For finished tablets, 10 whole tablets were measured their hardness (kg), and thickness (mm) individually by a tablet hardness, thickness and diameter tester PTB 311E model, Pharmatest, Germany). The mean hardness, and also thickness of 10 tablets were calculated.

Tablet friability

For tablets with a unit mass less than 650 mg, take a sample of whole tablets corresponding to 6.5 g. The tablets should be carefully dedusted prior to testing. Accurately weigh the tablet sample, and place the tablets in the drum of a friabilator (Roche model, Pharmatest, Germany). Rotate the drum 100 times for 4 min, and remove the tablets. Remove any loose dust from the tablets as before, and accurately weigh. The tests are run in triplicate, maximum mean weight loss from the 3 samples of not more than 1.0% friability is considered acceptable under the title of tablet friability test USP 40 [20].

Disintegration time (DT)

DT was defined as the interval required for complete disappearance of a tablet or its particles from a sieve of the testing basket. Place each tablet of 6 BJJ tablets in a glass tube of six tubes of the basket [21]. Operate a disintegration apparatus A (USP Model A, Pharmatest, Germany) using purified water maintained at $37 \pm 2^\circ$ [21]. The DT of 6 tablets were recorded and calculated for their mean.

Chemical identification (ID) tests of BJJ tablets compared with the juice sample

Chemical ID tests of BJJ extract was done by a technique of a thin layer Chromatography (TLC) as follows: (1) the 30.00 g juice sample (equivalent to oral dose of 50 g BJJ bunches [1, 2], and (2) the fine crushed powder from 2 tablets of BJJ tablet Rx 12 which was already mixed and shaken with 30.0 ml purified water, were mixed and shaken with 120.0 ml methanol for 48h for extracting the various active components [22]. The methanolic solutions were filtrated through a filter paper (grade 5, Whatman®) on a Buchner funnel of a vacuum suction flask. The entire filtrate was concentrated under reduced pressure in a rotary vacuum evaporator (Hei-VAP Value Rotary Evaporator, Heidolph, Germany). The

concentrated extract was air dried to a constant weight at room temperature [22]. Each portion of 0.100 g of dried residue was dissolved with 5.0 ml methanol, only 0.1 ml with a glass capillary was spotted as a point on a level of 1.0 cm from bottom of a 5 x10 cm glass TLC plate (TLC Silica gel 60 F₂₅₄, Merck KGaA, Darmstadt, Germany) and the two point was 0.7-cm wide and each point was 1.0-cm wide from the adjacent rim of the plate. Waiting until 2 spots on TLC plate were dried, the 100.0 ml mobile phase was prepared by mixing 81.0 ml ethyl acetate, 11.0 ml methanol, and 8.0 ml water in chromatographic tank with the height of 1.0 cm for 1 h to obtain the vapor saturation of mobile phase. The bottom TLC plate was immersed in the mobile phase of such tank. For next 10 min, various components on TLC plate were detected under UV-light wave-length of 254 nm and 365 nm and were detected latterly by spraying and reacting with p-anisaldehyde-sulfuric acid (a solution of freshly prepared 0.5 ml p-anisaldehyde in 50.0 ml glacial acetic acid and 1.0 ml conc. sulfuric acid) and were dried at 105°C. The value of hR_F , was the retarding factor, R_F multiplied with 100 as follow:

$$hR_F = \frac{\text{distance moved by solute} \times 100}{\text{distance moved by solvent}} \quad \text{Equation (3)}$$

Statistical data analysis of physicochemical properties among groups

Analysis of variances test (ANOVA Test)

The single one-way ANOVA was detected for its pooled variance, s^2 by an Xlstat Designer ver. 2018.1® statistic program for observing whether there are some significant differences in the physicochemical properties among those of various BJJ tablet Rx's and/or spray dried BJJ powder formulas at $p < 0.01$ with the confidence level of 99.0%.

Multiple comparison by least significant difference (LSD) procedure

The mean physicochemical parameters among various groups were significantly ranked by the LSD procedure at 1.0% allowance, two-tailed ($\alpha = 0.01$, 2-tailed) as follow [23]:

$$1.0\% \text{ allowance} = t \sqrt{s^2(1/n_i + 1/n_j)} \quad \text{Equation(4)}$$

where t is critical t value at α of 0.01 which depends upon the degree of freedom within group; s^2 is the pooled variance detected from previous ANOVA test; and n_i and n_j are the number of members between groups.

Results and Discussion

Spray dried BJJ powder

The results of spray drying of BJJ juices were depicted in Table 1. For BJJ juice without filler, the mean percentage yield of BJJ product of the 3 values was 0.31 % which was too small to handle the tablet formulations. Two soluble carriers, namely, maltodextrin (MDX) and lactose mixtures was added 4.0% by weight into the BJJ juice to provide enough larger mass for such purpose. The percentage yield was then changed to 4.31% by weight. For spray dried BJJ powder formulas 1- 4 as shown in figure 2, and as depicted in table 1, provided the collection efficiency of around 78% -82%. This might be resulted from the sticky effects of BJJ itself and added fillers that provided some loss of spray dried powder attached to the wall of the heating and the collection chambers.

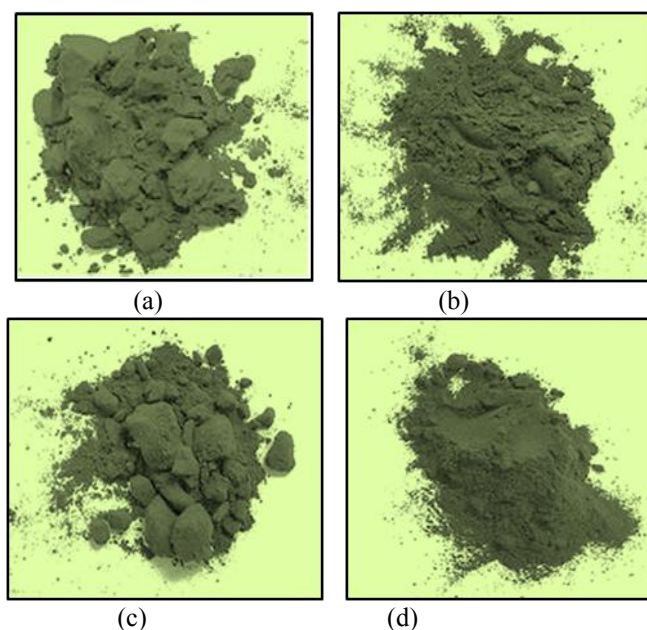


Figure 2. Various spray dried BJJ powders: (a) formula I, (b) formula II, (c) formula III, and (d) formula IV.

Determination of oral dosage mass unit

The LD₅₀ in white rat has been found to be 120g/ 1 kg body weight that is 300-time of human oral dose [2]. The oral dose of 0.400 g BJJ juice/1 kg body weight for human has provided very safely continuous treatment for 3 months in white rats without any acute [1-2] and chronic [7] toxic effects. If the human body weights are 60 - 90 kg (75 ± 15 kg), the BJJ juice oral dose is calculated to be 24 - 36 g which is equivalent to 1035 - 1553 mg spray dried BJJ power formulas I- IV or 2- 3 tablets containing 500 mg BJJ powder, bid., for 5-6 days and the medication is stopped for 4-5 days before the same new medication. The treatment purposed for immunological modulation should be not more than 3 months under the physician supervisions.

Moisture content of spray dried BJJ powder, formulas I - IV

From ANOVA test of LOD data as depicted from table 9, there were some significant differences in % LOD data of spray dried BJJ powder among formulas I- IV ($p < 0.01$). From LSD procedure ($\alpha = 0.01$, 2-tailed), 1.0% allowance was 0.13% LOD as depicted in table 5 and as shown in figure 3. The moisture contents of various formulas were significantly ranked as follows: % LOD of BJJ powder formula I > that of formula II > that of formula III > that of formula IV. The increased ratio of MDX to lactose in spray dried BJJ powder formulas

would play important role in order to significantly adsorb higher moisture from ambient to spray dried BJJ powder by MDX. Since the moisture from the atmosphere could be well adsorbed by BJJ powder, high moisture content would cause BJJ powder to form a hard cake, because of high interparticular adhesiveness. The spray dried BJJ powder of all formulas should be kept in the pouches of aluminum foil laminated with a low density polyethylene (LDPE, for sealing purpose) to protect spray dried BJJ powder from the moisture transmission from ambient and the photolysis of components in BJJ powder from light.

Table 5. The percentage loss on drying spray dried BJJ powder formulas I – IV.

Spray Dried BJJ Powder	% LOD			
	1	2	3	Mean
Formula I	6.38	6.42	6.45	6.42
Formula II	6.17	6.23	6.26	6.22
Formula III	5.99	6.06	6.08	6.04
Formula IV	5.76	5.87	5.92	5.82

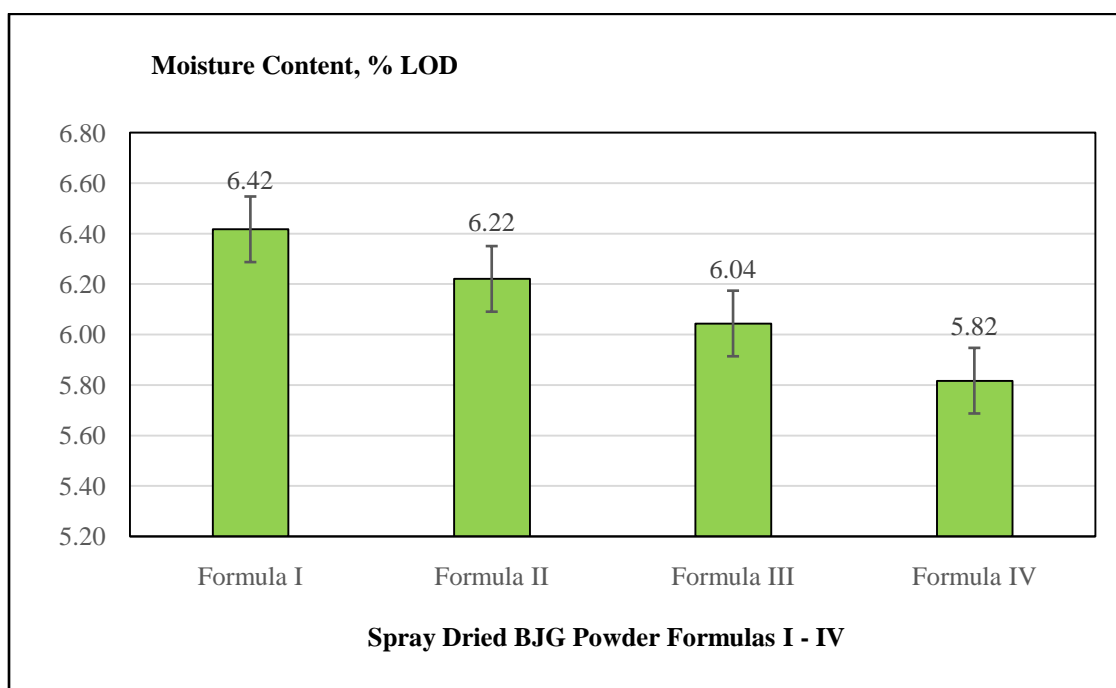


Figure 3. Moisture content in spray dried BJJ powder formulas I - IV ± 0.13 % ($\alpha = 0.01$, 2-tailed).

Microbiological examination of spray dried BJJ powder

From the requirements of Supplement to Thai Herbal Pharmacopoeia [16], the previous certified laboratory unit has reported that there was no contamination of *Clostridium spp.*, *Salmonella spp.* and *E. coli* in our sample of spray dried BJJ powder. This indicated that the pathogenic microorganisms might be destroyed by the high inlet air temperature of 180°C during the spray drying process.

Development of BJJ tablet formulations

For formulas I - IV, spray dried BJJ powder along with microcrystalline cellulose (MCC, Avicel® PH 102) and lactose were weighed and well mixed to prepare the wet granulations of 400 tablets of 500 mg BJJ formulations (Rxs) as follows: BJJ powder of formulas I -IV plus (i) MCC at 6% w/w of each BJJ powder for Rx 1-4, (ii) MCC at 10% w/w for Rx 5- 8, and (iii) MCC at 16% for Rx 9-12, respectively. MCC is used to facilitate the wet granulation process by reducing the sensitivity of wet mass to over-wetting, faster drying, fewer screen blockages, case hardenings, and faster disintegration [24-

26]. MCC is used in high moist granules as it binds the excess moisture keeping the granules dry and free flowing [24, 25]. The granulations compress quickly and produce tablets without hardening with age. MCC functions as tablet disintegrant in a concentration of 5-20% by the mechanism of wicking and swelling action [24-26]. Lactose is used as an inert diluent to make a tablet weight of 600 mg. For extragranular excipients, croscarmellose sodium, magnesium stearate, and fumed silica were used as a disintegrant, a glidant, and a lubricant at 2.5%, 0.25%, and 0.50% by weight of tablet formulation, respectively.

Volume and concentration of ethanol used in wet granulation

From a preliminary run when exposing to purified water as a wet granulating liquid into BJG powder of all formulas, the sticking adverse effect of both MDX and spray dried BJG itself increased remarkably, and consequently the obtained hard cake could not be passed through a sieve. The 100 ml of ethanol solution was used for each Rx of 400 tablets. For spray dried BJG powder formula I - IV, the concentrations (v/v) of ethanol were used as follows: (I) 90% for Rx 1, Rx 5, and Rx 9, (II) 80% for Rx 2, Rx 6, and Rx 10, (III) 70% for Rx 3, Rx 7, and Rx 11, and (IV) 60% for Rx 4, Rx 8, and Rx 12, respectively as depicted in table 4. High concentration by volume of ethanol is the appropriate granulating liquid for solving such problem case of hardening. Furthermore, the decreased ratio of MDX to lactose in BJG powder from formula I to II, III, and latterly IV would decrease the conc. of ethanol used from 90% to 80%, 70%, and latterly 60% v/v, respectively.

Preformulation studies: Flowability of excipients, BJG spray dried powder and dry BJG granules with and without extragranular excipients

The Carr's Compressibility Index and flow characters predicted of 6 tablet excipients along with 4 formulas of spray dried BJG powder, as well as various granulations without and with external excipients were depicted in table 6 and table 7, respectively. For tablet excipients, MDX, magnesium stearate, and fumed silica possessed poor flowability, whereas lactose and croscarmellose sodium possessed very poor flowability. However, MCC showed fair flow character. For various spray dried BJG powder, all formulas possessed poor flowability.

For dried granulations without external excipients, all formulation showed fair to passable flowability whereas those with such excipients possessed good flowability. These indicated that the wet granulation method of all formulas of spray dried BJG could improve the flowability of spray dried BJG powder. Furthermore, the addition of external excipients could improve the flowability of such dried granulations of all tablet Rxs 1 - 12.

Tablet weight variation

From the requirements of dietary supplements, USP 40 [19], mean weight of all tablet formulations were 599.0 mg - 609.3 mg which were more than 324 mg, were all met the requirements because no tablets differed from the mean weight by more than 5.0% as depicted in table 8. Tablet Rx 1 provided the highest CV of 1.69% with highest maximum deviation of -3.91% from mean tablet weight. These might be caused by the highest ratio of MDX to lactose of BJG powder formula I which provided sticky granulations with lowest flowability.

Table 6. The flow characteristics of various powders predicted by Carr's Compressibility Index.

Items	Materials	Tapped Density, g/ml (SD*)	Loose Density, g/ml (SD*)	% Carr's Compressibility Index (SD*)	Flow Character
Excipients	MCC	0.419 (0.002)	0.333 (0.003)	20.45 (0.23)	Fair
	Lactose	0.761 (0.001)	0.474 (0.002)	37.74 (0.40)	Very Poor
	Maltose	0.667 (0.008)	0.480 (0.004)	27.99 (1.25)	Poor
	Croscarmellose Na	0.740 (0.010)	0.429 (0.002)	42.02 (0.68)	Very Poor
	Mg stearate	0.317 (0.003)	0.233 (0.002)	26.57 (1.21)	Poor
	Fumed Silica	0.061 (0.002)	0.045 (0.001)	26.63 (0.53)	Poor
Spray Dried BJG Powder	Formula I	0.718 (0.005)	0.489 (0.005)	31.88 (0.92)	Poor
	Formula II	0.888 (0.009)	0.612 (0.004)	31.04 (0.88)	Poor
	Formula III	0.936 (0.006)	0.648 (0.005)	30.79 (0.93)	Poor
	Formula IV	0.961 (0.003)	0.678 (0.004)	29.46 (0.32)	Poor

*(SD): standard deviation.

Table 7. The flow characteristics of various granules with and without external excipients predicted by Carr's Compressibility Index.

Item	Tablet Formulation	Tapped Density, g/ml (SD*)	Loose Density, g/ml (SD*)	% Carr's Compressibility Index (SD*)	Flow Character
Without External Excipients	Rx 1	0.528 (0.006)	0.422 (0.002)	19.95 (0.80)	Fair
	Rx 2	0.575 (0.008)	0.461 (0.005)	19.78 (1.12)	Fair
	Rx 3	0.636 (0.007)	0.494 (0.003)	22.38 (0.48)	Passable
	Rx 4	0.650 (0.007)	0.501 (0.004)	22.93 (0.80)	Passable
	Rx 5	0.528 (0.003)	0.427 (0.002)	19.11 (0.34)	Fair
	Rx 6	0.574 (0.004)	0.462 (0.003)	19.45 (0.58)	Fair
	Rx 7	0.572 (0.011)	0.464 (0.017)	18.92 (2.11)	Fair
	Rx 8	0.584 (0.012)	0.451 (0.008)	22.68 (0.52)	Passable
	Rx 9	0.510 (0.003)	0.427 (0.002)	16.22 (1.99)	Fair
	Rx 10	0.577 (0.005)	0.461 (0.005)	20.06 (1.22)	Fair
	Rx 11	0.583(0.004)	0.478 (0.002)	18.02 (1.41)	Fair
	Rx 12	0.571 (0.002)	0.450 (0.007)	21.09 (1.72)	Passable
With External Excipients	Rx 1	0.500 (0.004)	0.430 (0.003)	13.04 (0.44)	Good
	Rx 2	0.566 (0.002)	0.489 (0.003)	13.61 (0.76)	Good
	Rx 3	0.621 (0.008)	0.539 (0.007)	13.29 (0.37)	Good
	Rx 4	0.625 (0.008)	0.545 (0.012)	12.80 (0.37)	Good
	Rx 5	0.488 (0.004)	0.433 (0.005)	11.30 (1.49)	Good
	Rx 6	0.566 (0.002)	0.489 (0.003)	13.61 (0.75)	Good
	Rx 7	0.628 (0.003)	0.544 (0.003)	13.41 (0.33)	Good
	Rx 8	0.662 (0.002)	0.574 (0.008)	13.31 (1.22)	Good
	Rx 9	0.477 (0.005)	0.401 (0.007)	10.31 (1.72)	Good
	Rx 10	0.565 (0.002)	0.493 (0.003)	12.78 (0.26)	Good
	Rx 11	0.632 (0.011)	0.549 (0.011)	13.21 (0.22)	Good
	Rx 12	0.642 (0.007)	0.552 (0.006)	13.90 (0.18)	Good

*(SD): standard deviation

Table 8. Weight variation of 500 mg BJB tablets, batch tablet formulations 1 - 12.

Tablet Rx	Spray dried BJB Formula	Mean Weight, mg	Deviation from mean < 5.0% (USP)		Coefficient of Variation, %
			Maximum	Passed/ Failed	
1	I	605.1	-3.91%	Passed	1.69
2	II	605.8	-2.94%	Passed	1.17
3	III	606.6	-3.07%	Passed	1.11
4	IV	606.1	-2.99%	Passed	1.03
5	I	608.1	+2.46%	Passed	0.79
6	II	609.3	+2.26%	Passed	0.91
7	III	609.0	+2.14%	Passed	0.92
8	IV	601.5	+1.26%	Passed	0.58
9	I	599.0	-2.17%	Passed	1.27
10	II	605.4	-2.87%	Passed	1.00
11	III	603.4	+1.79%	Passed	0.88
12	IV	602.1	+2.01%	Passed	0.95

* CV is Coefficient of Variation = (Standard Deviation/Mean) x100.

Effects of content of MCC and formula of spray dried BJB powder on the hardness and thickness of BJB tablets

For tablet compression of BJB tablets from Rx 1-4 with MCC content of 6.0% of BJB powder formulas I-IV, respectively, the BJB tablets provided the mean hardness of around 5 kg with smooth and shiny surface. To obtain such smooth and shiny surface of tablets without any rough surface, BJB granules of Rx 5-8 (MCC content at 10.0%), and of Rx 9-12 (MCC content at 16.0%) must be compressed to provide harder BJB tablets of around 7 kg. From ANOVA test for hardness data of all BJB tablet Rxs as depicted in table 9, there were some significant differences among groups ($p < 0.01$). From LSD procedure of all hardness data as depicted in table 9 and as shown in Figure 4, Rx 1-4 containing MCC at 6.0 % of BJB powder formula 1-4, respectively, provided the mean hardness values which were significantly lower than those of Rx 5-12. Among Rx 5-8 and Rx 9-12, there was no significant difference among groups. This showed that the content of MCC when was changed from 6.0% to 10.0%

or 16.0%, could significantly increase the mean hardness of BJB tablets. However, among the BJB powder formulas I - IV containing fixed content of MCC, the change from any formula of sprayed dried BJB powder to another one, could not significantly affect the mean hardness of the BJB tablets.

From ANOVA test among thickness data of BJB tablets as depicted in table 9, there were some significant differences among groups ($p < 0.01$). From LSD procedure of all thickness data as depicted in table 9, and as shown in Figure 5, Rx 1-4 containing 6.0 % MCC of BJB powder formula 1-4, respectively, provided the mean thicknesses which were significantly higher than those of Rx 5-12. Among Rx 5-8 and Rx 9-12, there was no significant difference among groups. This showed that the content of MCC when was changed from 6.0 % to 10.0 % or 16.0%, could significantly decrease the mean thickness of BJB tablets. However, among the BJB powder formula I - IV containing fixed content of MCC, the change from any formula of BJB powder to another one, could not affect the mean thickness of the BJB tablets.

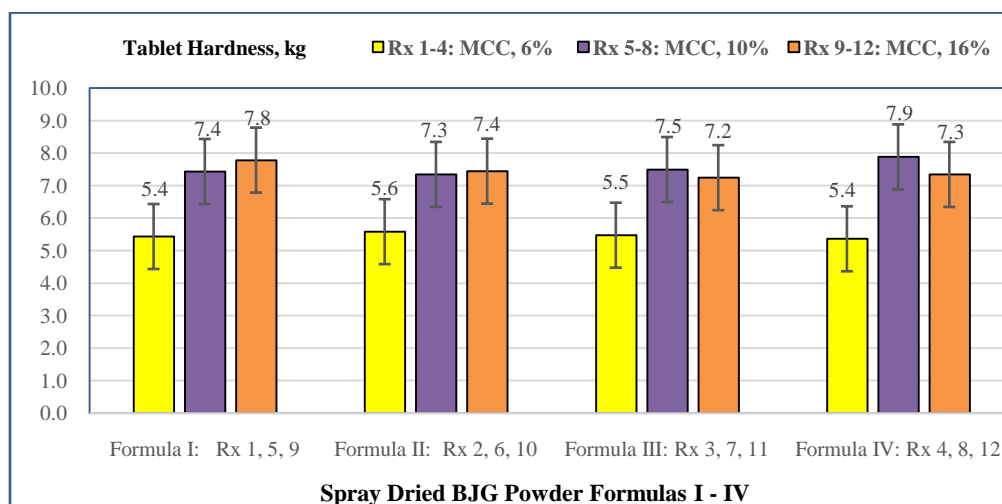


Figure 4. The mean tablet hardness of all Rxs containing various content of MCC in BJB powder formulas I- IV ± 0.94 kg ($\alpha = 0.01$, 2-tailed).

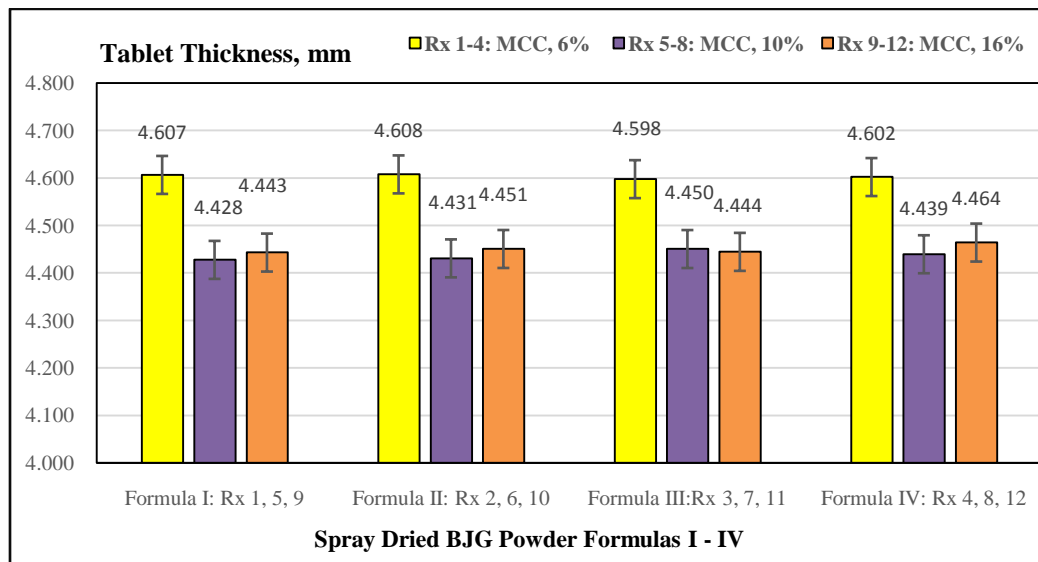


Figure 5. The mean tablet thickness of all Rxs containing various content of MCC in BJB powder formulas I- IV ± 0.037 mm ($\alpha = 0.01$, 2-tailed).

Effects of content of MCC and formula of spray dried BJB powder on the friability of BJB tablets

From ANOVA test among friability data of BJB tablets of all Rxs as depicted in table 9, there were some significant differences in the mean tablet friability among tablet Rxs ($p < 0.01$). From LSD procedure ($\alpha = 0.01$, 2-tailed) of friability data of BJB tablets of each formula of BJB powder as depicted in table 9, and as shown in Figure 6, the content of MCC could not affect the mean friability of BJB tablets of such formula. When the BJB tablets of BJB powder formula I or II were changed to formula III

and IV, the mean tablet friability would be increased significantly from 0.02-0.04 % to 0.14-0.16% and to 0.22-0.25%, respectively. However, there was no significant difference in mean tablet friability among Rxs of BJB formula I and II. These results might be caused by the reduced ratio of MDX to lactose from BJB powder formula I or II to III and IV that would reduce the interparticular bonding within BJB tablets. Furthermore, all BJB Rxs provided tablet friability of not more than 1.0% that still met the requirement of the tablet friability test, USP 40 [20].

Table 9. The necessary statistical parameters detected from ANOVA test ($p < 0.01$) and calculated from LSD procedure ($\alpha = 0.01$, 2-tailed) among various physicochemical properties of various BJB tablet formulations and BJB formulas.

Descriptions		BJG Powder Parameters	BJG Tablets Parameters			
Statistical Parameters		LOD, %	Hardness, kg	Thickness, mm	Friability, %	Disintegration time, min
p-value from ANOVA test		1.6×10^{-6} ($p < 0.01$)*	8.3×10^{-42} ($p < 0.01$)*	3.1×10^{-18} ($p < 0.01$)*	4.5×10^{-18} ($p < 0.01$)*	2.1×10^{-5} ($p < 0.01$)*
Pooled Variance, s^2		0.00215	0.6430	0.0010	0.000244	1.142
Degree of Freedom	Between group	3	11	11	11	11
	Within group	8	108	108	24	60
t (with degree of freedom of within) from t table		3.355	2.620	2.620	2.797	2.660
$1/n_i + 1/n_j$		$1/3 + 1/3$	$1/10 + 1/10$	$1/10 + 1/10$	$1/3 + 1/3$	$1/6 + 1/6$
1.0% Allowance ($\alpha = 0.01$, 2-tailed)		0.13%	0.94 kg	0.037 mm	0.036%	1.71 min

*Very highly significant.

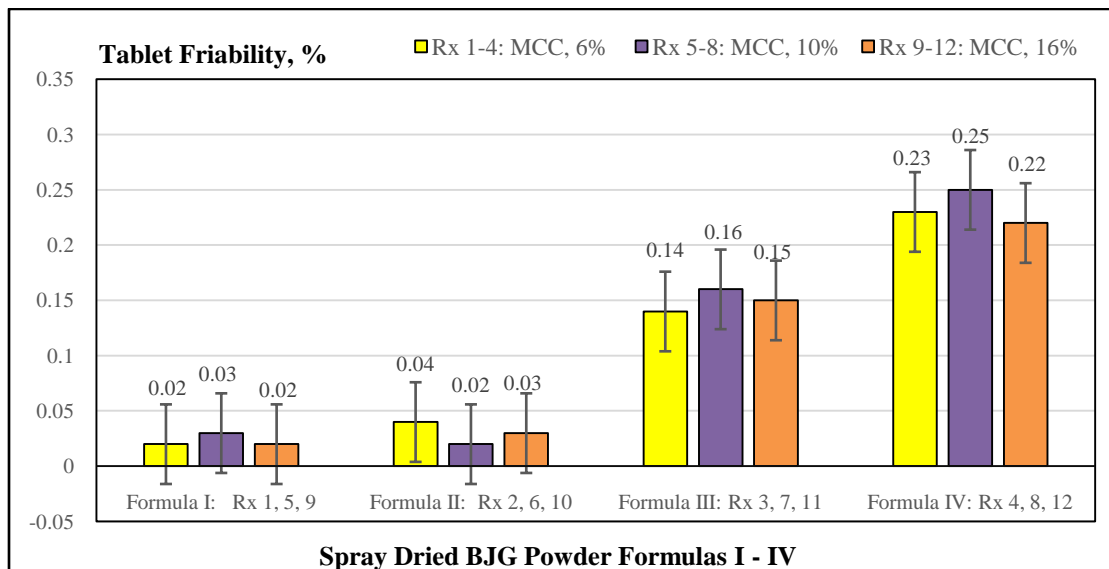
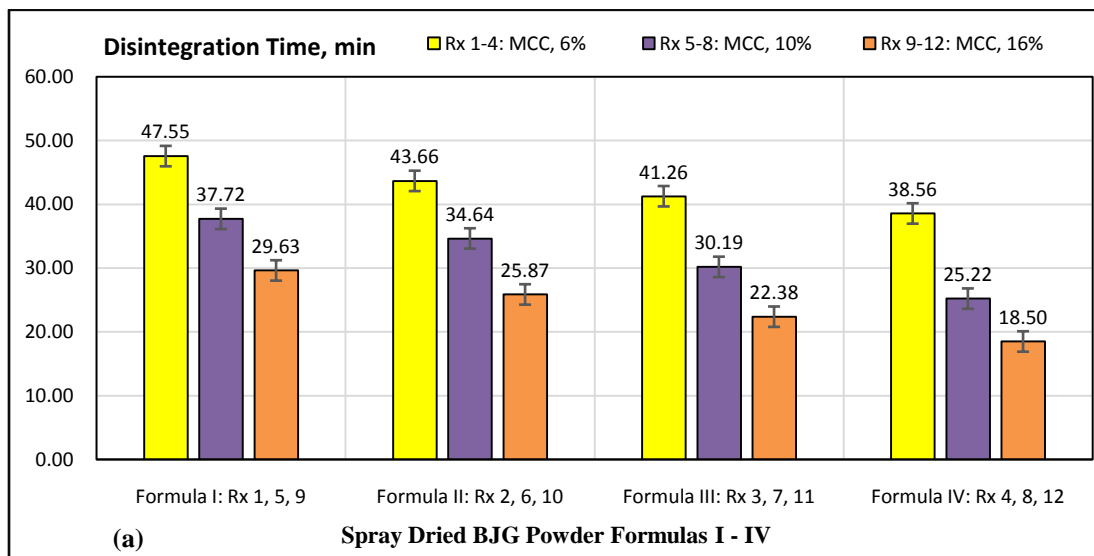


Figure 6. The mean tablet friability of all Rxs containing various content of MCC in BJJ powder formulas I- IV $\pm 0.036\%$ ($\alpha = 0.01$, 2-tailed).

Effects of content of MCC and formula of spray dried BJJ powder on the disintegration time of BJJ tablets

From ANOVA test among disintegration time (DT) data of BJJ tablets of all Rxs as depicted in table 9, there were some significant differences in mean DT among tablet Rxs ($p < 0.01$). From LSD procedure ($\alpha = 0.01$, 2-tailed) for each formula of spray dried BJJ powder as depicted in table 9, and as shown in figure 7(a), when the content of MCC was increased from 6.0 % to 10.0% and 16.0% of any BJJ powder formulas I-IV, the mean DTs were significantly decreased. This effect might be caused by the MCC was the effective intergranular disintegrant by the mechanism of diffusion, capillary, and swelling action [25-26]. Tablet Rxs containing the MCC content of 16% of BJJ formula I-V provided significantly shorter DTs

compared with those containing MCC of 6.0 %, or 10%. Furthermore, for the content of MCC was fixed at 6.0 %, 10.0 % or 16.0 % as depicted in table 9, and as shown in Figure 7(b), the change in BJJ powder formula from I to formula containing increased ratio of lactose and decreased ratio of MDX, i.e., BJJ powder formula II to IV, would significantly reduce the mean DT of BJJ tablets. This effect might be caused by lactose which is more readily to be hydrated by water and be freely dissolved in the disintegration medium, and consequently the BJJ tablet provided the faster disintegration. This indicated the best of spray dried BJJ powder was formula IV with the MCC content at 16.0%, would provide fastest mean DT of 18.50 min which met the requirement of disintegration test of dietary supplements for uncoated tablets (≤ 30 min), USP 40 [21].



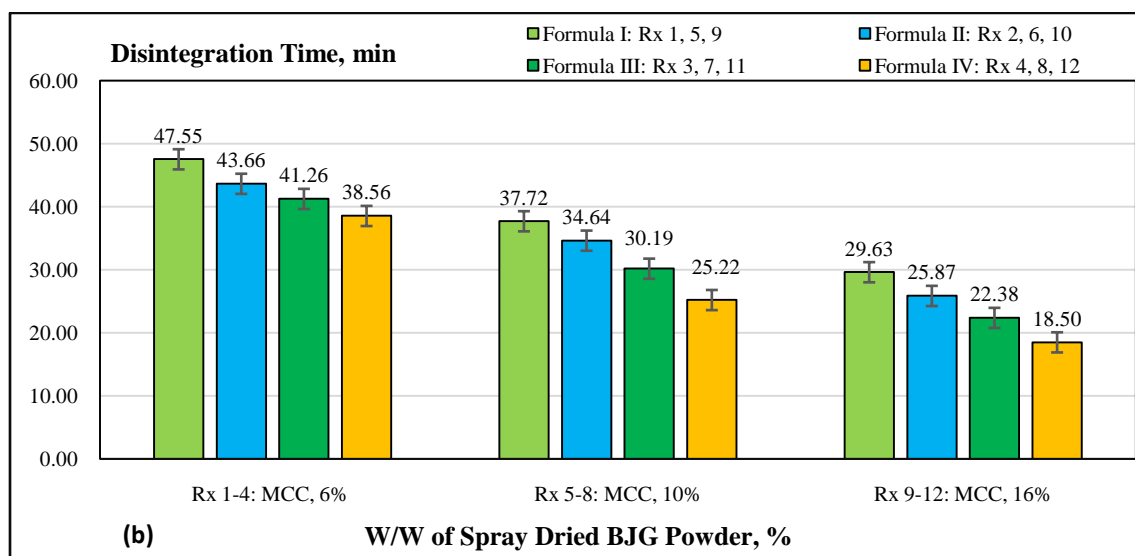


Figure 7. The mean disintegration time of all Rx's containing various content of MCC in BJG powder formulas I-IV ± 1.71 min ($\alpha = 0.01$, 2-tailed): (a) effect of content of MCC on the mean DT, (b) effect of BJG powder formula on the mean DT.

Chemical ID for BJG tablets compared with the juice sample

The hR_F of various components on TLC plate were detected under UV-light wavelength of 254 nm and 365 nm and then by spraying and reacting with p-anisaldehyde-sulfuric acid and drying at 105°C. The

results of chemical ID for fine crushed powder of BJG Rx 12 compared with sample of BJG juice without fillers were as shown in figure 8, and depicted in table 10, this result indicated that our BJG tablets Rx 12 are the same chemical ID compared with the BJG juice.

Table 10. The hR_F values of various components in BJG tablet compared with those in BJG juice sample.

Item	Fraction	hR_F	Detection		
			UV-254 nm	UV-365 nm	p-Anisaldehyde- H ₂ SO ₄
BJG Tablet Rx12	1	25	-	Soft green	Pink violet
	2	37.50	-	Violet	-
	3	45	-	Red	Pink Violet
	4	58.75	-	Fuchsia	-
	5	65	-	Soft Green	-
	6	75	-	Violet	Pink Violet
	7	80	-	Pink	Soft Yellow
	8	83.75	-	Black	Green
	9	87.50	-	Fuchsia	Brown
Sample of BJG Juice	1	25	-	Soft Green	Pink Violet
	2	37.50	-	Violet	-
	3	45	-	Red	Pink Violet
	4	58.75	-	Fuchsia	-
	5	65	-	Soft Green	-
	6	75	-	Violet	Pink Violet
	7	80	-	Pink	Soft Yellow
	8	83.75	-	Black	Green
	9	87.50	-	Fuchsia	Brown

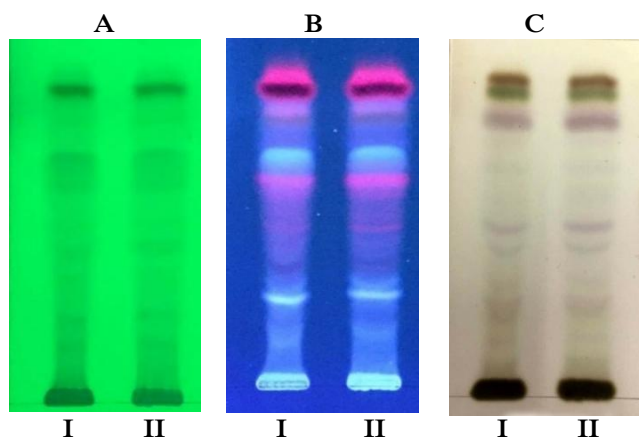


Figure 8. Chromatogram showing the ID of various components in Beijing grass by TLC method with mobile phase of ethyl acetate: methanol: water =81:11:8. (A) UV light: 254.0 nm, (B) UV light: 365.0 nm, and (C) spraying with p-anisaldehyde-sulfuric acid, (I) BJJ tablet Rx 12, and (II) sample of BJJ juice.

Conclusion

Murdannia loriformis (Hassk.) Rolla Rao *et* Kammathy, namely Beijing grass (BJG) is the favourite herb utilized to treat various types of cancer and to improve the quality of life for the patients. The purposes of this study are aimed to develop BJJ tablet formulation and spray dried BJJ powder. From spray drying, the 3 samples of BJJ juice provided mean yield of 0.31 % which was necessary to add 4.0% appropriate soluble carriers, i.e., MDX and lactose to obtain the theoretical yield of 4.31%. BJJ juice formulas I- IV with 4 different ratios of MDX: lactose, i.e., 3:1, 2:2, 1:3, and 4:0, provided spray dried BJJ powders formulas I- IV with the similar collection efficiency of around 80%, and with the LOD of 6.42%, 6.22%, 6.04, and 5.82%, respectively. The decrease in MDX content and the increase in lactose content with less hygroscopicity in the formulas would significantly reduce the LOD from formula I to IV of spray dried BJJ powder.

BJG tablets were prepared for 12 Rx by the wet granulation technique for previous spray dried BJJ powder formulas I- IV. MCC was used as the binder and disintegrant at the content of 6.0% for Rx 1- 4, 10.0% for Rx 5- 8, and 16.0 % for Rx 9 – 12 of each formula of spray dried BJJ powder, respectively. Croscarmellose sodium, magnesium stearate, and fumed silica, were used at 2.50%, 0.50%, and 0.25% by weight of tablet as the external disintegrant, lubricant and glidant, respectively. All Rx with the tablet weight of around 600 mg met the weight variation criteria of Dietary Supplements USP. Rx 1- 4 provided tablets with 5 kg hardness and greater thickness whereas Rx 5-12 provided tablets with significantly higher hardness of 7 kg and thinner thickness. For tablet friability, the decreased content of

MDX in spray dried BJJ powder of formula III (Rx 3, 7, and 11) and formula IV (Rx 4, 8, and 12) would significantly cause more friable tablets than that of formula I (Rx 1, 5, and 9) and formula II (Rx 2, 6, and 10), whereas the increase in content of MCC could not significantly affect the friability of BJJ tablets. For tablet disintegration, both the increase in content of lactose in spray dried BJJ powder and in MCC content in tablet formulation could significantly reduce the disintegration time (DT). Rx 12 with MCC at 16% in the BJJ powder formula IV provided shortest DT of about 19 min.

From chemical identification tests, similarly, the same results were obtained from BJJ tablet Rx 12 compared with the sample of BJJ juice without carrier. Each TLC plate showed all 9 isolated spots under UV-light wavelength of 254 nm and 365 nm, and when spraying and reacting with p-anisaldehyde-sulfuric acid, the 3 pink violet spots with the R_F of 25, 45, and 75 might be various glycosphingolipids with the same molecular structures of BJJ tablet compared with the sample of BJJ juice.

Acknowledgment

The authors are very thankful to the Research and Development Institute, Bansomdejchaopraya, Rajabhat University for providing a research fund for this study. I would also like to sincerely thank Dr. Satit Puttipipatkachorn, Assoc. Prof., the head of Department of Manufacturing Pharmacy, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand, for providing laboratory facilities and instrumentation. The last acknowledgement is given to Miss Petnumpung Rodpo, the instructor at the Program in Thai Traditional Medicine, Faculty of Science and Technology, Bansomdejchaopraya Rajabhat University, Bangkok 10600, Thailand for her nice service in first authentication of BJJ with the certain herbarium number of identification.

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