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INTRODUCTION

Mangosteen (*Garcinia mangostana* Linn.) pericarps have been used as a traditional medicine for treatment of skin infection for many years. Mangostins, gartanin, garcinone E are xanthones that occurs naturally in mangosteen[1)]. Due to biological activities of mangostins, they are popularly used in herbal cosmetic products[2)]. However, the stability data of mangostins has been lacking in the literature. In order to develop a suitable mangostins solution, inactive ingredients affecting the stability of total mangostins need to be identified. Design of experiments (DOE) has been used widely for formulation development. The major advantage of DOE for product development is that it allows all factors to be evaluated simultaneously, systematically, and quickly. The Plackett-Burman experimental design (PBED) has been used for screening independent factors that effect on potency of dependent factor. The results of PBED show a ranking of the independent factors and signs of effects to indicate that an increasing of a factor value is advantage or not[3)]. In the present study, PBED was used to evaluate the effect of inactive ingredients on the stability of total mangostins in preparation.

MATERIALS AND METHODS

Plant materials: The dried pericarps of *Garcinia mangostana* Linn. were purchased from Jao-Kom-Per Co., Ltd. (Bangkok, Thailand). The pericarps of mangosteen were identified and verified with organoleptic testing. They were washed 3 times with distilled water and dried at 40-50 °C in an oven. The clean and dried pericarps were ground with the cutting mill SM 100 (Retsch®, Germany). The dried mangosteen powder was stored at room temperature in a dark and dry place.

Extraction of mangosteen powder: The dried mangosteen powder was extracted with 95% ethanol by a maceration method. Extraction was carried out for 9 days with 3 cycles. The extracts were collected and filtrated by a filter paper (Whatman® no.1) with vacuum suction pump. The ethanol extract was evaporated to dryness with vacuum rotary evaporator (Buchi Rotavapor® model R-210, Switzerland). The dried extract powder was preserved in a refrigerator at 4 °C before analysis.

Quantitative analysis of total mangostins: The quantitative analysis of mangostins was determined by a UV-Vis spectrophotometer (model EX30, Varian, USA)[4)]. The wavelength was set at 320 nm. The content of total mangostins, calculated as α–mangostin, in dried extract was 71.83 ± 0.28%.

Preparation of dried extract solutions and stability study: The master formulation of dried extract preparation consisted of 1%w/v dried extract powder, 90%v/v propylene glycol, 5%v/v glycerol and deionized water. In this study, six independent variables (pH of the solution, butylated hydroxytoluene, disodium EDTA, sodium metabisulfite, sodium sulfite and sodium thiosulfate) were chosen. The total number of experimental formulations was eight. For example, experimental formulation no. 1 (Table 1) comprised disodium EDTA, butylated hydroxytoluene, sodium sulfite and pH at 7.5. Dried extract solutions were prepared by dissolving dried extract powder in propylene glycol. Ingredients as listed in formulation were dissolved in glycerol and mixed with a magnetic stirrer. The final volume was adjusted to 100 ml with deionized water. The dried extract preparations were kept in amber glass bottles, stored at 45°C for 10 days. The remaining amount of total mangostins was assayed by UV-Vis spectrophotometer (n=3). The percentage of the remaining amount of total mangostins was calculated as a relative percentage of initial amount of total mangostins at initial time to amount of total mangostins at final time.

Experimental design: The PBED of eight formulations with seven variables, is given in Table 1. Six ingredients (independent variables) and one dummy variable (Table 2) were used to evaluate the relative importance of various ingredients. The factors significant at 95% level (p-value < 0.05) were considered. A statistical analysis was performed using a multiple linear regression with the Microsoft® Excel 2007 software.

Table 1: Plackett-Burman experimental design of eight experimental formulations with seven variables. High and low levels are indicated as (+) and (-), respectively.

Experimental formulation no.	X_1	X_2	X_3	X_4	X_5	X_6	X_7	Percentage of total mangostins remaining
1	+	+	+	-	+	-	-	46.32
2	-	+	+	+	-	+	-	68.95
3	-	-	+	+	+	-	+	54.87
4	+	-	-	+	+	+	-	45.38
5	-	+	-	-	+	+	+	53.17
6	+	-	+	-	-	+	+	55.89
7	+	+	-	+	-	-	+	51.43
8	-	-	-	-	-	-	-	57.39

Table 2: High and low levels of independent variables.

Independent variables (symbol code)	Low level (-)	High level (+)
pH of solution (X_1)	5.5	7.5
Disodium EDTA (X_2)	0	0.1%
Butylated hydroxytoluene (X_3)	0	0.002%
Sodium metabisulfite (X_4)	0	0.2%
Sodium sulfite (X_5)	0	0.2%
Sodium thiosulfate (X_6)	0	0.2%
Dummy 1 (X_7)	-	-

RESULTS

The effect of ingredients on the stability of total mangostins in preparation was examined in the PBED with eight different experimental formulations. The formulation number of eight represented only the effect of an acidic environment. The highest and lowest percentage of total mangostins remaining were observed for formulation no. 2 and 4, respectively. The main effect of each ingredient on the percentage of the remaining total mangostins was calculated (Table 3). The main effect with negative sign indicates that the high concentration of this ingredient destabilized total mangostins. On the other hand, the positive sign showed that the high concentration of ingredient stabilized total mangostins. In this experiment, disodium EDTA, butylated hydroxytoluene, sodium metabisulfite and sodium thiosulfate showed a positive effect whereas the pH of solution and sodium sulfite had a negative effect. The statistical analysis (t-value) demonstrated that the pH of solution had a significantly negative influence on the stability of total mangostins.

Table 3: The main effect, t-value and *p*-value for the determination of variable significance in PBED

Independent variables	Main effect	t-value	*p*-value (* significant at 95% confidential interval)
pH of solution	-8.84	-13.19	0.0482*
Disodium EDTA	1.59	2.37	0.2546
Butylated hydroxytoluene	4.67	6.96	0.0908
Sodium metabisulfite	1.97	2.93	0.2092
Sodium sulfite	-8.48	-12.66	0.0502
Sodium thiosulfate	3.35	4.99	0.1258

DISCCUSION

The results indicate that the pH of solution is the most effective variable on stability of total mangostins, based on the criterion of *p*-value less than 0.05. When the pH of solution was increased, the stability of total mangostins was decreased. The result obtained was similar to those of Lourith et al[5]. Mangostins are polyphenolic compounds that showed potent antioxidant activity[6]. The antioxidant activity of mangostins is based on the redox property of compounds which allows it to act as reducing agents[1]. Polyphenols can be oxidized through to quinone forms easily. In solution the oxidation reaction is more a higher pH, due to a higher percentage of the phenolate that reacts with oxygen[7].

CONCLUSION

The PBED was recommended for preformulation compatibility evaluation because it offers an efficient method to identify the significant parameter that should be considered for the formulation study. In the present study, the lower pH of solution identified by PBED is the important parameter for improving the stability of total mangostins in preparation. The other ingredients, including disodium EDTA, butylated hydroxytoluene, sodium metabisulfite, sodium thiosulfate and sodium sulfite did not a effect the stability of total mangostins.

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