Journal of Current Science and Technology, January-April 2021 JCST Vol. 11 No.1, pp. 100-113

ISSN 2630-0656 (Online)

Cite this article: Suksaeree, J., & Monton, C. (2021, January). Evaluation of the interaction of phenolic compounds contained in the Trisamo recipe using simplex lattice design. *Journal of Current Science and Technology, 11*(1), 100-113. DOI:

Journal of Current Science and Technology

Journal homepage: https://jcst.rsu.ac.th

Evaluation of the interaction of phenolic compounds contained in the Trisamo recipe using simplex lattice design

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Received 13 October 2020; Revised 16 December 2020; Accepted 23 December 2020; Published online 29 January 2021

Abstract

This work sought to apply the simplex lattice design to determine the interaction between *Terminalia chebula* Retz. var. *chebula*, *Terminalia arjuna* Wight and Arn., and *Terminalia bellirica* (Gaertn.) Roxb., which are found in the traditional Trisamo recipe. The phenolic compounds gallic acid, corilagin, chebulagic acid, and chebulinic acid were analyzed using validated high-performance liquid chromatography. The results showed that a broader range of positive interaction was found in the decoction samples as opposed to the infusion samples. Moreover, it was determined that the original Trisamo recipe, which boasts an equal weight ratio of all three *Terminalia* plants, obtained from the decoction, exhibited a 250% increase in total content of the phenolic compounds as compared with the effects of any of the plants individually, while the original Trisamo recipe obtained from the infusion group revealed a 200% increase in total content of the phenolic compounds when compared with the results of an individual plant. Data from this work could be used to describe the synergism of plant compositions of Trisamo based on the chemical interactions, by enhancing extraction efficiency of total phenolic compounds. Moreover, they may support that the use of the Trisamo with an equal weight ratio distribution of the three *Terminalia* plants is in fact already appropriate.

Keywords**:** *chemical interaction; optimization; phenolic compound; simplex lattice design; Terminalia plants; Trisamo.*

1. Introduction

Trisamo is an herbal recipe that has been used for many centuries in Thai traditional medicine. Trisamo means "three *Terminalia* plants," with "Tri" meaning three and "Samo" meaning *Terminalia* plant. As such, Trisamo recipe consists of three *Terminalia* plants: *Terminalia chebula* Retz. var. *chebula* (*Samo Thai* in the Thai language), *Terminalia arjuna* Wight and Arn. (*Samo Thet*), and *Terminalia bellirica* (Gaertn.) Roxb. (*Samo Phiphek*). In Thai traditional medicine, Trisamo is used as an antipyretic and expectorant, with the ability to promote general good health and being particularly good for relieving abdominal bloating (Nanna, Jaijoy, Soonthornchareonnon, & Sireeratawong, 2015). A water extract stemming from Trisamo was shown previously to have antipyretic and analgesic properties in animal models. Antipyretic activity is related to the inhibition of prostaglandins, while analgesic activity may inhibit the biosynthesis and/or the release of some pain mediators (Nanna et al., 2015). The ethanolic extract of Trisamo exhibits antibacterial activity against *Escherichia coli* and *Salmonella* spp. with minimum inhibitory concentrations of 256 µg/mL and 512 µg/mL, respectively (Asae, Meemak, Poonthananiwatkul, Hemtrakoonwong, & Sama-ae, 2019). Further,

several phenolic compounds typically hold important roles as antioxidants (Li, Wu, & Huang, 2009; Piluzza & Bullitta, 2011; Turumtay et al., 2014; Vamanu & Nita, 2013). The *Terminalia* plants contain several phenolic compounds (Charoenchai, Pathompak, Madaka, Settharaksa, & Saingam, 2016; Deshmukh, Pawar, Tapre, & Deshmukh, 2019; Mahajan & Pai, 2010; Sheng, Zhao, Muhammad, & Zhang, 2018), meaning that this recipe exhibits a high level of antioxidant activity.

Two or more drugs that can produce similar effects individually will sometimes exhibit superior effects when given in combination with one another. When a combined effect of at least two components in a system is greater than that of an individual component, this is known as synergism (Tallarida, 2011). Synergism can enhance therapeutic or biological activities due to positive interactions among the different components contained in a combination (Save & Choudhary, 2017).

Combining herbal plants in traditional medicines is usually expected to boost the therapeutic effects and reduce side effects. Synergism in a traditional herbal recipe can occur in various ways, such as synergism among herbs or among other components in the prescription—for example, among the bioactive compounds of herbs (Yuan et al., 2017). In this case, chemical interaction can be used to describe the synergism of herbal recipe. The performance of a response surface analysis can provide a full explanation of any combinatory effects over a large number of doses (Foucquier & Guedj, 2015). So, the response surface analysis will be applied to the work to clarify the chemical interactions of the herbal recipe.

The phenolic compounds possesses antioxidant activity, several previous studies demonstrated that the total phenolic content correlates to the antioxidant activity (Annegowda, Nee, Mordi, Ramanathan, & Mansor, 2010; Aryal et al., 2019; Gan, Feng, He, Li, & Zhang, 2017; Rajurkar & Hande, 2011; Saha & Verma, 2016; Wanigasekera, Joganathan, Pethiyagoda, Yatiwella, & Attanayake, 2019). The data indicated that phenolic compounds derived from plants were effective natural antioxidants, and thus might contribute significantly to the protective effects in human (Rice-Evans, Miller, & Paganga, 1997). According to the interaction of plant chemical constituents, increasing phenolic compounds could increase antioxidant activity or other related activities.

Our previous work demonstrated the chemical interaction of Traditional herbal remedies, i.e., Triphala which was composed of equal mass ratios of *Phyllathus emblica*, *T. chebula* and *T. bellirica* (Monton, Wunnakup, Suksaeree, Charoenchai, & Chankana, 2020). We also found that decoction of Triphala gave more extraction yield and content of phenolic compounds. However, the chemical interaction of Trisamo obtained from the conventional extraction methods; decoction and infusion, was not previously reported.

2. Objectives

The objective of this study was determine the chemical interaction of the three *Terminalia* plants (*T. chebula*, *T. arjuna*, and *T. bellirica*) contained within the Trisamo recipe using a simplex lattice design to clarify how the plant mixture could increase the extraction efficiency of the active chemical constituents. It could be used to describe the synergism of the Trisamo recipe in terms of chemical analysis. In addition, the effect of two different extraction techniques—decoction and infusion—on the content of phenolic compounds were compared, to use as a guide for the selection of an extraction technique to maximize active chemical constituents. We hope that the results could support the synergism and the appropriate application of Trisamo in traditional medicine.

3. Materials and methods

3.1 Materials

The standards for the phenolic compounds gallic acid, corilagin, chebulagic acid, and chebulinic acid were purchased from Chengdu Biopurify Phytochemicals Ltd. (Chengdu, China). The four standards had their purity determined by high-performance liquid chromatography (HPLC) as 99.88%, 99.67%, 99.62%, and 98.42%, respectively. The solvents used in this work were of analytical and HPLC grades.

3.2 Preparation of the *Terminalia* plants

Dried fruits from the *T. chebula*, *T. arjuna*, and *T. bellirica* plants were purchased from Charoensuk Osod (Nakhon Pathom Province, Thailand). All of the plants were authenticated by Ajarn Nirun Vipunngeun, a plant taxonomist and lecturer at the College of Pharmacy at Rangsit

University, and were deposited at the college's Drug and Herbal Product Research and Development Center. The voucher specimens were coded as CM-TC001-1-04-2019 (*T. chebula*), CM-TA001-1-07-2019 (*T. arjuna*), and CM-TB001-1-04-2019 (*T. bellirica*). Any dried fruits that did not contain seeds were ground using a grinder and were stored in a dry place until use.

3.3 Simplex lattice experimental design for the extraction of plant samples

The simplex lattice design was applied during this work. The independent variables of this work were the weight ratio of *T. chebula* (X_1), *T. arjuna* (X_2), and *T. bellirica* (X_3). The weight ratio ranged from 0 to 1 and the summation of X_1, X_2, and X_3 was equal to 1. The coded and actual values of the simplex lattice design are shown in Table 1, where F1 to F3 involve a single-herb formula, F4 to F6 involve a two-herb formula, and F7 to F12 involve a three-herb formula. Additionally, F10 to F12 consisted of the same three-herb formula, which was repeated at the center point of the experimental design.

The formulas from the powder mixtures were extracted by either decoction or infusion. Decoction involved 6 g of the powder mixture of the *Terminalia* plants being added to a tea bag before being boiled in 50 mL of water for 15 minutes. This process was repeated three times. Next, the filtrates from each extraction round were filtered through Whatman® filter paper no. 1 before being pooled and freeze-dried at -60ºC for 24 h (SCIenergy Solution Ltd. Co., Nonthaburi, Thailand). The infusion process followed the same procedure, except that 50 mL of boiling water was added to the mixture, which was then left to stand for 15 minutes and repeated three times rather than being actively boiled.

The freeze-dried extract powder was kept in a desiccator until use. The contents of the four phenolic compounds were then analyzed by validated HPLC. The six dependent variables—extraction yield (Y_1), gallic acid content (Y_2), corilagin content (Y_3), chebulagic acid content (Y_4), chebulinic acid content (Y_5), and total content of the phenolic compounds (Y_6)—were monitored and used to produce the three-dimensional (3D) response surface using Design-Expert® 11.0. Additionally, the equations for the prediction of each dependent variable were also reported.

Table 1 Coded and actual values of the simplex lattice experimental design

Formula	Coded value			Actual value (g)		
	X_1	X_2	X_3	*T. chebula*	*T. arjuna*	*T. bellirica*
F1	1.00	0.00	0.00	6	0	0
F2	0.00	1.00	0.00	0	6	0
F3	0.00	0.00	1.00	0	0	6
F4	0.50	0.50	0.00	3	3	0
F5	0.50	0.00	0.50	3	0	3
F6	0.00	0.50	0.50	0	3	3
F7	0.67	0.17	0.17	4	1	1
F8	0.17	0.67	0.17	1	4	1
F9	0.17	0.17	0.67	1	1	4
F10	0.33	0.33	0.33	2	2	2
F11	0.33	0.33	0.33	2	2	2
F12	0.33	0.33	0.33	2	2	2

3.4 Preparation of standard phenolic compounds and plant extract solution

HPLC was used to analyze the four phenolic compounds of gallic acid, corilagin, chebulagic acid, and chebulinic acid. The stock solution of each phenolic compound in a concentration of 1 mg/mL was prepared using methanol as a solvent. Next, the samples were prepared as a mixed standard solution in concentrations of 10, 25, 50, 100, 150, and 200 μg/mL before being filtered and injected into the HPLC instrument. The calibration curves of each standard phenolic compound were then plotted between the peak area and concentration of the standard compounds. The aqueous solutions of the extract of F1 to F12 were prepared in a concentration of 2 mg/mL before being filtered and analyzed by HPLC. The content of each phenolic compound was calculated according to the calibration curve of each standard phenolic compound.

3.5 HPLC conditions for the analysis of phenolic compounds

The Agilent 1260 Infinity HPLC system (Agilent Technologies, Santa Clara, CA, USA) was used to analyze the phenolic compounds. The gradient elution was conducted on an ACE C18-PFP column (250 × 4.6 mm, internal diameter, 5 μm) and was controlled at 25ºC. The mobile phase was composed of acetonitrile (A) and 1% acetic acid aqueous solution (B).

The gradient system was based on the report by Charoenchai et al. (2016) and validated by Monton et al. (2020), and consisted of 5% A at zero to one minutes, 5% A to 10% A at one to four minutes, 10% to 15% at four to 12 minutes, 15% A to 35% A at 12 to 32 minutes, 35% A to 50% A at 32 to 35 minutes, 50% A to 100% A at 35 to 37 minutes, 100% A at 37 to 40 minutes, 100% A to 5% A at 40 to 41 minutes, and 5% A at 41 to 45 minutes. The mobile phase flow rate was 1 mL/min, while the injection volume was 10 μL and the detection wavelength was 270 nm.

3.6 Investigation of the chemical interaction of *Terminalia* plants contained in the Trisamo recipe

The chemical interaction of the specified *Terminalia* plants was investigated based on the response additivity approach (Foucquier & Guedj, 2015). Positive interaction occurs when the observed content of phenolic compounds from the combination plants is greater than the expected additive content of phenolic compounds given by the summation of the individual plants. In this study, the combination index (CI) was used as a tool for investigating chemical interaction (Zhou et al., 2016), as shown in Equation 1:

$$CI = \frac{E_A+E_B+E_C}{E_{combination}} \qquad \text{Eq.1}$$

where E_A, E_B, and E_C were the individual effects of *T. chebula*, *T. arjuna*, and *T. bellirica*, respectively, while $E_{combination}$ was the observed combination effect for *T. chebula*, *T. arjuna*, or *T. bellirica*. CI values of less than 1, equal to 1, and greater than 1 were considered to represent outcomes of positive interaction, additive effect, and negative interaction, respectively (Foucquier & Guedj, 2015; Zhou et al., 2016).

The CI values of the six dependent variables—extraction yield (Y_7), gallic acid content (Y_8), corilagin content (Y_9), chebulagic acid content (Y_{10}), chebulinic acid content (Y_{11}), and total content of phenolic compounds (Y_{12})—were also observed. The contour plots of each dependent variable were produced by Design-Expert® 11.0. The equations to predict each dependent variable were created and the predicted versus actual value plots were also produced.

The coefficient of determination (r^2) and adjusted r^2 were reported to describe the level of correlation between the predicted and actual values. Internally studentized residuals versus run plots were also produced in order to establish the level of data distribution. The contour plots of the desirability value of the optimal condition, which provided the minimum CI values of the total content of the phenolic compounds (Y_{12}), were created. In this case, the weight ratio of *T. chebula* (X_1), *T. arjuna* (X_2), and *T. bellirica* (X_3) were set to "in range" and the CI values of the total content of the phenolic compounds (Y_{12}) was set to "minimize". The overlay plots that revealed CI values of the total content of phenolic compounds (Y_{12}) of less than 0.5 and 0.4 were reported for the infusion and decoction groups, where the total phenolic compounds were presented as two and 2.5 times the effect of a single *Terminalia* plant, respectively, for the same two groups.

4. Results and discussion

4.1 Phenolic compounds of *Terminalia* plants in various proportions

The four phenolic compounds specified in this study were analyzed by validated HPLC and examples of the HPLC chromatograms of the decocted extract of *T. chebula* (F1), *T. arjuna* (F2), and *T. bellirica* (F3) and the Trisamo recipe (F10) are shown in Figure 1. The largest phenolic compounds found in the decoction extracts of *T. chebula*, *T. arjuna*, and *T. bellirica*, were chebulagic acid, chebulinic acid, and chebulagic acid, respectively. While the extraction yield from the decoction group was higher than that from the infusion group, larger amounts of the phenolic compounds were found in the infusion group. Prior reports have been made detailing the variation among phenolic compounds contained in the *Terminalia* plants. According to earlier research, the fruits of *T. chebula* contained gallic acid, methyl gallate, ethyl gallate, chebulagic acid, tetra-O-galloyl-β-D-glucose, ellagic acid, chebulinic acid, and penta-O-galloyl-β-D-glucose (Mahajan & Pai, 2010); the fruits of *T. bellirica* contained gallic acid,

corilagin, chebulagic acid, rutin, and chebulinic acid (Charoenchai et al., 2016); and the fruits of *T. chebula* contained gallic acid, rutin, 5-O-galloylshikimic acid, corilagin, 3,4,8,9,10-pentahydroxydibenzo [b,d] pyran-6-one, and ellagic acid (Sheng et al., 2018). Elsewhere, another study found that the fruits of *T. arjuna* contained gallic acid; the fruits of *T. bellirica* contained gallic acid, ellagic acid, and chebulinic acid; and the fruits of *T. chebula* contained gallic acid, chebulagic acid, ellagic acid, and chebulinic acid (Deshmukh et al., 2019).

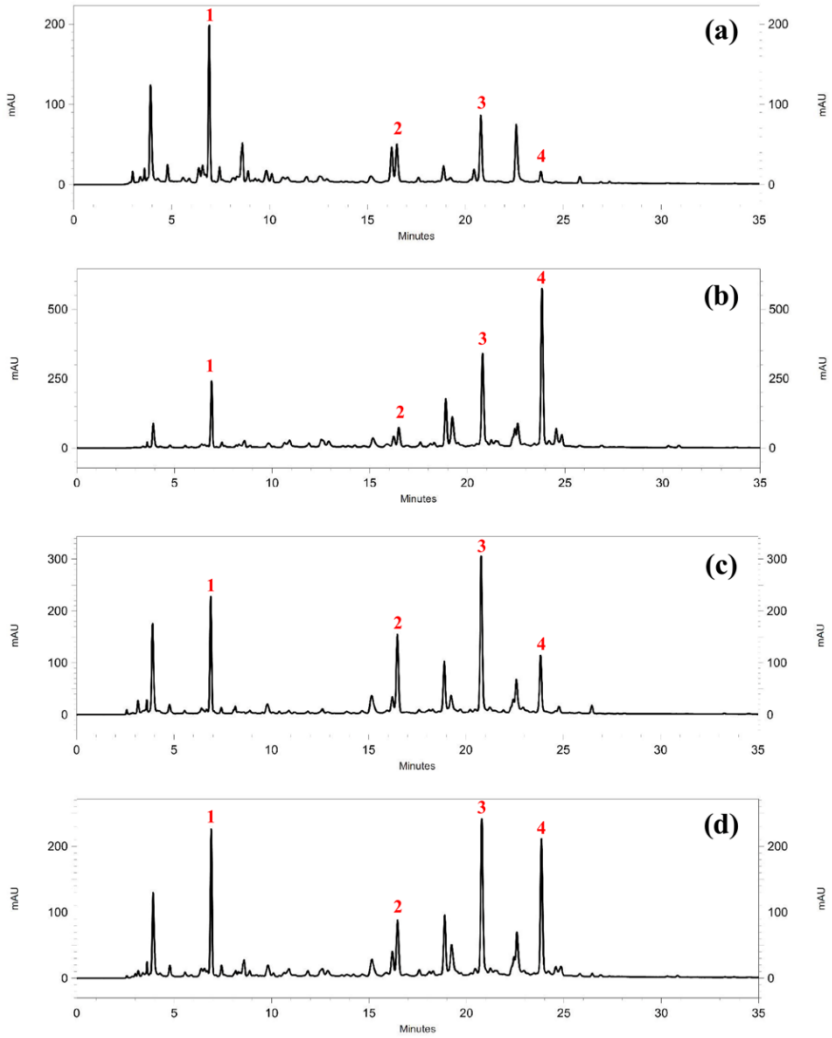

Figure 1 HPLC chromatograms of the decoction extract of (a) F1, (b) F2, (c) F3, and (d) F10 in a concentration of 2 mg/mL. The numbers (1) to (4) show the peaks of gallic acid, corilagin, chebulagic acid, and chebulinic acid, respectively

The mathematical equations for the prediction of the dependent variables (Y_1–Y_6) of the decoction and infusion groups are shown in Equations 2 to 13. The terms of the significant model and p-value for the independent variables are shown in Tables 2 and 3. According to the mathematic model of the variables, all dependent variables were fitted to the quadratic model, except for Y_1 in the decoction group and Y_5 in the infusion group, which were fitted to the linear model. This means that the terms X_1X_2, X_1X_3, and X_2X_3 did not affect Y_1 in the decoction group or Y_5 in the infusion group. Overall, p-values of less than 0.05 indicated that the model terms were significant. A significant model and non-significant lack of fit were required for the model to fit. In this case, the model was

significant for all dependent variables except for Y_1 in the infusion group. Moreover, the lack of fit was not significant for any dependent variables of the decoction and infusion groups. Conversely, the linear mixture was significant for various dependent variables except for Y_2 in the decoction group and Y_1, Y_2, and Y_3 in the infusion group. X_1X_2 was a significant term for all of the dependent variables except for Y_1 in the infusion group; X_1X_3 was a significant term for all dependent variables except for Y_5 in the decoction group and Y_1, Y_4, and Y_6 in the infusion group; and X_2X_3 was a significant term for all of the dependent variables except for Y_5 in the decoction group and Y_1 in the infusion group.

Decoction

$$Y_1 = 32.48X_1 + 64.31X_2 + 38.21X_3 \quad \text{Eq. 2}$$
$$Y_2 = 0.34X_1 + 0.37X_2 + 0.24X_3 + 1.57X_1X_2 + 1.53X_1X_3 + 1.42X_2X_3 \quad \text{Eq. 3}$$
$$Y_3 = 0.15X_1 + 0.21X_2 + 0.40X_3 + 0.98X_1X_2 + 1.85X_1X_3 + 1.63X_2X_3 \quad \text{Eq. 4}$$
$$Y_4 = 0.47X_1 + 1.93X_2 + 1.14X_3 + 7.54X_1X_2 + 4.85X_1X_3 + 5.70X_2X_3 \quad \text{Eq. 5}$$
$$Y_5 = 0.01X_1 + 2.62X_2 + 0.36X_3 + 8.09X_1X_2 + 0.24X_1X_3 + 2.53X_2X_3 \quad \text{Eq. 6}$$
$$Y_6 = 0.98X_1 + 5.14X_2 + 2.14X_3 + 18.19X_1X_2 + 8.47X_1X_3 + 11.28X_2X_3 \quad \text{Eq. 7}$$

Infusion

$$Y_1 = 23.03X_1 + 25.63X_2 + 25.83X_3 - 27.63X_1X_2 + 27.46X_1X_3 - 15.38X_2X_3 \quad \text{Eq. 8}$$
$$Y_2 = 0.29X_1 + 0.30X_2 + 0.30X_3 + 1.86X_1X_2 + 1.40X_1X_3 + 1.51X_2X_3 \quad \text{Eq. 9}$$
$$Y_3 = 0.14X_1 + 0.16X_2 + 0.26X_3 + 0.75X_1X_2 + 1.11X_1X_3 + 1.38X_2X_3 \quad \text{Eq. 10}$$
$$Y_4 = 0.64X_1 + 2.05X_2 + 1.23X_3 + 5.46X_1X_2 + 3.97X_1X_3 + 6.63X_2X_3 \quad \text{Eq. 11}$$
$$Y_5 = 0.74X_1 + 3.90X_2 + 0.80X_3 \quad \text{Eq. 12}$$
$$Y_6 = 1.29X_1 + 5.57X_2 + 2.33X_3 + 13.16X_1X_2 + 6.38X_1X_3 + 12.29X_2X_3 \quad \text{Eq. 13}$$

Table 2 Terms of the significant model and p-value for the independent variables (Y_1-Y_6) of decoction

Polynomial term	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6
Model	0.0003[a]	0.0011[a]	0.0018[a]	0.0002[a]	0.0002[a]	0.0002[a]
Linear mixture	0.0003[a]	0.2534	0.0233[a]	0.0026[a]	0.0001[a]	0.0006[a]
X_1X_2	-	0.0015[a]	0.0289[a]	0.0002[a]	0.0004[a]	0.0002[a]
X_1X_3	-	0.0017[a]	0.0017[a]	0.0024[a]	0.8430	0.0095[a]
X_2X_3	-	0.0025[a]	0.0032[a]	0.0010[a]	0.0729	0.0025[a]
Lack of fit	0.9274	0.3004	0.6673	0.7022	0.2971	0.6573

[a] Significant value

Table 3 Terms of the significant model and p-value for the independent variables (Y_1-Y_6) of infusion

Polynomial term	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6
Model	0.1879	0.0037[a]	0.0028[a]	0.0145[a]	0.0019[a]	0.0110[a]
Linear mixture	0.3209	0.8748	0.0686	0.0469[a]	0.0019[a]	0.0121[a]
X_1X_2	0.1032	0.0024[a]	0.0275[a]	0.0311[a]	-	0.0230[a]
X_1X_3	0.1049	0.0093[a]	0.0052[a]	0.0875	-	0.1918
X_2X_3	0.3261	0.0065[a]	0.0018[a]	0.0145[a]	-	0.0299[a]
Lack of fit	0.5985	0.5726	0.9053	0.7843	0.3035	0.6632

[a]Significant value

Figure 2 displays the 3D response surfaces of the model conditions of the dependent variables. The low, medium, and high weight ratios were based on the experimental design, for example the low, medium, and high weight ratios of each plant were approximately 1, 3, and 6 g, respectively. In the decoction group, the highest extraction yields were found at a low weight ratio for *T. chebula* and *T. bellirica* and in the high weight ratio for *T. arjuna.* Further, a high level of gallic acid content was found at an equal ratio for the three plants, while a high level of corilagin content was found at a low to medium weight ratio for *T. chebula* and *T. arjuna* and at a medium ratio for *T. bellirica.*

Separately, a high level of chebulagic acid content was found at a low to medium weight ratio for *T. chebula* and *T. bellirica* and at a medium ratio for *T. arjuna*, while a high level of chebulinic acid content was found at a low to medium weight ratio for *T. chebula*, at a medium to high ratio for *T. arjuna* and a low ratio for *T. bellirica.* In addition, a high total content level of the phenolic compounds was found at a low to medium weight ratio for *T.*

chebula and *T. bellirica* and at a medium to high ratio for *T. arjuna.*

In the infusion group, a high extraction yield was found at a medium weight ratio for *T. chebula* and *T. bellirica* and at a low ratio for *T. arjuna.* Conversely, a high level of gallic acid content was found at an equal ratio for all three plants, while a high level of corilagin content was found at a low to medium weight ratio for *T. chebula* and at a medium ratio for *T. arjuna* and *T. bellirica.* A high level of chebulagic acid content was found at a low to medium weight ratio for *T. chebula*, at a medium to high ratio for *T. arjuna*, and at a medium ratio for *T. bellirica.* A high level of chebulinic acid content was found at a low weight ratio for *T. chebula* and *T. bellirica* and at a high ratio for *T. arjuna.* Finally, a high level of total content of the phenolic compounds was found at a low to medium weight ratio for *T. chebula* and *T. bellirica* and at a medium to high ratio for *T. arjuna.*

Several previous studies demonstrated the high correlation between phenolic compounds and antioxidant activity (Annegowda et al., 2010; Aryal et al., 2019; Gan et al., 2017; Rajurkar & Hande, 2011; Saha & Verma, 2016; Wanigasekera et al., 2019). According to the interaction of plant chemical constituents, when phenolic compounds were increased, antioxidant activity or other related activities were also increased. The above data could indicate the importance of the increasing of phenolic compounds of Trisamo recipe to maximize biological or pharmacological activities.

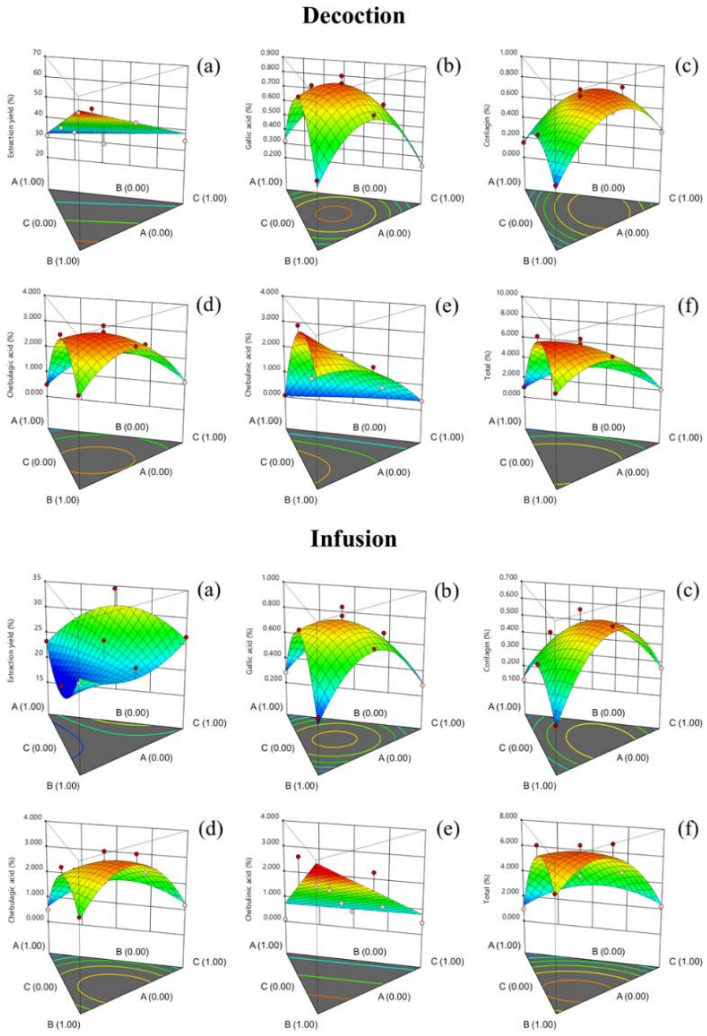

Figure 2 The 3D response surfaces of the model conditions of the (a) extraction yield, (b) gallic acid content, (c) corilagin content, (d) chebulagic acid content, (e) chebulinic acid content, and (f) total content of the phenolic compounds. A, B, and C show *T. chebula*, *T. arjuna*, and *T. bellirica*, respectively

4.2 Chemical interaction of *Terminalia* plants contained in the Trisamo recipe

The mathematical equations for predicting the dependent variables (Y_7 to Y_{12}) of the decoction and infusion groups are shown in Equations 14 to 25. The terms of the significant model and the p-values for the independent variables of Y_7 to Y_{12} are presented in Tables 4 and 5. The mean value of the model condition was a better predictor for Y_7 in the decoction groups, so there was no model term observed that affected Y_7 in the decoction group.

According to the mathematical models of the other dependent variables, Y_9 in the decoction group and Y_7, Y_8, Y_{10}, and Y_{12} in the infusion group were fitted to the quadratic model, while Y_{10} in the decoction group and Y_9 in the infusion group were fitted to the quadratic and special cubic models and Y_{11} and Y_{12} in the decoction group were solely fitted to the special cubic model. In addition, Y_8 in the decoction group was fitted to the quadratic model and the special quartic vs. quadratic models, while Y_{11} in the infusion group was fitted to the special cubic model and the special quartic vs. quadratic models, respectively. Therefore, the term $X_1X_2X_3$ did not affect Y_8 or Y_9 in the decoction group nor Y_7, Y_8, Y_{10}, Y_{11}, or Y_{12} in the infusion group. Further, the terms $X_1^2X_2X_3$, $X_1X_2^2X_3$, and $X_1X_2X_3^2$ only affected Y_8 in the decoction group and Y_{11} in the infusion group. In this case, the model was significant for all dependent variables except Y_7 and Y_{11} in the infusion group. Moreover, the lack of fit was not significant among all dependent variables in the decoction and infusion groups, and the linear mixture was not significant for all dependent variables. X_1X_2 and X_1X_3 were significant terms for all the dependent variables except Y_7 in the infusion group. X_2X_3 was a significant term for various dependent variables except for Y_7 and Y_{11} in the infusion group, while $X_1X_2X_3$ was a significant term for Y_{11} and Y_{12} in the decoction group and $X_1X_2^2X_3$ was a significant term for Y_8 in the decoction group. However, the terms $X_1^2X_2X_3$ and $X_1X_2X_3^2$ were not significant.

Decoction

$$Y_7 = 0.96 \quad \text{Eq. 14}$$

$$Y_8 = 1.00X_1 + 1.00X_2 + 1.00X_3 - 2.11X_1X_2 - 2.29X_1X_3 - 2.16X_2X_3 - 3.24X_1^2X_2X_3 + 15.95X_1X_2^2X_3 - 3.95X_1X_2X_3^2 \quad \text{Eq. 15}$$

$$Y_9 = 0.97X_1 + 1.01X_2 + 0.97X_3 - 1.97X_1X_2 - 2.31X_1X_3 - 1.97X_2X_3 \quad \text{Eq. 16}$$

$$Y_{10} = 0.98X_1 + 1.02X_2 + 0.98X_3 - 2.47X_1X_2 - 2.55X_1X_3 - 1.97X_2X_3 + 3.75X_1X_2X_3 \quad \text{Eq. 17}$$

$$Y_{11} = 0.99X_1 + 1.01X_2 + 1.02X_3 - 2.55X_1X_2 - 2.51X_1X_3 - 1.45X_2X_3 + 5.03X_1X_2X_3 \quad \text{Eq. 18}$$

$$Y_{12} = 0.98X_1 + 1.01X_2 + 0.99X_3 - 2.45X_1X_2 - 2.52X_1X_3 - 1.85X_2X_3 + 3.96X_1X_2X_3 \quad \text{Eq. 19}$$

Infusion

$$Y_7 = 1.02X_1 + 0.97X_2 + 1.02X_3 + 1.22X_1X_2 - 0.81X_1X_3 + 0.50X_2X_3 \quad \text{Eq. 20}$$

$$Y_8 = 0.97X_1 + 1.02X_2 + 0.97X_3 - 2.13X_1X_2 - 1.97X_1X_3 - 1.93X_2X_3 \quad \text{Eq. 21}$$

$$Y_9 = 0.97X_1 + 1.00X_2 + 1.00X_3 - 2.42X_1X_2 - 2.48X_1X_3 - 2.53X_2X_3 + 4.18X_1X_2X_3 \quad \text{Eq. 22}$$

$$Y_{10} = 0.94X_1 + 1.01X_2 + 0.97X_3 - 1.81X_1X_2 - 2.03X_1X_3 - 1.70X_2X_3 \quad \text{Eq. 23}$$

$$Y_{11} = 1.00X_1 + 1.00X_2 + 1.00X_3 - 2.09X_1X_2 - 2.36X_1X_3 - 1.60X_2X_3 - 11.69X_1^2X_2X_3 + 27.37X_1X_2^2X_3 + 9.81X_1X_2X_3^2 \quad \text{Eq. 24}$$

$$Y_{12} = 0.94X_1 + 1.02X_2 + 0.97X_3 - 1.71X_1X_2 - 1.97X_1X_3 - 1.46X_2X_3 \quad \text{Eq. 25}$$

Table 4 Terms of the significant model and p-value for the independent variables (Y_7-Y_{12}) of decoction

Polynomial term	Y_7	Y_8	Y_9	Y_{10}	Y_{11}	Y_{12}
Model	-	0.0017[a]	0.0004[a]	0.0004[a]	0.0002[a]	0.0003[a]
Linear mixture	-	0.1940	0.6346	0.3606	0.0519	0.2542
X_1X_2	-	0.0009[a]	0.0009[a]	0.0003[a]	< 0.0001[a]	0.0002[a]
X_1X_3	-	0.0007[a]	0.0004[a]	0.0002[a]	< 0.0001[a]	0.0001[a]
X_2X_3	-	0.0008[a]	0.0009[a]	0.0008[a]	0.0009[a]	0.0006[a]
$X_1X_2X_3$	-	-	-	0.0524	0.0066[a]	0.0310[a]
$X_1^2X_2X_3$	-	0.3905	-	-	-	-
$X_1X_2^2X_3$	-	0.0160[a]	-	-	-	-
$X_1X_2X_3^2$	-	0.3096	-	-	-	-
Lack of fit	0.9685	0.1658	0.2036	0.1754	0.4841	0.2592

[a]Significant value

Table 5 Terms of the significant model and p-value for the independent variables (Y_7-Y_{12}) of infusion

Polynomial term	Y_7	Y_8	Y_9	Y_{10}	Y_{11}	Y_{12}
Model	0.2442	0.0005[a]	0.0009[a]	0.0097[a]	0.1186	0.0182[a]
Linear mixture	0.5403	0.8449	0.9299	0.7641	0.4330	0.6755
X_1X_2	0.0674	0.0006[a]	0.0008[a]	0.0131[a]	0.0372[a]	0.0214[a]
X_1X_3	0.1901	0.0009[a]	0.0008[a]	0.0079[a]	0.0269[a]	0.0120[a]
X_2X_3	0.3908	0.0010[a]	0.0007[a]	0.0171[a]	0.0704	0.0392[a]
$X_1X_2X_3$	-	-	0.0733	-	-	-
$X_1^2X_2X_3$	-	-	-	-	0.4029	-
$X_1X_2^2X_3$	-	-	-	-	0.1075	-
$X_1X_2X_3^2$	-	-	-	-	0.4748	-
Lack of fit	0.7409	0.1692	0.3378	0.3484	0.8934	0.3202

[a]Significant value

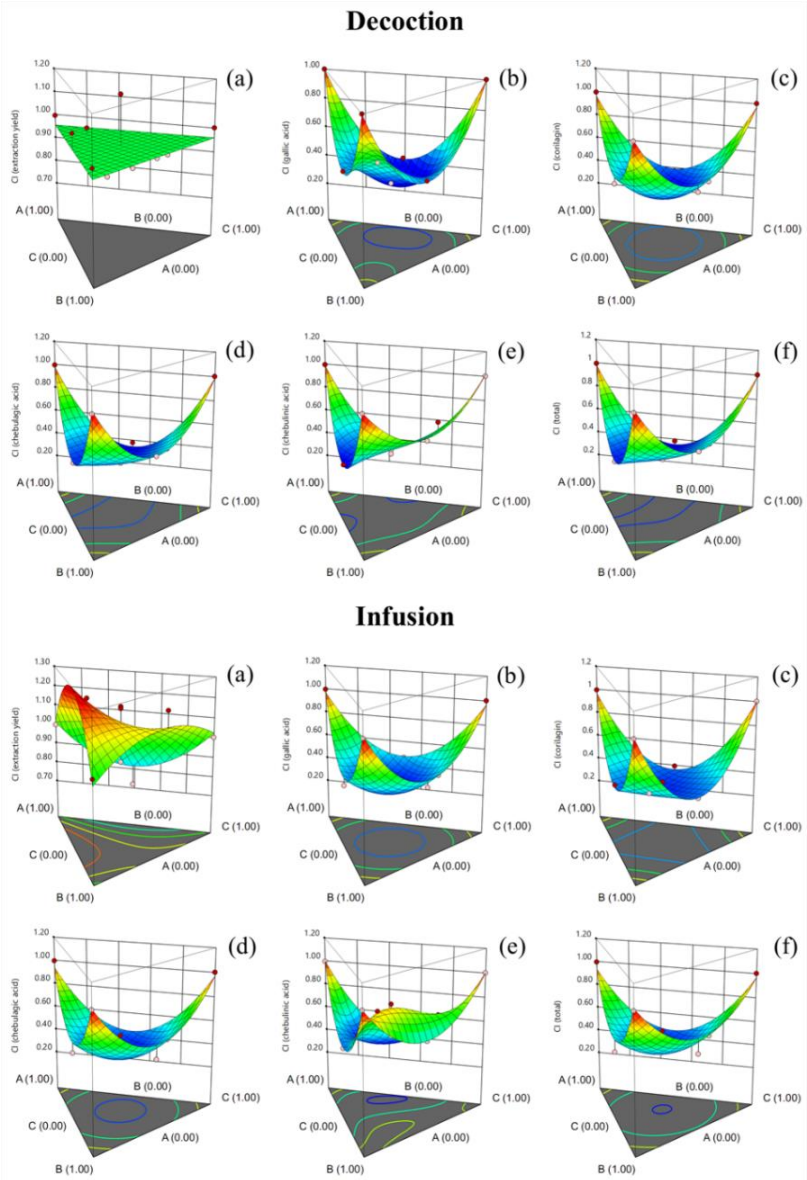

Figure 3 The 3D response surfaces of the model conditions of the combination index of the (a) extraction yield, (b) gallic acid content, (c) corilagin content, (d) chebulagic acid content, (e) chebulinic acid content, and (f) total content of the phenolic compounds. A, B, and C show *T. chebula*, *T. arjuna*, and *T. bellirica*, respectively

Figure 3 presents the 3D response surfaces of the model conditions of the CI of the extraction yield, gallic acid content, corilagin content, chebulagic acid content, chebulinic acid content, and total content of the phenolic compounds. The extraction yield for the decoction group could not be predicted in this case. The low CI value of gallic acid content was found to be at a medium weight ratio for *T. chebula* and *T. bellirica* and at a low to medium weight ratio for *T. arjuna.* Meanwhile, the low CI values of the individual contents of corilagin, chebulagic acid, chebulinic acid, and total content of the phenolic compounds were found in an equal ratio for all three plants. In the case of the infusion group, the low CI values of extraction yield and chebulinic acid content were found to be at a medium weight ratio for *T. chebula* and *T. bellirica* and a low ratio for *T. arjuna.* The low CI values of the individual contents of gallic acid, corilagin, chebulagic acid, and total content of the phenolic compounds were found in an equal ratio for all three plants.

Figure 4 shows the correlation plots between the predicted versus actual values of model conditions of the CI values of the extraction yield, gallic acid content, corilagin content, chebulagic acid, chebulinic acid, and total content of the phenolic compounds. All plots of the CI values of the gallic acid content, corilagin content, chebulagic acid, chebulinic acid, and total content of the phenolic compounds exhibited relatively high r^2 values (ranged from 0.9607–0.9959 for decoction and 0.8509–0.9726 for infusion), which indicated that the prediction of the Design-Expert® was precise and reliable except for the CI plots of the extraction yield in the decoction group. However, the plotting of the CI outcomes for extraction yield in the infusion group displayed a moderate r^2 value. According to the adjusted r^2 values of the CI values of the gallic acid content, corilagin content, chebulagic acid, chebulinic acid, and total content of the phenolic compounds were ranged from 0.9280–0.9848 for decoction and 0.7232–0.9397 for infusion. Figure 5 shows the distribution between the internally studentized residuals and the run numbers of model conditions of the CI values of the extraction yield, gallic acid content, corilagin content, chebulagic acid content, chebulinic acid content, and total content of the phenolic compounds. It was found that the data distributed within the red border lines were within the 95% confidence interval.

The above results revealed that the predictions made by the computer software program were reliable, in line with previous studies (Duangjit, Mehr, Kumpugdee-Vollrath, & Ngawhirunpat, 2014; Duangjit et al., 2012; Monton & Luprasong, 2019; Monton, Luprasong, & Charoenchai, 2019a, 2019b; Monton, Settharaksa, Luprasong, & Songsak, 2019).

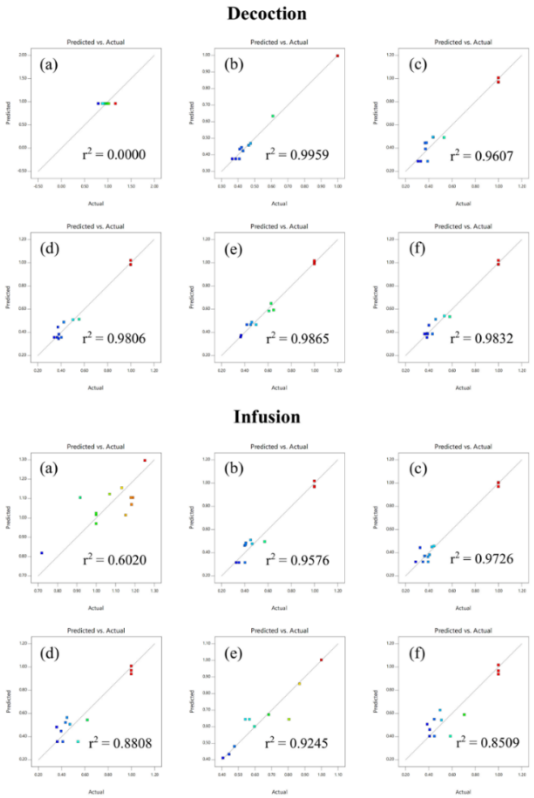

Figure 4 Predicted versus actual plots of model conditions of the combination index of the (a) extraction yield, (b) gallic acid content, (c) corilagin content, (d) chebulagic acid content, (e) chebulinic acid content, and (f) total content of the phenolic compound

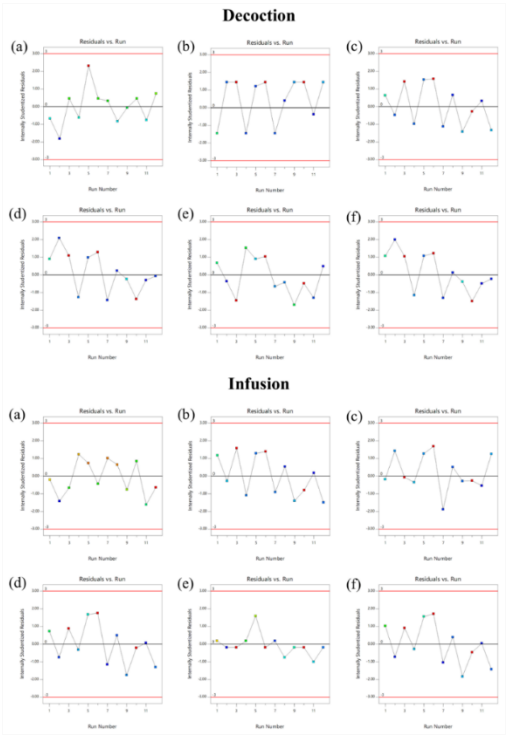

Figure 5 Residuals versus run plots of model conditions of the combination index of the (a) extraction yield, (b) gallic acid content, (c) corilagin content, (d) chebulagic acid content, (e) chebulinic acid content, and (f) total content of the phenolic compounds

Optimal conditions required the minimum CI value for the total content of the phenolic compounds. High desirability values were found in a broad range of the contour plots for both the decoction and the infusion groups (Figure 6a). These indicated that the original formula of Trisamo, with its equal weight ratio of the three *Terminalia* plants, had a high desirability value, meaning that a high level of positive interaction could occur in conjunction with this ratio. However, the chemical interaction pattern was slightly different among the various extraction techniques. Figures 6b and 6c present overlay plots wherein the yellow area is the area where the CI of the total content of the phenolic compounds was less than 0.5 and 0.4, respectively. In other words, the original Trisamo recipe had a CI for the total content of the phenolic compounds of at least 0.4 for the decoction group and at least 0.5 for the infusion group. From these results, it can be concluded that the mixture of the equal weight ratio for the three *Terminalia* plants in the Trisamo recipe exhibited a positive interaction that was at least 2.5 and two times greater for the decoction and infusion groups, respectively, when compared with the linear effect of the individual *Terminalia* plants. With regard to the Trisamo recipe, the *Terminalia* plants could promote chemical interactions among the active compounds within the parameters of the recipe as found in traditional Chinese medicine (Yuan et al., 2017). The mechanism of the synergism of pharmacological activity of herbal remedy was previously described (Yuan et al., 2017). The synergy could have occurred from pharmacodynamic synergism, pharmacokinetic synergism, elimination of adverse effects, and targeting disease resistance mechanisms (Caesar & Cech, 2019). However, there was limited number of publications that described the mechanism of how plant mixtures could increase the extraction efficiency of various chemical constituents. The chemical interactions of herbal mixtures was complex and difficult to identify. When working with a complex herbal remedy, chemical compositions responsible for activity are often not known. Furthermore, the plant extract constituents varied depending on how and where the plant material was grown, prepared, processed, and stored (Caesar & Cech, 2019). Our previous works demonstrated the chemical interaction of traditional herbal remedies, i.e., Triphala which was composed of an equal mass ratio of *Phyllathus emblica*, *T. chebula* and *T. bellirica* (Monton et al., 2020) and Chatuphalathika which was composed of equal mass ratio of *Phyllathus emblica*, *T. chebula*, *T. arjuna* and *T. bellirica* (Monton & Suksaeree, 2020). We found that the alteration of mass ratio of plants composed in the herbal recipe also altered content as well as extraction efficiency of chemical constituents. The extraction technique affected the total content of phenolic compounds. Thus, the higher content of phenolic compounds in the decoction group could contribute to the positive chemical interaction by 250%, which was higher than the 200% in the infusion group, compared to using a single plant. This data could be used to prove the usefulness of the Trisamo recipe in traditional medicine based on a chemical point of view.

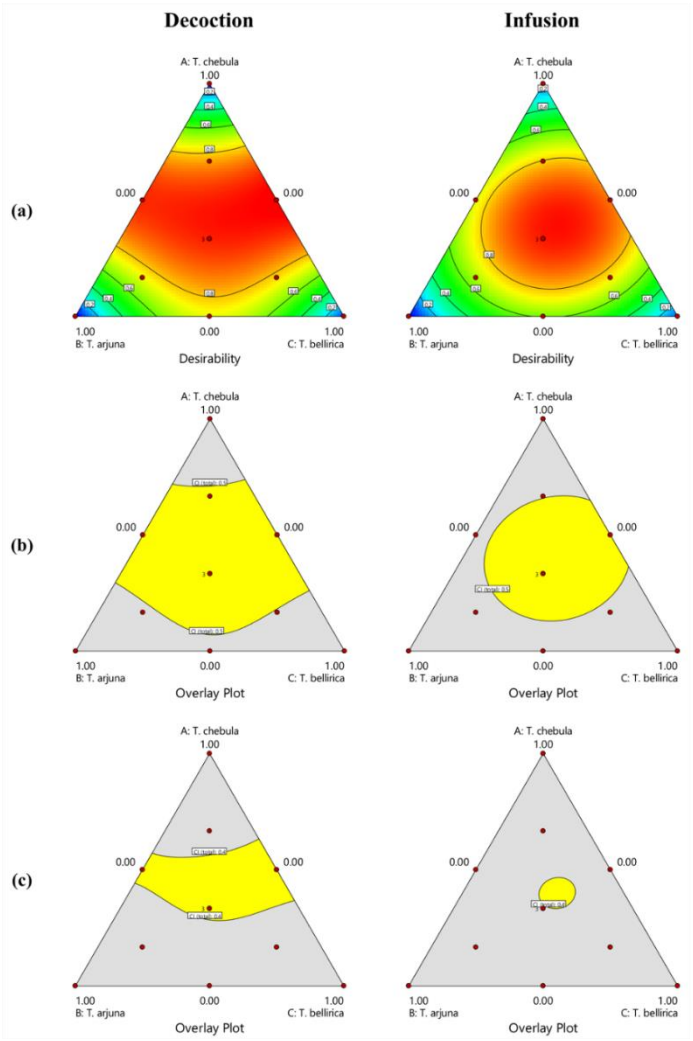

Figure 6 Contour plots of the desirability value (a) and the overlay plots (b and c) of the combination index of the total content of the phenolic compounds, where the value was lower than (b) 0.5 or (c) 0.4

5. Conclusion

This work applied the simplex lattice experimental design to determine the chemical interaction of three *Terminalia* plants—*T. chebula*, *T. arjuna*, and *T. bellirica*—contained in the Trisamo recipe. The levels of chemical interaction were determined based on the analysis of phenolic compounds including gallic acid, corilagin, chebulagic acid, and chebulinic acid. The computer software program used for this investigation was proven to be both reliable and accurate. A response surface analysis found that the original Trisamo recipe, with an equal weight ratio of all three *Terminalia* plants obtained from decoction and infusion groups, exhibited a level of chemical interaction that was at least 250% and 200% higher, respectively, than that achieved using a single plant. The data from this work could support the use of the Trisamo recipe with an equal weight ratio of the three *Terminalia* plants, which has been documented already in traditional medicine as an appropriate formula.

6. Acknowledgements

We would like to thank the College of Pharmacy, Rangsit University for allowing us to use their facilities. This paper was proofread and edited by Cambridge Proofreading LLC by the support of the Research Institute of Rangsit University.

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