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Optimization of herbal drink formulation based on aloe vera (*Aloe barbadensis* Miller) and java spices

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Abstract

This research aimed to determine the optimum combination of aloe vera and Java spices' herbal drink formulations. A Design Expert mixture D-optimal was used to analyze the optimal composite formulation. The optimum formula is determined through four stages, namely, the formula planning, formulation, analysis, and optimization stages. The selected formulation was determined based on the response to phenolics, flavonoids, antioxidant capacity tests, and pH. The formulations had pH values ranging from 4.452 to 4.636, the antioxidant capacity values ranged from 2.4796 to 12.5697 mg Ek vit C/100 g, the total phenolic content ranged from 0.0871 to 0.1883 mg GAE/g, and the total flavonoid content ranged from 0.1451 to 0.4210 mg QE/g. The most optimum response is obtained when the desirability value is close to one. The optimum formulation for aloe vera and Java spices herbal drinks consisted of 52.92% aloe vera, 11.58% ginger extract, 1.50% white turmeric extract, and 2.00% galangal extract. The formulation under investigation exhibits a pH of 4.502, an antioxidant capacity of 7.4638 mg ascorbic acid equivalent per 100 g, a total phenolic content of 0.1314 mg GAE/g, and a total flavonoid content of 0.3315 mg QE/g.

Keywords: *Aloe barbadensis* Miller; antioxidant capacity; herbal drink; java spices.

Practical Application: This article is relevant for precise formulation to produce herbal drink products from natural ingredients that can improve the immune system and have acceptable yet preferred characteristics.

1 INTRODUCTION

Herbal drinks are health drinks sourced from natural ingredients, including various plant components such as spices, roots, stems, leaves, flowers, or tubers. Herbal drinks have therapeutic characteristics that are useful for treating certain diseases (Ismiati, 2015). Aloe vera herbal drink is a commercially available drink, made from aloe vera and Javanese spices. Both types of ingredients have high antioxidant content. The antioxidant content in aloe vera herbal drinks has the potential to increase the body's immune response during the COVID-19 pandemic (Werdhasari, 2014; Wiedosari, 2007). The aloe vera plant contains flavonoid chemicals which are active antioxidants and can ward off free radicals in the human body (Siswanto & Ernawati, 2013; Sultana & Anwar, 2008).

The use of aloe vera in extract form is less practical, easily damaged, and has low acceptability. Therefore, aloe vera gel is processed into aloe vera drinks. However, the antioxidant levels in aloe vera can decrease during processing, so it is necessary to add other herbal ingredients that have high antioxidant levels (Riyanto & Wariyah, 2012). Apart from having a high antioxidant content, aloe vera peel extract is effective in regulating insulin and serum glucose levels in mice with type 2 diabetes (Christijanti et al., 2019). This effect can be attributed to the antioxidant activity of the aloin–peptide complex (Moosavi-Movahedi et al., 2015).

Ginger rhizome (*Zingiber officinale* R) is known to have potential as an antioxidant, antimicrobial, and proteolytic compound (Suryanti et al., 2014). The antioxidant activity and total phenolic content of red ginger (*Zingiber officinale* R)-based

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drinks have a total phenolic content value of 6,299 ppm (Widayat et al., 2018). The main ingredients used in the spiced drink are ginger, turmeric, white turmeric, sand ginger, lime, secang, rock sugar, brown sugar, and water, and it has an antioxidant activity of 78.642% (Dwiloka et al., 2020).

The optimization process is a normative approach to identifying the best solution to a problem. Optimization aims to minimize the effort required or operational costs and maximize the desired results (Ma'arif et al., 1989). Design Expert is a software for optimizing a process or formula for a product. The program can process four research designs: factorial design, combined design, mixed design, and response surface method design. To optimize the formula from a series of mixtures of components used, the mixture design can select a response surface methodology for optimizing biogas production (Nugraha et al., 2020; Nugroho, 2012). Formula optimization using the mixed design method has the advantage that the number of formula runs is less than the factorial and RSM methods (Hidayat et al., 2021).

The D-optimal mixture experimental design was applied to predict the optimal composite formulation. Experimental data were assessed to produce an empirical model to analyze the effect of component fractions on individual responses. The fitted model was checked statistically (via design standard errors), highly validated (via analysis of variance (ANOVA) and indicators of model adequacy), and verified experimentally (by estimating error, root-mean-square error, and standard error prediction (SEP) between predicted and actual values) (Dalanta et al., 2021). The D-optimal mixture design method has advantages compared with other optimization programs. Namely, it can automatically display the number of formulations, following predetermined limits. D-optimal mixture design will provide variations in the concentration of each ingredient with a predetermined number of formulas, making it easier for researchers to create formulas (Borhan et al., 2014). Functional drink formulation uses the D-optimal method mix Design Expert (Susilo, 2011; Januari, 2017).

The mass balance calculates all materials entering, accumulating, and leaving within a certain time. The mass balance shows the amount of material coming in and out. The basic principle is that if there is no accumulation of processing equipment in a process, then the amount of incoming material will be the same as the amount of output material (Maflahah, 2010). The mass balance is determined to calculate the amount of ingredients entering and leaving and the efficiency of herbal drink production (Wirakartakusumah, 1989). This research aims to produce an herbal drink made from aloe vera and Javanese spices, which has high levels of antioxidants to improve the immune system and has characteristics that are acceptable and liked by consumers through the application of Design Expert using the D-optimal mixture method, and to obtain the mass balance flow of the aloe vera herbal drink.

2 MATERIALS AND METHODS

2.1 Materials

The ingredients used in making herbal drinks were aloe vera (*Aloe barbadensis* Miller), red ginger (*Zingiber officinale* R), local varieties of white turmeric, local types of turmeric, white galangal varieties, honey, sugar, carboxymethyl cellulose (CMC), citric acid, Na-benzoate, salt, and plastic bottle packaging. The materials for analysis consisted of gallic acid p.a, methanol p.a, ethanol p.a, Folin–Ciocalteu reagent, Na2CO3 7%, aquadest, quercetin standard, AlCl3 10%, NaOH 1 M DPPH p.a powder, and buffer solutions with pH 4 and 7. The tools for producing aloe vera herbal drinks were basins, knives, glasses, blenders, pans, stoves, spoons, digital balances, bowls, plates, funnels, sealers, and wooden spatulas. The analytical tools used were a measuring flask, Erlenmeyer flask, beaker, dropper, measuring pipette, measuring pipette, micron pipette, ball filler, digital balance, spatula, weighing bottle, pH meter (SI Analytics Lab 865), Chromameter (3NH NH310), Pocket Refractometer PAL 1 Brix 0–53% (Atago), pycnometer, cuvette, and UV–Vis spectrophotometer.

2.2 Prepared aloe vera herbal drink

The process of making an aloe vera herbal drink begins with the process of peeling aloe vera using a knife to obtain aloe vera meat. Then the aloe vera meat is washed with clean water. Next, the materials were weighed using a digital balance. The weight of the aloe vera meat used is 55% of the base. The aloe vera meat is blended with water and CMC (0.02%) is added during the process. Mixing the ingredients is the most critical process in making an aloe vera herbal drink. The combined elements are 10% granulated sugar, 10% ginger juice, 2% white turmeric extract, 2% turmeric extract, 1% galangal juice, 5% honey, 0.03% salt, 0.03% citric acid, and 0.02% Na-benzoate. After that, the material is stirred until dissolved, and the pasteurization process is carried out at a temperature of 70–80°C for 30–45 min. The final stage of producing aloe vera herbal drinks involves packaging products in plastic bottles.

2.3 Methods

There were two research stages, namely, preliminary and leading research. Primary research is conducted to verify the formula used to make herbal drinks based on aloe vera and Java spices, as well as to determine the mass balance for the flow of the production process for making these herbal drinks. The preliminary research results will be used as a reference for the primary analysis. The main study used the Design Expert 13 software mixture D-optimal method to determine the formulation of the ingredients to be optimized, namely, aloe vera, ginger, white turmeric, and galangal. The responses to be tested include the analysis of bioactive components (phenolics and flavonoids), antioxidant capacity (as vitamin C), and pH. Table 1 shows the variables for mixture design.

Table 1. Variables for mixture design.

No	Variable	Low (%)	High (%)
1	Aloe vera	50	60
2	Ginger extract	5	15
3	White turmeric extract	0.5	1.5
4	Galangal extract	0.5	2

3 RESULTS AND DISCUSSION

3.1 Verification process of aloe vera herbal drinks

The verification process in the preliminary study aims to determine the characteristics of the product produced from the formula and procedures established by the Research Center Appropriate Technology team, with the responses being considered, namely, the antioxidant capacity (as vitamin C), total phenolic content, total flavonoid content, and pH. The results of the verification process for aloe vera herbal drinks are shown in Table 2.

The determination of the optimum formula consists of four stages: the formula planning stage, the formulation stage, the analysis stage, and the optimization stage. The first stage was to determine the formula/variable to be optimized, usually referring to the formula based on the existing research literature or the results of trial and error. Then the response to be tested will be selected, and each answer will be analyzed by Design Expert 13 to produce the D-optimal equation. Furthermore, Design Expert 13 will display the selected formulation according to the program, which is summarized based on the conclusions drawn from all responses. The number of runs or formulations given by Design Expert in 13 programs is 15. The number of runs is obtained automatically by the program. The matrix of the D-optimal mixture design of aloe vera herbal drinks is shown in Table 3.

Table 2. Results of the verification process for aloe vera herbal drinks.

Parameter	Value
Antioxidant capacity	7.0482 mg Ek.Vit C/100g
Total phenolic	0.1073 mg GAE/g
Flavonoid	0.1338 mg QE/g
pH	4.499

Table 3. Matrix of the D-optimal mixture design of aloe vera herbal drinks.

Formula	Aloe vera (%)	Ginger extract (%)	White turmeric extract (%)	Galangal extract (%)
1	53.63	12.62	0.79	0.96
2	57.62	8.13	1.29	0.96
3	50.00	14.50	1.50	2.00
4	60.00	7.00	0.50	0.50
5	60.00	6.00	1.50	0.50
6	60.00	5.00	1.00	2.00
7	60.00	6.25	0.50	1.25
8	52.00	15.00	0.50	0.50
9	51.00	15.00	1.50	0.50
10	55.75	10.75	1.00	0.50
11	55.25	10.25	0.50	2.00
12	52.62	12.38	1.29	1.71
13	50.70	15.00	1.00	1.30
14	50.50	15.00	0.50	2.00
15	55.08	10.08	1.50	1.33

The primary research aims to determine the optimum formulation of aloe vera herbal drink products using the Design Expert 13 software. The desired responses are pH, antioxidant capacity (vitamin C), total phenolic content, and flavonoid content.

3.2 pH

They obtained the highest pH value from eight formulations, with the highest composition of ginger extract at 15%, aloe vera at 52%, white turmeric extract at 0.5%, and galangal extract at 0.5%. The pH value of the 15 aloe vera herbal drink formulas ranged from 4.452 to 4.636. The polynomial model recommended by Design Expert for the pH response is linear. The ANOVA results show that at the 5% level, the recommended model has a significant value, with a p-value of "prob < F" less than 0.05, which is 0.0001. The pH response contour plot obtained from the research using the mixture design method is shown in Figure 1.

The pH of food and beverages is influenced by the acid content found in natural ingredients. In composition, the red ginger component is the second largest component used for manufacturing aloe vera herbal drinks (Fardiaz, 1997). The greater the concentration of ginger extract added to beetroot juice, the pH of the beetroot juice increases. This is because the H+ ions released are smaller. In addition, adding citric acid to aloe vera herbal drinks can also affect the pH, where the addition of citric acid serves as a regulator of acidity (Chasparinda et al., 2014; Winarno, 2008).

3.3 Antioxidant capacity

The antioxidant capacity values of the 15 aloe vera herbal drink formulas ranged from 2.4796 to 12.5697 mg Ek.vit C/100 g. They obtained the highest antioxidant capacity value from the six formulations containing 60% aloe vera, 5% ginger extract, 1% white turmeric extract, and 2% galangal extract. The polynomial model recommended by Design Expert for the antioxidant capacity response is linear. The ANOVA results show that at the 5% level, the recommended model has a significant value, with a p-value of "prob < F" less than 0.05, which is 0.0001. The graph of the contour plot of the antioxidant capacity response obtained from the research results using the mixture design method is shown in Figure 2.

The blue color on the contour plot shows the lowest antioxidant capacity response value, 2.4796 mg Ek.vit C/100 g. The red color indicates the highest antioxidant capacity response, 12.5697 mg Ek.vit C/100 g. The antioxidant properties of aloe vera herbal drinks and Java spices can come from the aloe vera and Java spices used because each ingredient has compounds that act as antioxidants. Aloe vera contains flavonol compounds, such as kaempferol, quercetin, and myricetin; these compounds are included in the polyphenol group, which is believed to have antioxidant properties (Sultana & Anwar, 2008). Ginger contains gingerol and shagaol compounds, and these compounds have the ability to act as primary antioxidants against lipid radicals.

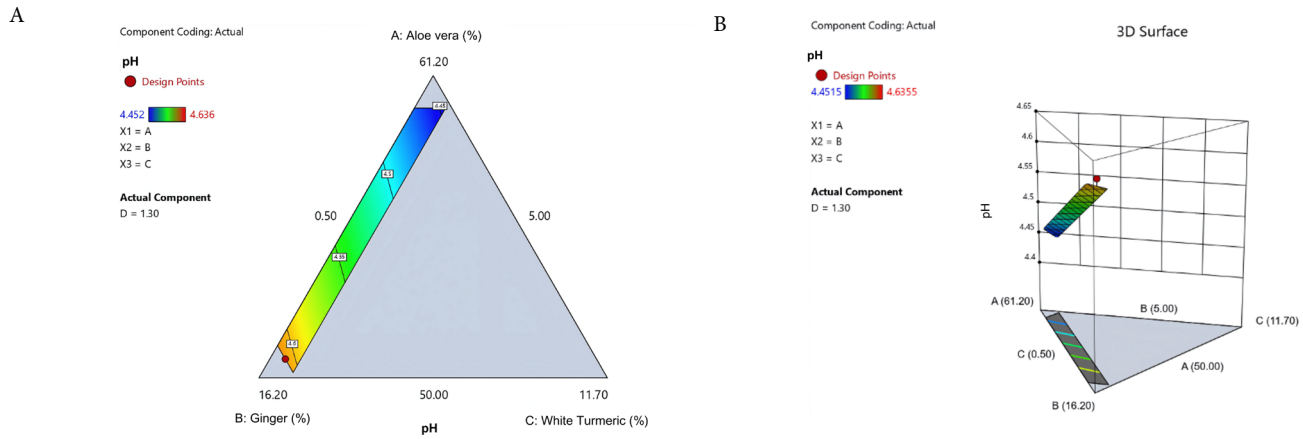

Figure 1. (a) Contour plot graph and (b) 3D drawing of the pH response test.

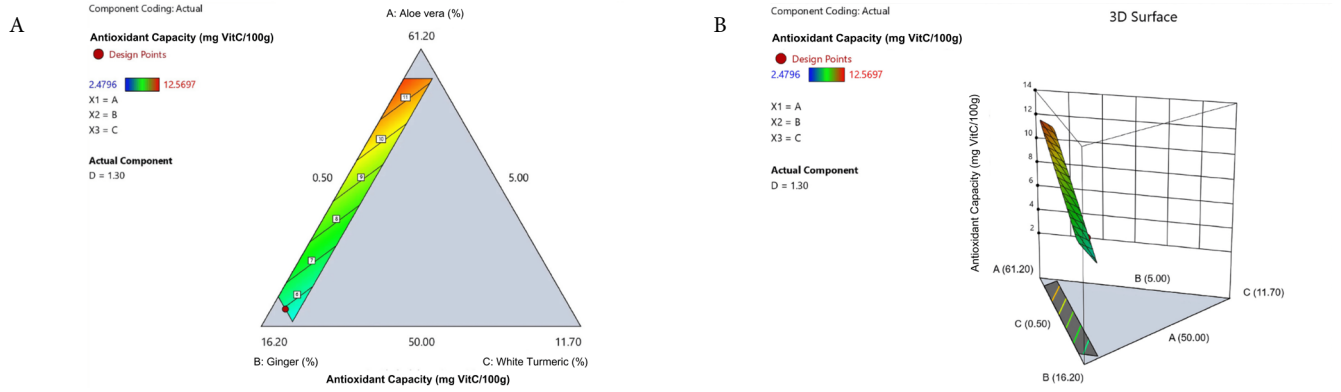

Figure 2. (a) Contour plot graph and (b) 3D drawing of antioxidant capacity.

3.4 Total phenolic level

The total phenolic content of the 15 aloe vera herbal drink formulas ranged from 0.0871 to 0.1883 mg GAE/g. It obtained the most considerable total phenolic value from the 14 formulations with the most significant component of ginger extract at 15%, aloe vera at 50.50%, white turmeric extract at 0.5%, and galangal extract at 2%. The polynomial model recommended by Design Expert for total phenolic content response is linear. ANOVA results show that at the 5% level, the recommended model has a significant value, with a p-value of "prob < F" less than 0.05, which is 0.0005. The contour plot graph of the response to total phenolic levels obtained from the research results using the mixture design method is shown in Figure 3.

The blue color on the contour plot shows the lowest response value for total phenolic content, which is 0.0871 mg GAE/g. In contrast, the red color indicates the response value of the highest total phenolic content, 0.1883 mg GAE/g. Total phenolic levels are thought to increase with the addition of red ginger. Phenolic compounds have one (phenol) or more (polyphenol) phenol rings, namely, hydroxy groups attached to aromatic rings, so they are easily oxidized by donating hydrogen atoms to free radicals. Its ability to form stable phenoxy radicals in oxidation reactions causes phenolic compounds to be very potent as antioxidants (Dhurhania & Novianto, 2018). Red ginger, emprit ginger, and elephant ginger contain oleoresin (4.0–7.5%) (Gelgel et al., 2016).

3.5 Total flavonoid

Table 4 shows the total flavonoid content of the 15 aloe vera herbal drink formulas ranging from 0.1451 to 0.4210 mg QE/g. It obtained the highest total flavonoid value from the 6th formulation with a composition of 60% aloe vera, 5% ginger extract, 1% white turmeric extract, and 2% galangal extract. The polynomial model recommended by Design Expert for the response to total flavonoid levels is quadratic. The ANOVA results show that at the 5% level, the recommended model has a significant value, with a p-value of "prob < F" less than 0.05, which is 0.0001. The contour plot graph of the response to total flavonoid levels obtained from the research results using the mixture design method is shown in Figure 4.

3.6 Determination of optimum formulation

The formula optimization process is carried out to obtain a formula with the most optimum response using Design Expert. The most optimum response is received with a desirability value close to one (Wulandhari, 2007). The desirability value close to one indicates that the product formula has reached the optimum procedure according to the desired response variable. In contrast, if the desirability index is close to zero, it indicates that the formulation is difficult to reach the optimum point based on the response variable (Wahyudi, 2012). The optimized components,

target values, limits, and importance at the formula optimization stage using the Design Expert 13 programs are shown in Table 4.

Vitamin C, phenolic compounds, and flavonoids, known for their antioxidant activities, are functional components in herbal beverages. Table 4 shows that the responses of antioxidant capacity, total phenolic content, and total flavonoid content had a level of importance of 5 (+++++). Java spices have high antioxidant content because of the presence of various phytochemicals. The Design Expert 13 program recommends an optimum formula solution, as seen in Table 5.

The optimum formula solution resulting from the Design Expert program optimization has a desirability value of 0.585, which means that the formula will produce a product with characteristics following the optimization target of 58.50%.

3.7 Validation for verification

Validation for verification of the optimum formula is carried out to determine the actual value of the optimum formula so that it can be compared with the predictions given by the Design Expert 13 programs. The results of the prediction and verification of formulas for making aloe vera herbal drinks are shown in Table 6.

Based on the results of the formula verification, the data from the verification results are still the predictions made by the Design Expert 13 programs. This is indicated by the response values of pH, antioxidant capacity (as vitamin C), total phenolic content, and total flavonoid content, which met the 95% Prediction Interval (PI) predicted by the Design Expert 13 programs.

4 CONCLUSION

The results of the optimization of the formula of aloe vera herbal drinks obtained using the D-optimal mixture method yielded a desirability value of 0.585 consisting of 52.92% aloe vera, 11.58% ginger extract, 1.50% white turmeric extract, and 2.00% galangal extract. The results of the main verification analysis of the optimum formulation predicted by the D-optimal mixture method, namely, the pH value of 4.502, the antioxidant capacity value of 7.4638 mg of vitamin C/100 g of ingredients, the total phenolic content of 0.1314 mg GAE/g, and the total flavonoid content of 0.3315 mg QE/g, for all responses, meet 95% PI low and 95% PI high.

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Table 4. Optimized components and responses, targets, limits, and importance at the formula optimization.

Components/responses	Targets	Low	High	Importance
Aloe vera	*In range*	50	60	3 (+++)
Ginger extract	*In range*	5	15	3 (+++)
White turmeric extract	*In range*	0.5	1.5	3 (+++)
Galangal extract	*In range*	0.5	2	3 (+++)
pH	*Maximize*	4.452	4.636	3 (+++)
Antioxidant capacity	*Maximize*	2.4796	12.5697	5 (+++++)
Total phenolic	*Maximize*	0.0871	0.1883	5 (+++++)
Total flavonoid	*Maximize*	0.1451	0.4210	5 (+++++)

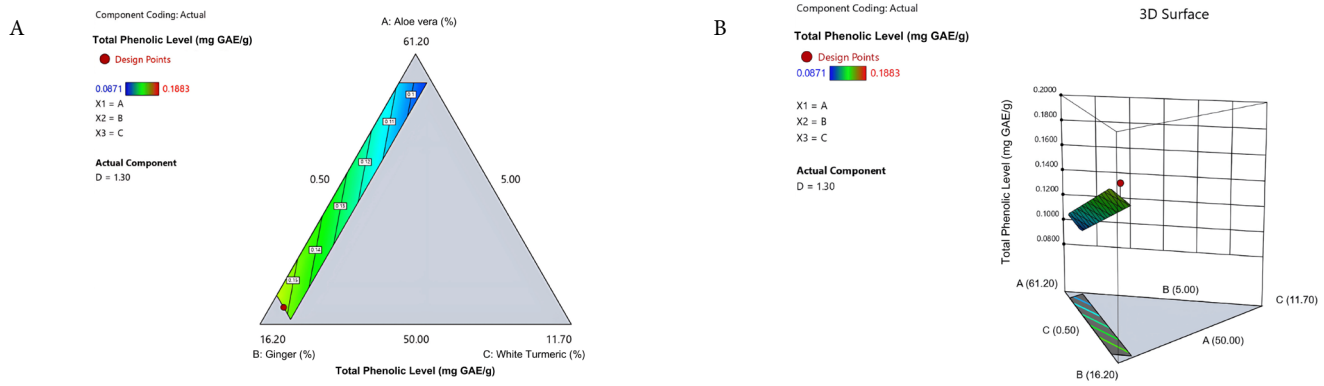

Figure 3. (a) Contour plot graph and (b) 3D drawing of the total phenolic level.

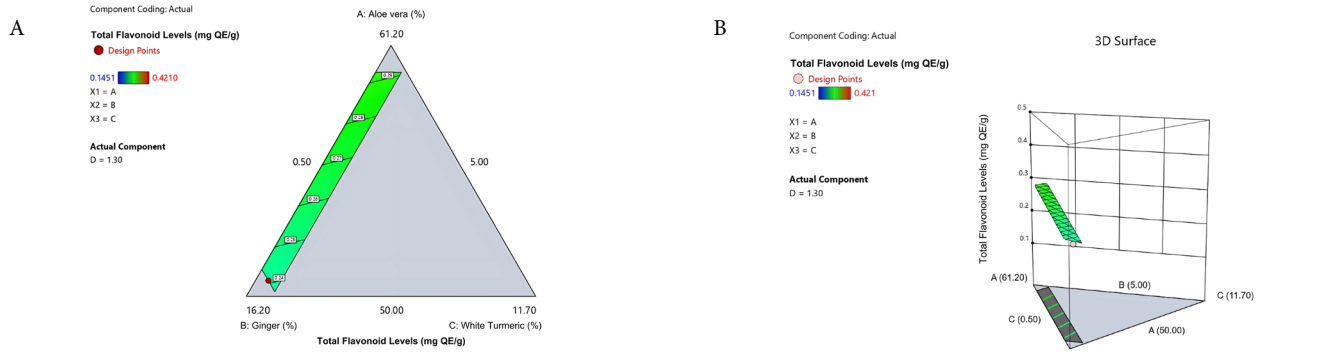

Figure 4. (a) Contour plot graph and (b) 3D drawing of total flavonoid levels.

Table 5. Optimum formula solution.

Aloe vera (%)	Ginger extract (%)	White turmeric extract (%)	Galangal extract (%)	Desirability
52.92	11.58	1.50	2.00	0.585

Table 6. Predicted and verification values for the optimized formula.

Responses	Results		95% PI Low	95% PI high
	Prediction	Verification		
pH	4.5410	4.5020	4.4830	4.5990
Antioxidant capacity	6.5495	7.4638	3.8078	9.2912
Total phenolic	0.1309	0.1314	0.0993	0.1624
Total flavonoid	0.3513	0.3315	0.3087	0.3938

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