International Journal of Pharmaceutical Sciences and Drug Research, 2024;16(4):625-633

Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

journal home page : https://ijpsdronline.com/index.php/journal

Research Article

Novel Development of Phytomedicine and its Innovative Formulation of Guava Leaf Extract Chocolates for Various Incredible Therapeutic Activities

T Prabhakar[1*], D Baggiya Selvi[1], Chandanam Sreedhar[2], Deepak Kumar Jha[3]

[1]*Department of Pharmacognosy, Karnataka College of Pharmacy, Bangalore, Karnataka, India.*
[2]*Department of Pharmaceutical Analysis, Karnataka College of Pharmacy, Bangalore, Karnataka, India.*
[3]*Department of Pharmacology, Karnataka College of Pharmacy, Bangalore, Karnataka, India.*

ARTICLE INFO

Article history:
Received: 04 May, 2024
Revised: 22 May, 2024
Accepted: 25 May, 2024
Published: 30 July, 2024

Keywords:
Guava, Chocolate,
Response surface methodology,
Physico chemical

DOI:
10.25004/IJPSDR.2024.160410

ABSTRACT

Incorporating guava leaf powder into chocolate is a creative way to boost its nutritional profile and potentially add a unique flavor. Guava leaf powder, being rich in soluble dietary fiber and vitamin C, can indeed enhance the health benefits of chocolate. Soluble dietary fiber can aid in digestion and promote gut health. Thus, the present study was to achieve an innovative formulation, i.e., guava leaf chocolates containing guava leaf extract and effective ingredients, which could be a way to entrap all the benefits of the guava leaf into an easy-to-consume formulation by increasing patient compliance and therapeutic outcomes. Response surface methodology (RSM) has been used here to optimize the formulation. RSM allows us to systematically vary the levels of these ingredients and analyze their impact on sensory attributes such as taste, texture, aroma, hardness, and overall acceptability. The use of various batches (F1–F15) suggested a systematic approach to formulation adjustment, likely involving tweaking ingredient proportions and processing parameters to achieve the desired sensory attributes. The inclusion of physico chemical analysis confirmed the stability of the formulation, ensuring product quality and consistency. The results were felt within a satisfactory range, which indicates that the formulation was stable and suitable for its intended purpose, potentially for therapeutic applications. Furthermore, highlighting the eco-friendliness, ease of handling, and positive sensory attributes such as sweetness and flavor can contribute to the overall appeal of the product. By meeting these criteria, the formulation not only meets functional requirements but also aligns with consumer preferences and environmental considerations.

Introduction

Guava leaves *(Psidium guajava L.,* Myrtaceae) have been used for centuries due to their various health benefits, including immunostimulant,[1] anti-hyperglycemic, anti-hyperlipidemic,[2] antioxidant,[3] etc. The leaves contain compounds such as flavonoids, tannins, and other phytochemicals that inhibit the growth of *Staphylococcus aureus* bacteria and have antimicrobial properties that can help prevent infections and promote healing. They are often used to treat wounds and cuts. Guava leaves are used to reduce inflammation and relieve pain. They contain anti-inflammatory compounds that can help with conditions like arthritis and other inflammatory diseases. Guava leaf extract is known to help lower blood sugar levels, making it beneficial for people with diabetes. It can help regulate glucose metabolism and improve insulin sensitivity. Drinking guava leaf tea is believed to help with weight loss by preventing complex carbohydrates from converting into sugars. They can help treat acne, black spots, and other skin problems due to their antiseptic properties. Also, used to treat bronchitis and coughs. They help open up the lungs, loosen mucus, and reduce the symptoms of respiratory ailments.[4] These traditional uses of guava leave highlight their potential as a natural

***Corresponding Author:** Dr. T Prabhakar
Address: Department of Pharmacognosy, Karnataka College of Pharmacy, Bangalore, Karnataka, India.
Email ✉**:** pharmplants@gmail.com
Tel.: +91-9845230384

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

remedy for a variety of health issues. However, while there is anecdotal evidence supporting these benefits, more scientific research is needed to fully understand their efficacy and potential side effects. It's fascinating how guava leaves have been utilized across diverse cultures for various health purposes. In India, China, Pakistan, and Bangladesh, decoctions, infusions, and boiled preparations are commonly consumed to address a range of ailments, including rheumatism, diarrhea, diabetes mellitus, and cough.[5-8] This tradition reflects a long-standing belief in the medicinal properties of guava leaves and their potential to alleviate these health issues. In Southeast Asia,[4,6,7] guava leaf decoctions are used as gargles for mouth ulcers,[6,7] likely due to their perceived antibacterial and soothing properties. Similarly, in Nigeria, the decoction serves as an antibacterial agent, indicating a widespread belief in its antimicrobial efficacy. Moreover, the application of guava leaf poultices for skin and wound care in Mexico, Brazil, the Philippines, and Nigeria demonstrates the versatility of this plant in traditional medicine. The poultices are applied externally, suggesting a belief in the leaves' ability to promote wound healing and alleviate skin ailments.[6-9]

These diverse traditional uses of guava leave highlight their importance in folk medicine across different regions, showcasing the plant's potential therapeutic value beyond just its nutritional benefits. However, it's crucial to approach traditional remedies with caution and to seek guidance from healthcare professionals for appropriate usage and dosage. The use of chewing sticks for oral care in Nigeria[7] is a traditional practice that has persisted for generations. These sticks, often obtained from certain trees with medicinal properties, are chewed to clean the teeth and freshen the breath. They're believed to have antimicrobial properties that can help prevent dental diseases. With the increasing interest and potential health benefits, it's indeed a growing field of research globally. Guava leaves, for example, have drawn attention due to their rich content of phenolic compounds, which are known for their antioxidant properties and potential health benefits. In Japan, guava leaf products, including teas and supplements, have become available due to their purported ability to modulate blood sugar levels. These products are marketed as food for specified health use, indicating that the Japanese government has approved them for their health benefits.[8,9] Phenolic compounds found in guava leaves are believed to play a role in regulating blood glucose levels, which could be beneficial for individuals managing conditions like diabetes. Research into the specific mechanisms and efficacy of these compounds is ongoing, but they hold promise as natural alternatives or supplements for maintaining health.[10] Guava leaves have been known for their health benefits, so incorporating them into chocolate could add an interesting twist. Enriching chocolate with essential nutrients could make it not only a delicious treat but also a healthier option. This project has the potential to create innovative chocolate products that offer both indulgence and health benefits, catering to a diverse audience.

The pharmaceutical advantage of an herbal chocolate formulation incorporating guava leaf extracts lies in its unique combination of therapeutic benefits and consumer appeal. The presence of fats in chocolate can enhance the bioavailability of fat-soluble compounds in guava leaves, such as certain flavonoids and terpenoids, improving their absorption and efficacy. Rich in bioactive compounds, they possess antioxidant properties, antimicrobials that reduce oxidative stress and inflammation, astringent properties, and can help in treating gastrointestinal issues and contributing to overall health. The formulation will improve patient compliance in terms of palatability and convenience. The formulation combines the pleasure of eating chocolate with the health benefits of guava leaves, appealing to health-conscious consumers looking for functional foods that offer more than just basic nutrition. The herbal chocolate formulation represents an innovative approach in the nutraceutical and functional food markets, differentiating it from conventional products. In summary, the pharmaceutical advantage of this herbal chocolate formulation lies in its ability to deliver the medicinal benefits of guava leaves in a highly palatable and convenient form, potentially improving compliance and expanding the therapeutic applications of herbal medicines.

Thus, the present work is to explore the medicinal potential of guava leaves by integrating their active constituents, such as flavonoids, terpenoids, and tannins, into a novel chocolate formulation. Guava leaves have been traditionally used to treat various human ailments due to their numerous bioactive compounds, but no previous studies have developed chocolate formulations incorporating guava leaf extracts. This research aims to: identify and extract bioactive compounds; develop chocolate formulations; evaluate health benefits; conduct sensory analysis; and analyze stability. The integration of guava leaf extracts into chocolate could offer a delicious and health-promoting alternative for consumers, thereby enhancing the therapeutic application of traditional medicinal plants.

Materials And Methods

Plant Material

The Guava leaves were collected during May 2023, Mandya, Mysore road, Karnataka. The sample was identified by the Botanist, Research Officer, Central Ayurveda Research Institute, Bangalore- 560109 and the reference no. Authentication/SMPU/CAIR/BNG/2023-24/407.

Ethanolic Extraction of Guava Leaf and Preliminary Identification of Phytochemical(s) of Extract Guava Leaves

Preparation of guava leaf powder

Chopping and drying: Fresh guava leaves were chopped and dried under shade at room temperature. Powdering and Sieving: Once dried, the leaves were powdered and sieved to obtain a uniform consistency using a coarse sieve (10/40 mesh).

Extraction process

- *First method*

Each 100 g of the sieved powder was used for extraction with 1 liter of ethanol in a reflux condenser, which involves heating the solvent to create vapor, which then condenses and drips back into the extraction flask, enhancing the extraction efficiency. The extraction was performed for three cycles, with each cycle lasting 7 hours. The process was continued until the volume of the solvent was reduced to half.

- *Second method*

The 100 g sieved powder was dissolved in 1000 mL ethanol and stirred overnight using a magnetic stirrer (200 rpm) at room temperature.

Filtration and evaporation

After extraction, the extract was filtered through muslin cloths to remove any solid residues. The filtered extract was then evaporated to dryness.

Storage

The dried extract was stored at -20°C until further use. Storing the extract at low temperatures helps preserve its stability and bioactive properties over time.

This extraction method allows for the concentration of bioactive compounds present in guava leaves, which can then be incorporated into various formulations, such as chocolates, to enhance their nutritional and health benefits. It's a systematic process that ensures the efficient extraction and preservation of valuable phytochemicals from the plant material.[11]

Development and formulation of guava leaf extract chocolates

Extract of guava leaves, milk powder, sugar (D Glucose), cocoa powder, flavoring agents (Citrus Flavor, Strawberry Flavor), normal saline, cocoa butter

Preparation of chocolate formulation

All ingredients, including cocoa powder, milk powder, sugar, cocoa butter, herbal drug extract (Guava), strawberry, and citrus flavoring agent, are weighed accurately.

In one beaker, a mixture of cocoa, milk powder, and sugar (30%) was prepared and mixed thoroughly. In another beaker, cocoa butter (30%) is melted. The melted cocoa butter is then added to the powder mixture, and the two are blended together using an electric blender until a fine consistency is achieved. The chocolate mixture is manually tempered on a cool surface to ensure proper texture and appearance. Accurately measured herbal drug extract (guava) is added to the chocolate mixture and thoroughly incorporated. Strawberry and citrus flavoring agents (0.03%) are added to enhance the taste of the chocolate. The prepared chocolate mixture containing the herbal extract is poured into molds. The molds are then placed in a freezer and left overnight to set. Once set, the guava chocolate is properly packaged in aluminum foil to maintain freshness. The packaged chocolate is stored at refrigeration to preserve its quality.

Optimizations of formulation of guava leaf extract chocolates by using RSM

RSM was used to optimize the formulation of guava milk chocolate. RSM involves creating regression equations to understand the relationships between input parameters, independent variables, and product properties, as well as dependent variables like color, texture, flavor, graininess, and hardness. A Central Composite Design (CCD) with three independent variables (guava, milk, and cocoa powder) was performed using Design-Expert DX 13.[12] CCD is a common experimental design method for RSM, as it allows for studying the curvature of the response surface. The independent variables were varied at different levels within a predetermined range, and the experimental design with the actual levels was outlined. Then, the responses to the product properties (color, texture, flavor, etc.) were measured at these different levels of the independent variables. The goal of this experimentation was likely to be to understand how independent variables were affected by changes in the amounts and the characteristics of the chocolate formulation and, ultimately, to optimize these variables to achieve the desired product properties.

Physico chemical analysis and sensory evaluation of guava chocolates[13,14]

Analysis of guava candies, specifically in relation to their moisture, protein, fat, ash content, and ascorbic acid content. Each of these analyses is crucial for assessing the nutritional composition and quality of the guava candies, ensuring they meet regulatory standards and consumer expectations. And the method was outlined in the AOAC, 2000 (Association of Official Agricultural Chemists). Sensory evaluation was done by using 9-point Hedonic scale (Lawless and Heymann, 2010). Texture Analyzer was used to measure the samples of chocolate's hardness (Bourne *et al*., 1978)

Statistical Analysis

A one-way ANOVA was used to determine if there were any statistically significant differences between the means of different groups, which in this case could be different formulations of the guava leaf chocolate. Data visualization and analysis were done by Microsoft Excel, which was employed for initial data entry, basic statistical calculations, and preliminary data visualization. Prism 5.0 and Design-Expert DX 13 software were used for more advanced statistical analysis and detailed graphical representation of the data.

Table 1: Build information

File version	*13.0.5.0*		
Study type	Response surface	Subtype	Randomized
Design type	Central composite	Runs	15.00
Design model	Quadratic	Blocks	No Blocks
Build time (ms)	375.00		

Results And Discussions

Ensuring the consistent extraction and standardization of bioactive compounds from guava leaves is crucial for the effectiveness of the final product. Variability in the concentration of active ingredients can affect the therapeutic efficacy and consumer experience. Previously, a study was done with the guava plant and reported the various phytoconstituents, which could be the reason for the type of therapeutic activity. Similarly, in the current study, the ethanolic extract of guava was subjected to testing for various phytoconstituents and found to contain alkaloids, flavonoids, tannins, phenols, carbohydrates, saponins, cardiac glycosides, and triterpenoids; however, steroids and anthraquinones were found to be absent. Flavonoids, tannins, phenols, saponins, and terpenoids are the active constituents. These various combined phytochemicals and nutrients were used for the further development and formulation of chocolates. The yield of ethanolic extract of guava by reflux condenser 1.6%, and by magnetic stirrer extract overnight at RT: 2.8%. The optimization of the formulation was done by using the factorial design file version 13.0.5.0 for a total of 15 runs (Table 1). The formulation of chocolates was mentioned in Table 2; factors 1 to 3 were dependent variables, and responses 1 to 5 were independent variables. The independent variables were analyzed by the ANOVA method, and the mean results (Table 3) were found as follows: body texture (6.57), graininess (5.63), color (7.24), flavor (6.98), and hardness (19.79). In Response 1, body texture factor coding was coded, and the model F-value was 6.79. This was the F-statistic for the overall significance of the regression model. A larger F-value indicates a more significant model (Table 4). About 2.42% chance of occurring due to noise: This was the significance level, or *p-value*, associated with the F-value. It indicates the probability of obtaining an F-value as large as observed, assuming that the null hypothesis is true (i.e., assuming that the model has no predictive power).

Table 2: Formulation of chocolates by factorial model

Std.	*Run*	*Row status*	*Factor 1*	*Factor 2*	*Factor 3*	*Response 1*	*Response 2*	*Response 3*	*Response 4*	*Response 5*
			A:Guava	*B:Milk*	*C:Coca*	*Body Texture*	*Graininess*	*Colour*	*Flavour*	*Hardness*
			%	*%*	*%*					*lbf*
1	1	Normal	7.5	7	7.5	6.2	6.12	6.19	6.17	14.09
9	2	Normal	4.94	10.5	11.25	7.1	5.13	7.26	5.64	13.65
6	3	Normal	15	7	15	5.8	4.88	7.47	7.24	19.79
11	4	Normal	11.25	4.61	11.25	4.9	5.76	7.39	6.57	18.3
10	5	Normal	17.55	10.5	11.25	6.1	5.89	6.12	7.9	18.55
3	6	Normal	7.5	14	7.5	7.8	4.78	7.93	5.68	15.94
2	7	Normal	15	7	7.5	4.6	6.01	6.45	7.08	16.45
8	8	Normal	15	14	15	7.3	6.87	6.09	7.89	17.89
5	9	Normal	7.5	7	15	5.2	5.49	7.98	6.45	19.25
15	10	Normal	11.25	10.5	11.25	6.9	4.98	8.48	6.98	17.45
13	11	Normal	11.25	10.5	4.94	8.1	5.45	7.92	6.97	12.51
14	12	Normal	11.25	10.5	17.55	8.2	5.78	8.19	7.86	18.64
7	13	Normal	7.5	14	15	7.4	5.67	7.32	6.75	12.95
12	14	Normal	11.25	16.38	11.25	6.8	5.78	6.64	7.92	17.54
4	15	Normal	15	14	7.5	6.2	5.79	7.18	7.58	18.56

Table 3: Summary of ANOVA for measured responses

Response	*Name*	*Units*	*Observations*	*Minimum*	*Maximum*	*Mean*	*Std. Dev.*	*Ratio*
R1	Body Texture		15.00	4.6	8.2	6.57	1.13	1.78
R2	Graininess		15.00	4.78	6.87	5.63	0.5404	1.44
R3	Colour		15.00	6.09	8.48	7.24	0.7872	1.39
R4	Flavour		15.00	5.64	7.92	6.98	0.7752	1.40
R5	Hardness	lbf	15.00	12.51	19.79	16.77	2.39	1.58

Table 4: Response 1, body texture

Source	*Sum of squares*	*df*	*Mean square*	*F-value*	*p-value*	
Model	16.46	9	1.83	6.79	0.0242	significant
A-Guava	1.41	1	1.41	5.22	0.0711	
B-Milk	7.46	1	7.46	27.70	0.0033	
C-Coca	0.0835	1	0.0835	0.3101	0.6016	
AB	0.0613	1	0.0613	0.2273	0.6536	
AC	1.71	1	1.71	6.35	0.0532	
BC	0.0313	1	0.0313	0.1160	0.7473	
A^2	0.3864	1	0.3864	1.43	0.2847	
B^2	1.62	1	1.62	6.02	0.0576	
C^2	0.5280	1	0.5280	1.96	0.2204	
Residual	1.35	5	0.2694			
Cor Total	17.81	14				

Table 5: Response 2, Graininess

Source	*Sum of squares*	*df*	*Mean square*	*F-value*	*p-value*	
Model	3.99	9	0.4430	21.91	0.0017	significant
A-Guava	0.5611	1	0.5611	27.75	0.0033	
B-Milk	0.0303	1	0.0303	1.50	0.2752	
C-Coca	0.0429	1	0.0429	2.12	0.2052	
AB	1.07	1	1.07	53.08	0.0008	
AC	0.0120	1	0.0120	0.5942	0.4756	
BC	1.74	1	1.74	86.03	0.0002	
A^2	0.2310	1	0.2310	11.43	0.0197	
B^2	0.4996	1	0.4996	24.71	0.0042	
C^2	0.3272	1	0.3272	16.18	0.0101	
Residual	0.1011	5	0.0202			
Cor Total	4.09	14				

A low *p-value* (such as 0.0242 in this case) suggests that the observed F-value was unlikely to occur by chance alone and thus indicates that the regression model was significant. The *p-value* less than 0.0500 indicates model terms are significant. The final equation in terms of coded factors of body texture was = +7.01-0.3208 (A) + 0.7392 (B) + 0.0782 (C) - 0.0875 (AB) + 0.4625 (AC) + 0.0625 (BC) - 0.2527 (A2) -0.5178 (B2) + 0.2953 (C2). The factor coding of the actual body texture of the 3D graph is represented in Fig. 1. Coding factors in this manner allows us to use a standardized way to analyze the effects of different factors on the response variable. It simplifies the interpretation and comparison of factor coefficients, making it easier to identify which factors have a larger impact on the response variable. Similarly, in response 2, graininess factor coding was coded, and the model F-value of 21.91

Table 6: Response 3, color

Source	*Sum of squares*	*df*	*Mean square*	*F-value*	*p-value*	
Model	8.35	9	0.9283	14.46	0.0045	significant
A-Guava	1.26	1	1.26	19.62	0.0068	
B-Milk	0.0506	1	0.0506	0.7884	0.4152	
C-Coca	0.1791	1	0.1791	2.79	0.1557	
AB	0.3741	1	0.3741	5.83	0.0605	
AC	0.1953	1	0.1953	3.04	0.1415	
BC	2.54	1	2.54	39.61	0.0015	
A^2	2.64	1	2.64	41.09	0.0014	
B^2	1.80	1	1.80	28.03	0.0032	
C^2	0.1907	1	0.1907	2.97	0.1453	
Residual	0.3209	5	0.0642			
Cor Total	8.68	14				

Table 7: Response 4, flavor

Source	*Sum of squares*	*df*	*Mean square*	*F-value*	*p-value*	
Model	7.79	9	0.8659	6.98	0.0228	significant
A-Guava	5.34	1	5.34	43.06	0.0012	
B-Milk	0.7641	1	0.7641	6.16	0.0557	
C-Coca	0.8055	1	0.8055	6.49	0.0514	
AB	0.2245	1	0.2245	1.81	0.2364	
AC	0.0968	1	0.0968	0.7803	0.4175	
BC	0.1105	1	0.1105	0.8903	0.3887	
A^2	0.1425	1	0.1425	1.15	0.3328	
B^2	0.0013	1	0.0013	0.0103	0.9232	
C^2	0.0337	1	0.0337	0.2716	0.6245	
Residual	0.6203	5	0.1241			
Cor Total	8.41	14				

implies the model was significant (Table 5). There was only a 0.17% chance that an F-value this large could occur due to noise. The final equation of graininess was: + 4.97 + 0.2027 (A) + 0.0471 (B) + 0.0560 (C) - 0.3663 (AB) - 0.0388 (AC) + 0.4662 (BC) + 0.1954 (A2) + 0.2873 (B2) + 0.2325 (C2). The factor coding of the actual graininess of the 3D graph is represented in Fig. 1. In response 3, color factor coding was coded, and the model F-value of 14.46 implies the model was significant (Table 6). There was only a 0.45% chance that an F-value this large could occur due to noise. The final equation of color was = + 8.50 - 0.3037 (A) - 0.0609 (B) + 0.1145 (C) - 0.2162 (AB) - 0.1562 (AC) - 0.5637 (BC) - 0.6601 (A2) - 0.5452 (B2) - 0.1775 (C2). The factor coding of the actual graininess of the 3D graph is represented in Fig. 1. In response 4, flavor factor coding was coded, and according to Table 7, the model's F-value of 6.98 indicates that it was significant. The probability that an F-value this great may be the result of noise is merely 2.28%. The final equation of flavor was = + 7.04 + 0.6254 (A) + 0.2365 (B) + 0.2429 (C) + 0.1675 (AB) - 0.1100 (AC) + 0.1175 (BC) - 0.1534 (A2) + 0.0145 (B2) + 0.0746 (C2). The factor coding of the actual graininess of the 3D graph is represented in Fig. 1. In response 5, hardness factor coding was coded, and according to Table 8, the model's F-value of 6.98 indicates that it was significant. The probability that an F-value this great may be the result of noise is merely 2.28%, and the final equation of hardness was = + 17.38 + 1.37 (A) – 0.4041 (B) + 1.11 (C) + 0.5825 (AB) + 0.0625 (AC) – 1.52 (BC) – 0.3740 (A2) + 0.2695 (B2) – 0.5596 (C2). The factor coding of the actual graininess of the 3D graph is represented in Fig. 1. The desirability of a response variable or outcome against the levels of input variables was plotted and represented (Fig. 2). The graph represents the combination of input variables that maximizes the

Table 8: Response 5, hardness

Source	*Sum of squares*	*df*	*Mean square*	*F-value*	*p-value*	
Model	72.33	9	8.04	5.08	0.0442	significant
A-Guava	25.61	1	25.61	16.18	0.0101	
B-Milk	2.23	1	2.23	1.41	0.2886	
C-Coca	16.81	1	16.81	10.62	0.0225	
AB	2.71	1	2.71	1.72	0.2473	
AC	0.0313	1	0.0313	0.0197	0.8937	
BC	18.48	1	18.48	11.68	0.0189	
A^2	0.8467	1	0.8467	0.5350	0.4973	
B^2	0.4395	1	0.4395	0.2777	0.6207	
C^2	1.90	1	1.90	1.20	0.3237	
Residual	7.91	5	1.58			
Cor Total	80.24	14				

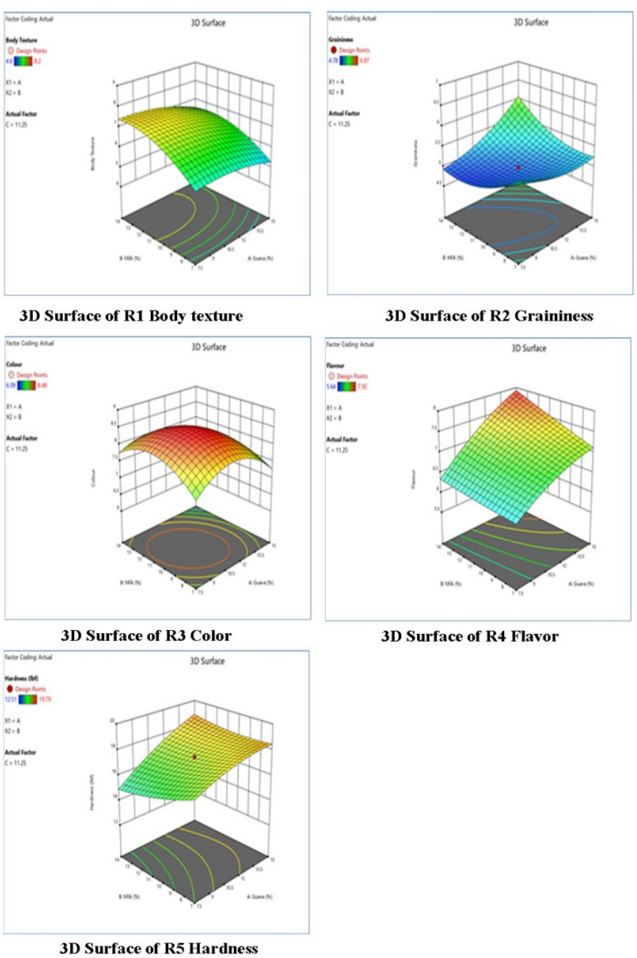


Fig. 1: Optimization 3D graph response 1 to 5

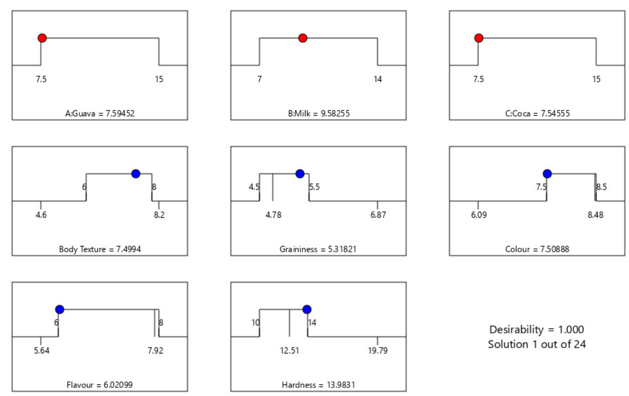


Fig. 2: Optimization desirability graph

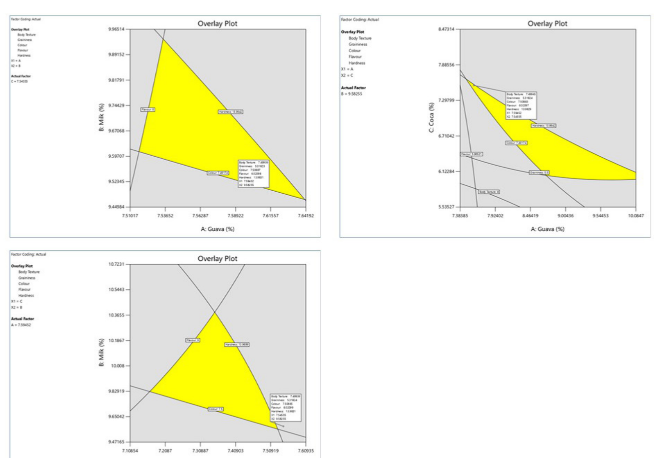
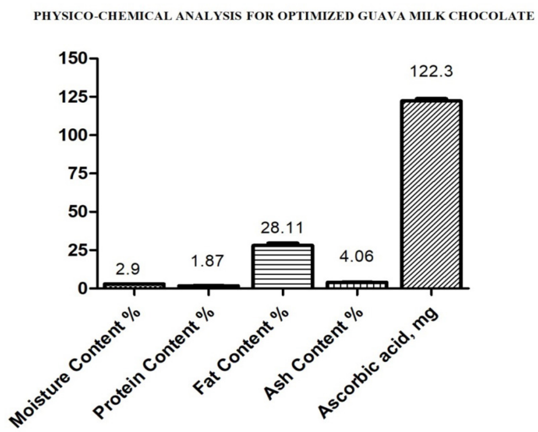


Fig. 3: Overlay plot of dependent variables *vs* independent variables

Table 9: Coefficients table

	Intercept	*A*	*B*	*C*	*AB*	*AC*	*BC*	A^2	B^2	C^2
Body texture	7.00593	-0.320849	0.739219	0.0782156	-0.0875	0.4625	0.0625	-0.252662	-0.517827	0.295346
p-values		0.0711	0.0033	0.6016	0.6536	0.0532	0.7473	0.2847	0.0576	0.2204
Graininess	4.97423	0.202694	0.0471291	0.0560152	0.36625	-0.03875	0.46625	0.195362	0.287286	0.232486
p-values		0.0033	0.2752	0.2052	0.0008	0.4756	0.0002	0.0197	0.0042	0.0101
Color	8.4997	-0.303675	-0.0608738	0.114527	-0.21625	-0.15625	-0.56375	-0.660121	-0.545216	-0.17752
p-values		0.0068	0.4152	0.1557	0.0605	0.1415	0.0015	0.0014	0.0032	0.1453
Flavor	7.03722	0.625389	0.236542	0.242867	0.1675	-0.11	0.1175	-0.15343	0.0145078	0.0746118
p-values		0.0012	0.0557	0.0514	0.2364	0.4175	0.3887	0.3328	0.9232	0.6245
Hardness	17.3754	1.36933	-0.404058	1.10929	0.5825	0.0625	-1.52	-0.374006	0.269461	-0.559621
p-values		0.0101	0.2886	0.0225	0.2473	0.8937	0.0189	0.4973	0.6207	0.3237

p-value shading: $p < 0.05$ $0.05 \le p < 0.1$ $p \ge 0.1$



Values are expressed in Mean ± SEM, n = 3

Fig. 4: Physico-chemical analysis of proximate composition of optimized guava milk chocolate

desirability of the outcome. With these settings it helps to lead to the most desirable outcomes and make informed decisions to optimize the process. Similarly, the overlay plot was a share of all data series to ensure that the data was aligned properly for comparison (Fig. 3). This allows for a more comprehensive analysis of how the variables relate to each other and behave under different conditions. A coefficient representation of the coefficients was estimated in a statistical model, typically from regression analysis. It displayed the estimated coefficients, their standard errors, and *p-values* (Table 9). It has interpreted the results of the regression analysis and the relationships between variables. Physicochemical analysis was done for the optimized guava chocolate formulation, and the results were shown as 2.9% moisture, 1.877% protein, 28.111% fat, and 4.06% ash. The level of ascorbic acid was 122.3 mg in 100 mL of the guava chocolate formulation (Fig. 4).

Crude drug standardization through pharmacognostic studies is essential for the accurate identification and quality assurance of medicinal plants. Realizing the potential of herbal chocolate formulations, further research is needed in several areas. Studies should focus on specific health outcomes, such as antioxidant levels, inflammatory markers, and microbial activity. Further research on optimizing the extraction process, concentration, and integration of guava leaf extracts will enhance the efficacy and consumer acceptability of the product. Exploring different types of chocolate and additional functional ingredients can create a range of products tailored to various health needs and preferences.

Conclusion

In summary, the conclusion drawn would typically involve identifying the factors with the most significant coefficients and their directionality to understand their relative importance in predicting the response variable. The herbal formulation of chocolates underwent a successful optimization process using factorial design. The results seem promising, with satisfactory outcomes in terms of various parameters like body texture, graininess, color, flavor, and hardness. Additionally, the physico-chemical analysis yielded results within acceptable ranges, further validating the success of the formulation. This suggests that the herbal chocolates could be well-received by consumers, given their desirable qualities and adherence to quality standards.

Acknowledgment

The present work was made possible through the generous support and resources provided by several key departments. The infrastructure for conducting the research was extended by Dr. K. Ramesh, Director of the Karnataka College of Pharmacy in Bangalore, to whom the authors express their sincere gratitude. Additionally, the study was funded by the Advanced Research Wing at Rajiv Gandhi University of Health Sciences in Bangalore, India, under Grant No. 19PHA311. This financial support was crucial in facilitating the various stages of the

research, from the preparation and extraction of guava leaf compounds to the development and analysis of the chocolate formulations.

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HOW TO CITE THIS ARTICLE: Prabhakar T, Selvi DB, Sreedhar C, Jha DK. Novel Development of Phytomedicine and its Innovative Formulation of Guava Leaf Extract Chocolates for Various Incredible Therapeutic Activities. Int. J. Pharm. Sci. Drug Res. 2024;16(4):625-633. **DOI:** 10.25004/IJPSDR.2023.160410