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Research Paper

Development And Evaluation of Guava Leaf Extract Enriched Chocolate as a Nutraceutical Product

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ABSTRACT

Nutraceutical products have gained considerable importance because of their nutritional and potential health-promoting properties. The present investigation focused on the development and evaluation of nutraceutical guava leaf chocolate using guava leaf extract as a herbal ingredient. Guava leaves (Psidium guajava) contain bioactive phytoconstituents including flavonoids, tannins and phenolic compounds and are reported to possess antioxidant and other pharmacological activities. In the present study, an aqueous guava leaf extract was prepared and incorporated into chocolate formulations at different concentrations to obtain F1, F2 and F3. The formulations were evaluated for organoleptic properties, weight variation, hardness, melting point, pH, moisture content, antioxidant activity using the DPPH assay, and stability. The reported antioxidant inhibition values were 62%, 74% and 85% for F1, F2 and F3, respectively. F2 provided the most favorable overall balance of taste, texture, hardness, stability and antioxidant potential and was therefore identified as the optimized formulation. The study supports the feasibility of incorporating guava leaf extract into chocolate as a potential functional food formulation, while further safety, shelf-life and advanced characterization studies are required.

INTRODUCTION

1.1 Introduction to Nutraceuticals

The term nutraceutical is derived from the combination of "nutrition" and "pharmaceutical." Nutraceuticals are products that provide nutritional as well as therapeutic advantages to the human body[1]. These products are widely used for the prevention and management of various diseases and are considered an important part of modern healthcare systems. Nutraceuticals include dietary supplements, functional foods, herbal products, fortified foods, probiotics, and medicinal beverages.

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In recent times, the demand for nutraceutical products has increased significantly because people are becoming more health conscious and prefer naturally derived remedies over synthetic medicinal products. Herbal nutraceuticals are especially popular due to their safety, lower side effects, and therapeutic value. Medicinal plants contain several bioactive compounds such as flavonoids, tannins, alkaloids, and phenolic compounds that exhibit antioxidant, antimicrobial, anti-inflammatory, and antidiabetic properties.

Modern pharmaceutical research is has emphasized the development of nutraceutical products in novel delivery forms to enhance user compliance and acceptability. As a result, chocolates, gummies, herbal candies, and chewable products are increasingly being developed as nutraceutical delivery systems. Chocolate-based formulations are particularly preferred because they possess excellent taste, uniform smooth texture, and flavor masking properties.

1.2 Concept of Herbal Chocolate

Chocolate is one of the most widely consumed confectionery products worldwide because of its agreeable taste, aroma, and broad consumer acceptance. Traditional chocolates are mainly prepared from cocoa solids, cocoa butter, milk solids, sugar, and flavoring agents. Recently, the concept of herbal chocolate has gained importance in nutraceutical and pharmaceutical industries.[2]

Herbal chocolates are formulations in which medicinal plant extracts or herbal powders are added into chocolate bases to offer therapeutic advantages together with nutritional benefits. These formulations are considered excellent alternatives to conventional dosage forms such as tablets and capsules because they improve palatability and patient compliance.

Chocolate itself possesses antioxidant potential because cocoa contains flavonoids and polyphenolic compounds. These compounds are reported to help counter oxidative stress and provide cardiovascular benefits. When herbal ingredients are incorporated into chocolate, the resulting formulation may show enhanced nutraceutical properties due to synergistic therapeutic effects.

Herbal chocolates are particularly useful for pediatric and geriatric patients because they are convenient to consume and can help conceal bitter herbal flavors of herbal ingredients. thus, chocolate-based herbal formulations are gaining increasing attention in the development of functional foods and nutraceutical products.

1.3 Botanical Profile of Guava Leaves

Guava (Psidium guajava) belongs to the family Myrtaceae and is extensively grown in tropical and subtropical regions. The plant is recognized for its nutritional fruits and medicinal leaves. Different parts of the guava plant including fruits, leaves, bark, and roots are traditionally used in herbal medicine for the treatment of various disorders.[3]

Taxonomical Classification

Guava leaves possess numerous phytoconstituents such as flavonoids, tannins, phenolic compounds, glycosides, terpenoids, and essential oils. These phytochemicals are associated with multiple pharmacological activities including antioxidant, antimicrobial, anti-inflammatory, and antidiabetic effects.

Because of the presence of these bioactive compounds, guava leaves are considered highly suitable for nutraceutical and functional food applications.

1.4 Medicinal Importance of Guava Leaves

Guava leaves possess significant medicinal and therapeutic properties and have been used traditionally for the treatment of diarrhea, dysentery, diabetes, cough, oral infections, and

gastrointestinal disorders. The medicinal value of guava leaves is mainly attributed to the presence of flavonoids, tannins, and polyphenolic compounds.[4]

One of the major therapeutic properties of guava leaves is antioxidant potential. Antioxidants help neutralize harmful free radicals and reduce oxidative stress in the body. Oxidative stress is associated with several chronic diseases such as diabetes, cardiovascular disorders, cancer, and aging-related complications.

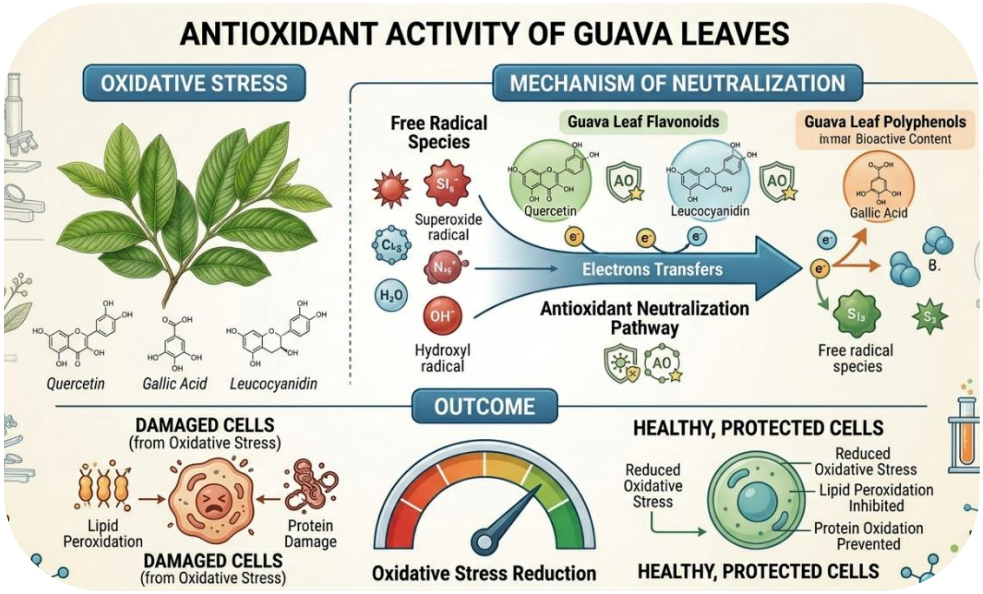
Guava leaves also exhibit antimicrobial activity against various pathogenic microorganisms. The antimicrobial action is mainly due to tannins and flavonoids present in the leaves, which interfere with microbial proliferation and metabolism.

In addition to antioxidant and antimicrobial effects, guava leaves are reported to possess anti-inflammatory, antidiabetic, gastroprotective, and hepatoprotective properties. Due to these multiple health-related benefits, guava leaves are widely used in herbal formulations and nutraceutical preparations.

1.5 Antioxidant Activity of Guava Leaves

Antioxidants are compounds that protect biological systems against oxidative damage caused by reactive oxygen species and free radicals. Excessive production of free radicals may lead to cellular damage, inflammation, aging, and chronic diseases.

Guava leaves are rich in natural antioxidants such as quercetin, catechin, gallic acid, and other polyphenolic compounds. These compounds exhibit strong free-radical-scavenging capacity and help protect cells from oxidative stress.[5]



The antioxidant potential of guava leaves can be evaluated using different laboratory methods including:

DPPH radical scavenging assay

Ferric reducing antioxidant power assay

Hydrogen peroxide scavenging assay

Incorporation of guava leaf extract into chocolate formulations may improve the antioxidant profile of the resulting product and increase its nutraceutical value.

1.6 Antimicrobial Properties of Guava Leaves

Guava leaves possess significant antibacterial and antifungal activities against several pathogenic microorganisms. Natural antimicrobial agents obtained from medicinal plants are becoming

increasingly important because of rising microbial resistance to synthetic antibiotics.

The antimicrobial properties of guava leaves are mainly due to the presence of flavonoids, tannins, and essential oils. These phytochemicals inhibit microbial proliferation by damaging microbial cell membranes and interfering with protein synthesis. Studies have shown that guava leaf extracts exhibit activity against microorganisms such as:

- Escherichia coli
- Staphylococcus aureus
- Salmonella species
- Candida albicans

Due to these antimicrobial properties, guava leaf formulations may help support gastrointestinal health and improve overall immunity.

1.7 Health Benefits of Nutraceutical Guava Leaf Chocolate

Nutraceutical guava leaf chocolate combines the nutritive value of chocolate with the medicinal properties of guava leaves. Such formulations may provide several health benefits including antioxidant protection, digestive support, immune enhancement, and reduction of oxidative stress.[6] The presence of cocoa flavonoids along with guava leaf phytoconstituents may produce synergistic therapeutic effects. Chocolate formulations also improve the palatability of herbal products and increase patient compliance.

Some important health benefits of nutraceutical guava leaf chocolate include:

- Antioxidant activity
- Improved consumer acceptability
- Herbal nutritional supplementation
- Better patient compliance
- Functional food benefits
- Digestive health support

Thus, nutraceutical herbal chocolates can serve as effective and consumer-friendly functional food products.

1.8 Need for Nutraceutical Confectionery Products

Modern lifestyle patterns, unhealthy food habits, stress, and environmental pollution have increased the occurrence of chronic diseases and nutritional deficiencies. As a result, consumers are increasingly demanding products that provide both nutritional and health-related benefits.

Conventional pharmaceutical dosage forms such as tablets and capsules may show poor patient compliance, particularly among pediatric and geriatric populations. Nutraceutical confectionery products such as herbal chocolates provide a more palatable and convenient alternative dosage form.

Advantages of nutraceutical chocolates include:

- Pleasant taste
- Easy administration
- Better patient compliance
- Attractive dosage form
- Improved palatability
- Convenient consumption

These advantages make herbal chocolates promising products for nutraceutical and functional food applications.

1.9 Problem Statement

while guava leaves possess excellent medicinal properties, their direct consumption is limited due to bitter taste and poor palatability. Most herbal preparations are available in the form of powders, capsules, and decoctions, which may reduce patient acceptability.

There is a need to develop an innovative nutraceutical dosage form that can effectively deliver guava leaf phytoconstituents while maintaining good taste and consumer acceptance. Chocolate-based herbal formulations may effectively mask bitterness and improve the overall acceptance of herbal products.

Thus, the current investigation focuses on the development and evaluation of nutraceutical

guava leaf chocolate as a novel functional food formulation.

1.10 Scope of the Study

The current investigation has considerable importance in pharmaceutical and nutraceutical fields because it focuses on the development of a herbal chocolate formulation containing guava leaf extract.[7]

The scope of The investigation includes:

- Formulation of nutraceutical guava leaf chocolate
- Evaluation of physicochemical parameters
- Assessment of antioxidant activity
- Stability studies
- Improvement of herbal product palatability
- Development of consumer-friendly nutraceutical dosage forms

The developed product may have potential future applications in nutraceutical industries, functional food sectors, herbal pharmaceutical industries, and health supplement markets.

REVIEW OF LITERATURE

2.1 Overview of Literature Review

Literature review is an important part of pharmaceutical and nutraceutical research because it provides scientific background regarding previously conducted studies, formulation approaches, medicinal plant applications, antioxidant activities, and evaluation methods. The present review focuses on nutraceuticals, herbal chocolates, guava leaves, antioxidant properties, antimicrobial activity, and functional food development.

Several researchers have reported that medicinal plants containing polyphenols, flavonoids, and tannins possess significant therapeutic activities and can be successfully incorporated into nutraceutical products. Chocolate-based herbal formulations have also gained increasing attention because of their improved palatability, patient compliance, and consumer acceptance.

2.2 Studies on Nutraceuticals and Functional Foods

Researchers introduced the concept of nutraceuticals and explained their role in disease prevention and health promotion. Nutraceutical products provide health-related benefits in addition to nutritive value and may reduce dependence on synthetic drugs.

Various studies reported that functional foods and nutraceutical products have become important components of preventive healthcare systems. Nutraceuticals containing natural antioxidants may reduce the risk of chronic diseases such as cardiovascular disorders, diabetes, and cancer.

Scientific investigations also highlighted the importance of bioactive plant constituents in improving human health. Nutraceutical products containing herbal ingredients can provide physiological and health-related benefits beyond basic nutrition.

The increasing global demand for nutraceutical products has encouraged pharmaceutical industries to develop innovative functional food formulations with improved stability, safety, and acceptability.

2.3 Studies on Herbal Chocolate Formulations

Herbal chocolates have gained considerable importance in recent times due to their nutritive value and improved patient compliance. Several studies have been conducted on incorporation of medicinal plant extracts into chocolate formulations.

Research on herbal chocolates prepared using medicinal plant extracts demonstrated good consumer acceptance and enhanced antioxidant potential. Chocolate can serve as an effective

carrier system for herbal ingredients because of its flavor masking properties and uniform smooth texture.

Studies on nutraceutical chocolate formulations demonstrated that incorporation of herbal extracts into chocolates improved the functional value of the product without significantly affecting organoleptic characteristics. The prepared formulations exhibited acceptable hardness, melting point, texture, and stability.

Research investigations have also indicated that herbal chocolates may be particularly useful in pediatric and geriatric populations due to ease of administration and pleasant taste. Chocolate formulations effectively mask the bitterness associated with herbal ingredients and improve patient adherence.

2.4 Studies on Guava Leaves

Guava (Psidium guajava) leaves have been extensively studied for their medicinal and pharmacological activities. The leaves contain important phytoconstituents such as flavonoids, tannins, phenolic compounds, glycosides, and essential oils responsible for various therapeutic effects.

Studies investigating the medicinal properties of guava leaves reported significant antioxidant, antimicrobial, antidiabetic, and anti-inflammatory activities. Researchers concluded that guava leaves possess high therapeutic potential and can be used in herbal pharmaceutical formulations.

Pharmacognostic investigations on guava leaves reported the presence of quercetin, catechin, gallic acid, and other polyphenolic compounds which support substantial antioxidant potential. Researchers emphasized the importance of guava leaves in functional food and nutraceutical applications.

Several studies have also demonstrated the antidiarrheal and gastroprotective effects of guava leaves. Traditional systems of medicine have used guava leaf preparations for the treatment of gastrointestinal disorders, oral infections, wounds, and diabetes.

2.5 Studies on Antioxidant Activity

Oxidative stress plays an important role in the development of chronic diseases such as diabetes, cardiovascular disorders, aging, and cancer. thus, antioxidant-rich nutraceutical products are gaining significant attention in pharmaceutical research.

Studies have shown that guava leaves possess strong antioxidant potential due to the presence of flavonoids and phenolic compounds. Guava leaf extracts exhibited excellent free-radical-scavenging capacity when evaluated using DPPH and ferric reducing antioxidant assays.

Cocoa used in chocolate formulations is also rich in flavonoids and polyphenols which contribute to antioxidant potential. thus, the combination of guava leaf extract and cocoa may produce synergistic antioxidant effects in nutraceutical chocolate formulations.

Several research studies have concluded that herbal formulations containing antioxidant-rich plant extracts may help reduce oxidative stress and improve overall health status.

2.6 Studies on Antimicrobial Activity

The antimicrobial activity of guava leaves has been widely investigated against various pathogenic microorganisms. Researchers have reported that guava leaf extracts exhibit antibacterial and antifungal activities due to the presence of tannins, flavonoids, and terpenoids.

Microbiological studies on guava leaf extract demonstrated inhibitory activity against microorganisms such as Escherichia coli, Staphylococcus aureus, Salmonella species, and Candida albicans. The studies concluded that guava leaves may serve as natural antimicrobial agents in herbal formulations.

Research investigations also suggested that plant-based antimicrobial agents are becoming increasingly important because of rising microbial resistance associated with synthetic antibiotics. Herbal nutraceutical products containing antimicrobial phytoconstituents may help support gastrointestinal health and immunity.

2.7 Studies on Chocolate Evaluation Parameters

Evaluation of chocolate formulations is an essential part of nutraceutical product development. Several researchers have studied physicochemical and organoleptic characteristics of chocolate formulations including hardness, melting point, texture, moisture content, taste, and stability.

Pharmaceutical evaluation studies reported that proper optimization of chocolate formulations is necessary to achieve suitable texture, appearance, and melting characteristics. Excessive moisture content may affect stability and microbial proliferation, whereas improper hardness may reduce consumer acceptance.

Researchers also emphasized the importance of sensory assessment studies for determining consumer preference and palatability of herbal chocolates. Parameters such as taste, odor, color, oral texture, and texture significantly influence the success of nutraceutical confectionery products.

2.8 Research Gap

While several studies have been conducted on guava leaves and nutraceutical formulations, limited investigation is available on the development of nutraceutical guava leaf chocolate formulations. Most available herbal products are prepared as capsules, powders, syrups, or decoctions, which may show poor patient compliance due to bitterness and unpleasant taste.

Very few studies have focused on incorporating guava leaf extract into chocolate formulations while evaluating physicochemical characteristics, antioxidant potential, sensory attributes, and stability parameters simultaneously. thus, the current investigation aims to develop and evaluate nutraceutical guava leaf chocolate as an innovative herbal functional food product with improved palatability, antioxidant potential, and consumer acceptance.

2.9 Chart of Review of Literature

Table 4.10 Summary Chart of Literature Review

Flow Chart of Literature Review

Nutraceutical Concept

↓

Functional Food Development

↓

Medicinal Importance of Guava Leaves

↓

Phytochemical Constituents

↓

Antioxidant and Antimicrobial Activity

↓

Development of Herbal Chocolate

↓

Evaluation of Chocolate Formulation

↓

Stability and Acceptability Studies

↓

Development of Nutraceutical Guava Leaf Chocolate

The reviewed literature indicates that guava leaves possess significant medicinal and antioxidant properties due to the presence of flavonoids, tannins, and polyphenolic compounds. Previous studies also demonstrate that chocolate formulations can effectively mask the bitterness of herbal ingredients while improving palatability and patient compliance.

The literature further suggests that nutraceutical products containing natural antioxidants may help

reduce oxidative stress and improve overall health status. However, limited studies are available regarding incorporation of guava leaf extract into chocolate formulations. thus, the present research was undertaken to develop and evaluate nutraceutical guava leaf chocolate as a novel herbal functional food product.

AIM AND OBJECTIVES

The current investigation entitled "Development and Evaluation of Nutraceutical Guava Leaf Chocolate" was carried out with the aim of developing a novel herbal nutraceutical formulation with improved therapeutic value, antioxidant potential, and consumer acceptance. The investigation focused on formulation development, physicochemical evaluation, antioxidant assessment, and stability analysis of guava leaf chocolate formulations. The major objectives of The investigation are as follows:

3.1 Primary Objective

To develop and evaluate nutraceutical guava leaf chocolate containing medicinally active guava leaf extract as a functional food formulation. [8]

3.2 Secondary Objectives

3.2.1 Collection and Authentication of Plant Material

To collect fresh guava leaves and perform proper cleaning, drying, and processing for formulation development.
To ensure the quality and suitability of guava leaves for nutraceutical applications. [9]

3.2.2 Preparation of Guava Leaf Powder/Extract

To prepare guava leaf powder or extract using suitable pharmaceutical processing methods.
To obtain maximum extraction of bioactive phytoconstituents responsible for antioxidant and therapeutic activities. [10]

3.2.3 Formulation of Nutraceutical Chocolate

To formulate herbal chocolate preparations using guava leaf extract in different concentrations.
To prepare various formulations such as F1, F2, and F3 for comparative assessment.
To develop a stable and palatable nutraceutical chocolate formulation. [11]

3.2.4 Evaluation of Organoleptic Properties

To evaluate the prepared chocolate formulations for color, odor, taste, texture, and appearance.
To study consumer acceptance and palatability of the developed formulations. [12]

3.2.5 Evaluation of Physicochemical Parameters

To evaluate physicochemical characteristics such as:

- Weight variation
- Hardness
- Melting point
- pH
- Moisture content
- Stability characteristics

To determine the quality and consistency of the prepared chocolate formulations. [13]

3.2.6 Evaluation of Antioxidant Activity

To assess the antioxidant potential of guava leaf chocolate formulations using suitable antioxidant evaluation methods.
To determine the free radical scavenging potential of the prepared nutraceutical product. [14]

3.2.7 Stability Study

To perform stability assessments under suitable storage environment.
To evaluate changes in texture, appearance, odor, and overall stability during storage duration. [15]

3.2.8 Comparative Evaluation of Formulations

To compare all prepared formulations (F1, F2, and F3) based on evaluation parameters.
To identify the best formulation with optimum physicochemical characteristics and antioxidant potential. [16]

3.2.9 Development of Functional Nutraceutical Product

To develop an innovative herbal nutraceutical chocolate with improved therapeutic value and consumer compliance.
To explore the pharmaceutical and functional food applications of guava leaf chocolate formulations. [17]

MATERIALS AND METHODS

4.1 Overview of Methodology

The current investigation was carried out for the development and evaluation of nutraceutical guava leaf chocolate using guava leaf powder/extract as a medicinal and functional ingredient. The methodology included collection and processing of guava leaves, preparation of extract, formulation of chocolate using different concentrations of guava leaf extract, and evaluation of prepared formulations for physicochemical, organoleptic, and antioxidant parameters.
The entire study was performed using standard pharmaceutical and nutraceutical formulation techniques under controlled laboratory conditions. [18]

4.2 Materials

The materials used in the preparation of nutraceutical guava leaf chocolate were of pharmaceutical or food grade quality. Fresh guava leaves were collected from local agricultural areas and all other ingredients were procured from authorized suppliers.

4.3 Collection and Authentication of Guava Leaves

Fresh and healthy guava leaves were collected from local cultivated plants during the morning hours to ensure maximum phytochemical content. Diseased, damaged, and contaminated leaves were removed carefully.
The collected leaves were washed thoroughly with distilled water to remove dirt, dust particles, and foreign contaminants. The cleaned leaves were authenticated based on their morphological characteristics such as shape, color, odor, and texture.
The authenticated leaves were then subjected to drying and pulverization for further processing. [19]

4.4 Preparation of Guava Leaf Powder

4.4.1 Drying of Leaves

The washed guava leaves were shade dried at ambient temperature for approximately 7–10 days. Shade drying was preferred to prevent degradation of heat-sensitive phytoconstituents and preserve antioxidant potential.
The leaves were spread uniformly on clean trays and protected from direct sunlight, dust, and moisture during the drying process.

4.4.2 Pulverization

After complete drying, the leaves became brittle and were pulverized using a mechanical grinder to obtain coarse powder. The powdered material was

sieved through suitable mesh size to ensure uniform particle distribution.

The prepared powder was stored in airtight containers protected from moisture and light until further use. [20]

4.5 Preparation of Guava Leaf Extract

The guava leaf extract was formulated using aqueous extraction method because of its simplicity, safety, and suitability for nutraceutical applications.

4.5.1 Extraction Procedure

- Approximately 50 g of dried guava leaf powder was weighed accurately.
- The powder was mixed with distilled water in a suitable extraction vessel.
- The mixture was heated at controlled temperature for 30–45 minutes with continuous stirring.
- The extract was cooled and filtered using muslin cloth followed by filtration through Whatman filter paper.
- The filtrate was concentrated using controlled heating until a semisolid extract was obtained.
- The prepared extract was stored in airtight containers under refrigerated storage environment for further formulation studies. [21]

4.6 Formulation of Nutraceutical Guava Leaf Chocolate

Nutraceutical guava leaf chocolates were formulated using standard chocolate manufacturing method. the various formulations were formulated by varying the concentration of guava leaf extract.

4.6.1 Formula for Chocolate Preparation

4.6.2 Method of Chocolate Preparation

Step 1: Preparation of Chocolate Base

Cocoa butter was melted using water bath method at controlled temperature of approximately 40–45°C. Cocoa powder and milk powder were added slowly with continuous stirring to obtain a uniform mixture.

Step 2: Addition of Guava Leaf Extract

Measured quantity of guava leaf extract was incorporated into the chocolate base according to formulation design (F1, F2, and F3). Continuous stirring was performed to ensure uniform distribution of extract throughout the formulation.

Step 3: Addition of Excipients

Sugar, lecithin, and vanilla essence were added to improve sweetness, texture, emulsification, and flavor characteristics.

Step 4: Molding Process

The prepared chocolate mixture was poured into suitable molds and allowed to cool at refrigerated temperature until solidification occurred.

Step 5: Packaging and Storage

Prepared chocolates were removed carefully from molds, wrapped in aluminum foil, and stored in airtight containers under cool and dry conditions for further evaluation studies. [22]

4.7 Evaluation Parameters

Prepared nutraceutical guava leaf chocolates underwent evaluation for various organoleptic, physicochemical, and stability parameters.

4.7.1 Organoleptic Evaluation

organoleptic characteristics of the prepared formulations underwent evaluation visually and manually.

Parameters Evaluated

- Color
- Odor
- Taste
- Appearance
- Texture

These parameters underwent evaluation to determine consumer acceptance and overall product quality. [23]

4.7.2 Weight Variation Test

Individual chocolates from each formulation batch were weighed separately using digital weighing balance.

Procedure

- Ten chocolates were selected randomly.
- Individual weight was recorded.
- Average weight was calculated.
- Percentage deviation was determined.
- The test was performed to ensure uniformity of formulation. [24]

4.7.3 Hardness Test

Hardness of chocolate formulations was determined using suitable hardness testing method.

Purpose

- To evaluate mechanical strength
- To determine handling properties
- To assess texture consistency

Proper hardness is essential for maintaining product stability and consumer acceptance. [25]

4.7.4 Determination of Melting Point

Melting point of chocolate formulations was determined using capillary method.

Importance

Indicates thermal stability

Helps determine storage conditions

Influences oral texture and texture characteristics

The melting characteristics of chocolates significantly affects sensory acceptance. [26]

4.7.5 Determination of pH

The pH of chocolate formulations was determined by dispersing sample in distilled water followed by pH measurement using digital pH meter.

Importance of pH Study

Determines formulation compatibility

Influences stability

Affects microbial proliferation and product quality

4.7.6 Moisture Content Determination

Moisture content was determined using drying method.

Importance

- Excess moisture may promote microbial proliferation
- Influences texture and shelf life
- Affects formulation stability

The moisture content of nutraceutical chocolate should remain within acceptable limits for maintaining quality. [27]

4.7.7 Antioxidant Activity Study

Antioxidant potential of guava leaf chocolate formulations was evaluated using DPPH free radical scavenging assay.

Principle

The method is based on reduction of DPPH radicals by antioxidant compounds present in guava leaf extract.

Importance

Determines antioxidant potential

Indicates nutraceutical value

Evaluates free-radical-scavenging capacity

Higher antioxidant potential indicates better therapeutic potential of the formulation. [28]

4.7.8 Stability Study

Stability assessments were carried out to determine the effect of storage environment on formulation quality.

Parameters Observed During Stability Study

- Change in color
- Odor variation
- Texture changes
- Appearance
- Melting characteristics

The formulations were stored under ambient temperature and refrigerated storage environment for evaluation of stability characteristics. [29]

RESULTS

5.1 Overview of Results

The prepared nutraceutical guava leaf chocolate formulations (F1, F2, and F3) were successfully developed and evaluated for various organoleptic, physicochemical, antioxidant, and stability parameters. The results obtained from evaluation studies indicated that incorporation of guava leaf extract influenced texture, taste, antioxidant potential, and overall formulation attributes.
comparative assessment of all formulations was carried out to identify the most suitable nutraceutical chocolate formulation with acceptable quality, stability, and therapeutic potential. [30]

5.2 Organoleptic Evaluation

Organoleptic evaluation was performed to assess the sensory attributes of prepared chocolate formulations including color, odor, taste, texture, and appearance.

Table 7.1 Organoleptic Evaluation of Nutraceutical Guava Leaf Chocolate

Parameters	F1	F2	F3
Color	Dark Brown	Dark Brown	Brownish Green
Odour	Pleasant	Pleasant	Slight Herbal Odor
Taste	Sweet and Acceptable	Slightly Bitter	Bitter
Texture	Smooth	Smooth	Slightly Granular
Appearance	Uniform	Uniform	Slightly Non-uniform

Observation

- F1 showed best taste and appearance.
- F2 exhibited acceptable sensory properties with mild herbal taste.
- F3 showed increased bitterness due to greater concentration of guava leaf extract. [31]

5.3 Weight Variation Test

The weight variation test was performed to determine uniformity among prepared chocolate formulations.

Table 7.2 Weight Variation Study

Formulation	Average Weight (g)	Percentage Deviation
F1	10.12	±1.2%
F2	10.18	±1.5%
F3	10.25	±1.8%

Observation

All formulations showed acceptable weight variation within permissible pharmaceutical standards indicating uniform distribution of ingredients during formulation process. [32]

5.4 Hardness Evaluation

Hardness evaluation was performed to determine physical strength and texture consistency of chocolate formulations.

Table 7.3 Hardness Study

Formulation	Hardness (kg/cm²)
F1	3.2
F2	3.5
F3	3.9

Observation

- Hardness increased with increase in concentration of guava leaf extract.
- F2 exhibited optimal hardness and suitable texture characteristics.
- F3 showed comparatively higher hardness which slightly affected oral texture. [33]

5.5 Melting Point Determination

Melting point determination was carried out to evaluate thermal stability and melting characteristics of formulations.

Table 7.4 Melting Point Study

Formulation	Melting Point (°C)
F1	33°C
F2	34°C
F3	35°C

Observation

All formulations showed appropriate melting characteristics for chocolate formulations. Increase in herbal extract concentration slightly increased melting point values. [34]

5.6 pH Determination

The pH values of formulations were determined to evaluate compatibility and stability.

Table 7.5 pH Study

Formulation	pH
F1	6.4
F2	6.2
F3	6.0

Observation

All formulations showed near neutral pH indicating suitability for nutraceutical consumption and formulation stability. [35]

5.7 Moisture Content Determination

Moisture content analysis was performed to evaluate stability and shelf-life characteristics.

Table 7.6 Moisture Content Study

Formulation	Moisture Content (%)
F1	1.8
F2	2.0
F3	2.3

Observation

- Moisture content increased slightly with increase in guava leaf extract concentration.
- All formulations remained within acceptable stability limits.
- Excessive moisture was not observed in any formulation. [36]

5.8 Antioxidant Activity Study

antioxidant potential was evaluated using DPPH free radical scavenging assay.

Table 7.7 Antioxidant Activity Study

Formulation	Percentage Inhibition (%)
F1	62%
F2	74%
F3	85%

Observation

- antioxidant potential increased with increase in guava leaf extract concentration.
- F3 exhibited greatest antioxidant potential due to greater phytochemical content.
- Presence of cocoa flavonoids may have contributed synergistically to antioxidant effects. [37]

5.9 Stability Study

stability assessments were carried out under ambient temperature and refrigerated storage environment for 30 days.

Table 7.8 Stability Study

Parameter	F1	F2	F3
Change in Color	No Change	No Change	Slight Change
Change in Odor	No Change	No Change	Slight Herbal Odor
Texture Stability	Stable	Stable	Slightly Hard
Appearance	Uniform	Uniform	Slightly Non-uniform

Observation

- F1 and F2 maintained stability throughout storage duration.
- F3 showed minor alterations in texture and appearance due to higher herbal concentration.
- Refrigerated storage environment provided better formulation stability. [38]

5.10 Comparative Evaluation of Formulations

Table 7.9 Comparative Evaluation Summary

Evaluation Parameter	Best Formulation
Taste	F1
Texture	F2
Hardness	F2

Antioxidant Activity	F3
Stability	F2
Overall Acceptability	F2

Overall Observation

Among all formulations, F2 showed the most favorable balance between antioxidant potential, texture, palatability, stability, and overall consumer acceptance. while F3 exhibited greatest antioxidant potential, increased bitterness and hardness reduced its sensory acceptance. F1 demonstrated excellent taste but comparatively lower antioxidant potential.

thus, F2 was considered the optimized nutraceutical guava leaf chocolate formulation. [39]

FUTURE SCOPE

Advanced phytochemical characterization and quantification of phenolic and flavonoid constituents.

Evaluation using additional antioxidant assays and detailed dose-response studies.

Microbial quality assessment and extended stability/shelf-life studies.

Optimization of packaging, processing conditions and scale-up manufacturing.

Nutritional profiling and assessment of consumer acceptability using an appropriately designed sensory study.

Further safety and toxicological evaluation before any health or therapeutic claims.

Assessment of commercial feasibility and reproducibility at pilot scale.

LIMITATIONS OF THE STUDY

The investigation was performed at laboratory scale using a limited number of formulations.

The reported stability observation period was 30 days; longer-term shelf-life studies are required.

The antioxidant evaluation reported in the source work used the DPPH assay; additional complementary assays may strengthen characterization.

Clinical efficacy and therapeutic outcomes were not established by the reported study.

Further microbial quality, safety, toxicological, nutritional and advanced phytochemical studies are required before commercial or therapeutic claims can be made.

DISCUSSION

The current investigation focused on the development and evaluation of nutraceutical guava leaf chocolate as a novel herbal functional food formulation. Different chocolate formulations containing varying concentrations of guava leaf extract were formulated successfully and subjected to evaluation for organoleptic, physicochemical, antioxidant, and stability parameters. The obtained results demonstrated that incorporation of guava leaf extract significantly influenced the quality attributes and nutraceutical properties of the prepared formulations. [40]

The sensory assessment indicated that all formulations possessed acceptable appearance and texture; however, differences were noted in taste and odor due to differences in concentration of guava leaf extract. Formulation F1 showed better sweetness and overall taste because of reduced concentration of herbal extract. F2 demonstrated balanced sensory properties with acceptable herbal taste profile and uniform smooth texture, whereas F3 showed comparatively higher bitterness and mild herbal odor due to greater concentration of guava leaf extract. These findings indicate that over-incorporation of herbal extract may

negatively influence palatability and consumer acceptance of nutraceutical chocolates. [41]

The weight variation studies established that all prepared formulations showed uniform distribution of ingredients and proper molding characteristics. The percentage deviations observed in all formulations were within acceptable pharmaceutical standards, indicating satisfactory preparation consistency and production uniformity.

Hardness evaluation demonstrated that increase in concentration of guava leaf extract resulted in increased hardness of chocolate formulations. F3 exhibited greatest hardness due to higher solid content contributed by the herbal extract. while higher hardness may improve physical strength and handling properties, excessive hardness can negatively influence oral texture and sensory acceptance. F2 exhibited optimal hardness with suitable textural characteristics, suggesting appropriate balance between structural strength and consumer preference. [42]

melting-point assessment showed slight increase in melting point values with increase in guava leaf extract concentration. The melting characteristics of chocolate is an important parameter because it has a direct influence on texture, storage stability, and oral texture. All prepared formulations showed appropriate melting characteristics within suitable range for chocolate products. The results indicated that incorporation of guava leaf extract did not substantially affect with the melting properties of chocolate base.

The pH values of all formulations stayed close to neutral, indicating compatibility of formulation ingredients and suitability for nutraceutical consumption. Stable pH values also suggest reduced likelihood of chemical deterioration and microbial instability during storage environment.

Moisture analysis indicated that all formulations contained low moisture content suitable for maintaining product stability and limiting microbial contamination. Slight increase in moisture content was observed with increasing concentration of guava leaf extract. Excessive moisture in chocolate formulations may lead to microbial proliferation, texture changes, and shortened shelf life; however, all prepared formulations remained within acceptable moisture limits. [43]

a major observation of the current investigation was the antioxidant potential exhibited by nutraceutical guava leaf chocolate formulations. antioxidant potential rose progressively from F1 to F3 due to greater concentration of guava leaf extract. Guava leaves are abundant sources of flavonoids, phenolic compounds, quercetin, and tannins, which support substantial free-radical-scavenging capacity. Cocoa present in chocolate formulations also contains flavonoids and polyphenolic compounds that may act synergistically to antioxidant effects. thus, incorporation of guava leaf extract successfully increased the nutraceutical value of chocolate formulations. [44]

stability assessments indicated that F1 and F2 formulations remained physically stable throughout the storage duration without marked changes in appearance, texture, or odor. F3 showed minor hardening and mild changes in appearance during storage due to greater concentration of herbal extract. Refrigerated storage environment were found to be more favorable for preserving formulation stability and sensory attributes.

comparative assessment of all prepared formulations demonstrated that F2 was the optimized preparation because it exhibited balanced organoleptic characteristics, acceptable hardness, good stability, and considerable antioxidant potential. while F3 showed greatest antioxidant potential, increased bitterness and hardness reduced overall acceptance. F1 exhibited superior taste but comparatively lower

nutraceutical activity due to lower herbal concentration.

The current investigation demonstrated successfully that guava leaf extract can be successfully incorporated into chocolate formulations to develop nutraceutical products with improved therapeutic value and acceptable sensory attributes. Chocolate proved to be an effective carrier system for herbal ingredients because it effectively masked bitterness and improved palatability. The developed nutraceutical chocolate may serve as a promising functional food product for consumers concerned with health and may have potential future applications in pharmaceutical and nutraceutical industries. [45]

CONCLUSION

The current investigation entitled "Development and Evaluation of Nutraceutical Guava Leaf Chocolate" was successfully carried out with the objective of developing a novel herbal nutraceutical formulation having therapeutic potential, antioxidant potential, and improved consumer acceptance. Guava leaf extract was successfully incorporated into chocolate formulations to prepare nutraceutical chocolates with functional-food potential. The investigation established that guava leaves contain important phytoconstituents responsible for antioxidant and medicinal activities. Incorporation of guava leaf extract into chocolate formulations significantly increased the nutraceutical value of the resulting product without producing major instability in formulation attributes.the various formulations (F1, F2, and F3) were formulated and evaluated for organoleptic characteristics, physicochemical parameters, antioxidant potential, and stability assessments. The results demonstrated that all formulations possessed acceptable quality attributes such as consistent appearance, appropriate melting characteristics, satisfactory hardness, suitable pH, and limited moisture content.Among all prepared formulations, F2 was identified as the optimized preparation because it exhibited the most favorable balance between taste, texture, antioxidant potential, stability, and overall consumer acceptance. while F3 demonstrated greatest antioxidant potential due to greater concentration of guava leaf extract, its increased bitterness and hardness slightly reduced sensory acceptance. F1 showed very good palatability but comparatively comparatively reduced antioxidant potential.The antioxidant studies established that nutraceutical guava leaf chocolate possesses significant free-radical-scavenging capacity, mainly due to the presence of flavonoids and polyphenolic compounds in guava leaves and cocoa. The developed formulation thus may help reduce oxidative stress and provide beneficial health effects.The investigation also demonstrated that chocolate can serve as an effective carrier system for herbal ingredients because it improves palatability, masks bitterness, and enhances patient compliance. Nutraceutical chocolates may thus represent a promising alternative to traditional herbal dosage forms such as powders, syrups, and capsules.overall, nutraceutical guava leaf chocolate may be regarded as a potential herbal functional food with potential applications in nutraceutical, pharmaceutical, and health-supplement sector. The developed formulation showed satisfactory stability, acceptable organoleptic characteristics, and enhanced antioxidant potential, making it suitable for future development as a commercial nutraceutical preparation.

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