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Design and Analysis of Antidiabetic Chocolate Containing *Mangifera indica* Extract

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ABSTRACT

Chocolate is one of the most widely consumed confectionery products across all age groups; however, its consumption is often restricted in individuals with diabetes, obesity, hypertension, and cardiovascular disorders due to its high sugar content. The present study aimed to formulate and evaluate a nutraceutical antidiabetic chocolate incorporating *Mangifera indica* (mango leaf) extract, a natural source of antioxidants, polyphenols, flavonoids, and vitamins known for their blood glucose-lowering potential. The medicated chocolate was formulated using a hydroalcoholic extract of *Mangifera indica* leaves, cocoa powder, cocoa butter, monk fruit sweetener, and erythritol in optimized proportions to provide a sugar-free and health-conscious alternative for diabetic individuals. The formulated chocolate was evaluated for various physicochemical parameters including appearance, taste, odour, consistency, blooming characteristics, physical stability, and drug content using UV spectrophotometric analysis. Furthermore, its safety and biological activity were assessed through an MTT assay using INS-1 832/3 pancreatic β -cell lines, with metformin employed as the reference standard. The formulation successfully incorporated mango leaf extract while effectively masking its undesirable taste and maintaining the desirable sensory attributes of chocolate, including a smooth texture and pleasant flavour. The MTT assay results demonstrated good cell viability and absence of significant cytotoxic effects, indicating the safety of the developed formulation. The study highlights the potential of *Mangifera indica*-based nutraceutical chocolate as a functional food product that may assist in maintaining blood glucose levels, promoting preventive healthcare, and supporting lifestyle management in individuals at risk of or living with diabetes.

Keywords: Antidiabetic chocolate, *Mangifera indica*, Mango leaf extract, Diabetes mellitus, Nutraceutical, Functional food, MTT assay, Cytotoxicity

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INTRODUCTION

Chocolate is one of the most widely most beloved treats, cherished for its velvety texture and it's mood enhancing properties. However, because it is typically having high sugar and fat content, often make it unsuitable for individuals with metabolic disorders like Diabetes Mellitus, Hypertension, Obesity and Coronary artery disease.[1] Diabetes in particular is a rapidly growing global health concern; it is a condition where the body struggles with chronic high blood sugar because it either cannot produce enough insulin or cannot use it properly.[3,5] According to the International Diabetes Federation, the number of adults living with diabetes is projected to rise significantly in the coming decade, which highlights how badly we need new ways to combine medicine with the food we actually enjoy eating.

Mangifera indica leaves possess a wide range of therapeutic benefits, making them an important candidate for functional food development. Rich in bioactive compounds such as mangiferin, quercetin, catechins, and various polyphenols, the leaves exhibit potent antioxidant, anti-inflammatory, and antidiabetic activities.[3] *Mangiferin*, the key xanthonoid, has been shown to enhance glucose uptake in peripheral tissues, inhibit α -amylase and α -glucosidase enzymes, and improve insulin sensitivity, thereby contributing to effective glycemic control.[7]

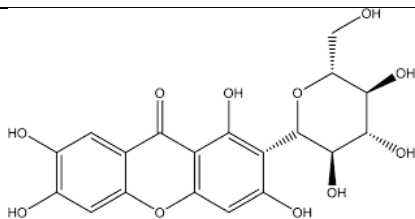
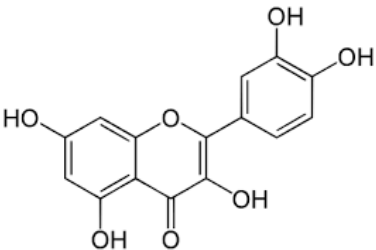
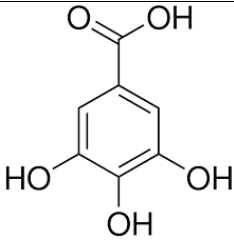
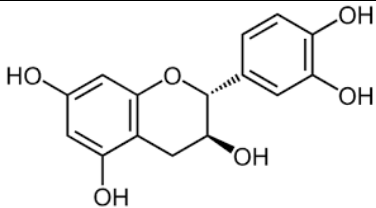
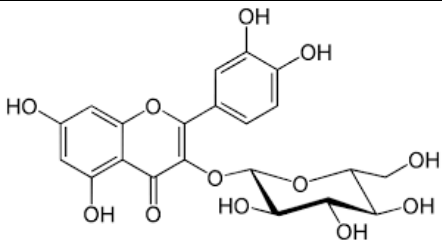
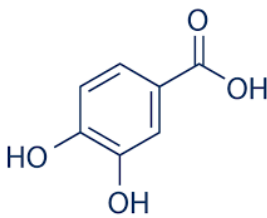
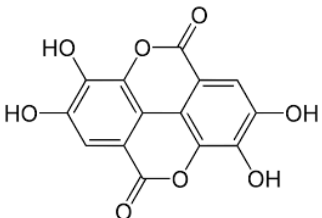
Despite these benefits the bitter taste and medicinal aroma of the leaf extract often pose challenges for direct consumption. Developing an antidiabetic chocolate enriched with *Mangifera indica* leaf extract presents a novel strategy to combine the enjoyment of chocolate with targeted health benefits. Such formulation could provide a palatable, patient friendly alternative to conventional medicinal preparations, potentially improving adherence to dietary recommendations among diabetic and pre-diabetic individuals. In addition to taste and texture optimization, it is essential to ensure that the antidiabetic chocolate maintains it's functional efficacy, stability and safety for consumption.[3]

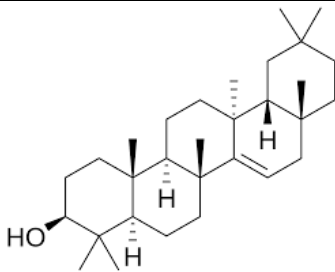
Mechanism:

Mangiferin is a natural compound found in various parts of mango family plants. One of its key actions is blocking a protein called PTP1B. [3]

PTP1B normally acts as a brake on insulin signalling by removing phosphate groups from the insulin receptor and IRS-1 (a protein needed for insulin signalling). When PTP1B is active, it reduces insulin's effectiveness, leading to insulin resistance. By inhibiting PTP1B, *Mangiferin* helps keep the insulin receptor and IRS-1 active, allowing insulin to work better. This improves glucose uptake and helps in managing diabetes.[3]

Table 1: Major Phytoconstituents in *Mangifera Indica*:

Sr.No	Phytoconstituents	Structure
1	<i>Mangiferin</i>	
2	<i>Quercetin</i>	
3	<i>Gallic Acid</i>	
4	<i>Catechin</i>	
5	<i>Isoquercetin</i>	
6	<i>Protocatechuic Acid</i>	
7	<i>Ellagic Acid</i>	

8	3-B-taraxerol	
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MATERIALS AND METHOD

- **Mango Leaves:**

Biological Source: Obtained from dried leaves of *Mangifera Indica*

Family: Anacardiaceae

- **Cocoa Powder:**

Biological Source: Obtained from fermented, roasted, and ground seeds of *Theobroma Cacao Linn.*

Family: Malvaceae

- **Cocoa Butter:**

Biological Source: Fat obtained from the seeds of *Theobroma Cacao Linn.*

Family: Malvaceae

- **Monk Fruit:**

Biological Source: Obtained from dried fruits of *Siraitia grosvenorii*

Family: Cucurbitaceae

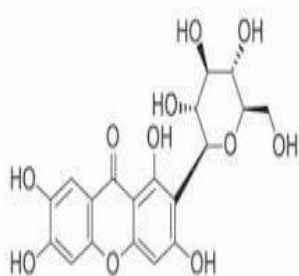
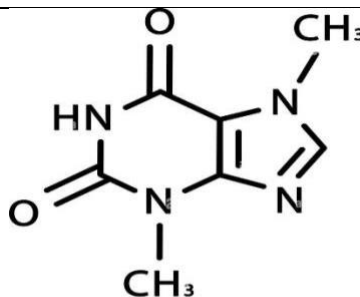
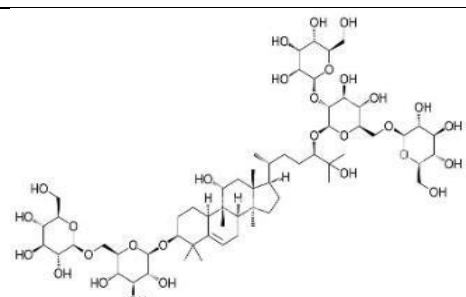
- **Erythritol**

Equipments: Beakers, Mortar Pestle, Conical flask, Measuring Cylinder, Funnel, Chocolate mould

Instruments: Water Bath, Refrigerator, sieve

Table 2: Taxonomical and Phytochemical profile of selected ingredients

	Mango Leaves	Cocoa Powder	Monk Fruit
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Scientific Name	<i>Mangifera indica</i>	<i>Theobroma cacao</i> L.	<i>Siraitia grosvenorii</i>
Kingdom	Plantae	Plantae	Plantae
Division	Magnoliophyta	Tracheophyta	Magnoliophyta
Class	Magnoliopsida	Magnoliopsida	Magnoliopsida
Order	Sapindales	Malvales	Cucurbitales
Family	Anacardiaceae	Malvaceae	Cucurbitaceae
Genus	<i>Mangifera</i>	<i>Theobroma</i>	<i>Siraitia</i>
Species	Indica	Cacao	Grosvenorii
Chemical Constituents			
	It includes <i>mangifera</i> , phenolic acids, benzophenones, flavonoids, carotenoids, quercetin, ascorbic acid and tocopherols.	It includes lipids, proteins, carbohydrates, and bioactive compounds like flavonoids (catechins, epicatechins), methylxanthines (theobromine, caffeine), and polyphenols.	It includes triterpene glycosides, flavonoids, carbohydrates, proteins, fats, vitamins, and minerals.
Chemical Structure			
	 Mangiferin	 Theobromine $C_7H_8N_4O_2$	 Mogroside V Esgoside $C_{60}H_{102}O_{23}$
Traditional Medicinal Uses [4]			
	1. Regulates diabetes 2. Lowers blood pressure and risk of hypertension 3. Dissolve gall and kidney stones 4. Cures respiratory problems 5. Provides relief from ear	1. Lowering blood pressure. Improved blood flow. 2. Can dilate coronary arteries, which can be beneficial for heart health. 3. Antioxidant and Anti-	1. Lowers risk of obesity and diabetes. 2. It works as an Anti-inflammatory and coolant. 3. Helps to treat and possibly prevent cancer. 4. Combats infections 5. Works as a natural

	aches 6. Treats restlessness 7. Cures dysentery 8. Heal burns 9. Prevents various stomach ailments 10. Cures hiccups	inflammatory properties. 4. Antimicrobial activity. 5. Mood boosting and stress reduction properties.	antihistamine. 6. Fights fatigue 7. Treats diabetes
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METHODS:

Preparation of Extract:

1. Fresh mango leaves were collected and thoroughly washed with water with water to remove dust and other impurities. The leaves were then shade-dried at room temperature.
2. Further, the dried leaves was subjected to pulverization to covert into the coarse powder using a grinder.
3. 50g of the dried leaf powder was macerated with 150mL of 70% hydroalcoholic solvent, the mixture was subjected to sonication at room temperature for 2 hours to enhance extraction efficiency/
4. The mixture was filtered using Whatman Filter paper and the filtrate was concentrated by evaporating the solvent under reduced pressure.
5. Further, the obtained extract was Standardized and incorporated into the chocolate formulation.[3]

Standardization of extract

1. Phytochemical screening:

Phytochemical screening of the plant extract was performed to identify the major bioactive constituents that contribute to its pharmacological activity. The qualitative analysis confirmed the presence of alkaloids, glycosides, carbohydrates, saponins, flavonoids and tannins using standard chemical tests namely Mayer's test, Keller–Killiani test, Molisch's test, foam test, Shinoda test and ferric chloride test respectively. These phytoconstituents are known to exhibit various biological activities such as antioxidant, antidiabetic, anti-inflammatory and protective effects. Alkaloids and glycosides play a significant role in regulating metabolic processes, while flavonoids and tannins contribute to free-radical scavenging and enzyme modulation. Saponins are associated with glucose-lowering and membrane-stabilizing properties and carbohydrates indicate the nutritional value of the extract. The presence of these compounds supports the therapeutic potential of the extract and justifies its selection for further formulation and evaluation as an antidiabetic preparation.

2. Ultra violet analysis of extract

Ultraviolet (UV) spectrophotometric analysis is an important analytical technique used for the standardization and quantitative estimation of phytoconstituents present in herbal extracts. In the present study, UV spectrophotometric analysis was carried out in the wavelength range of 200–800 nm to identify and quantify the selected biomarker-gallic acid. A standard solution of gallic acid was prepared using a hydroalcoholic solvent and the extract was analyzed following Beer–Lambert’s law within the concentration range expressed in µg/ml. The determination of gallic acid content helps in ensuring batch-to-batch consistency, quality and reproducibility of the extract. Since gallic acid is known for its antioxidant and antidiabetic properties, its estimation further supports the therapeutic potential and standardization of the extract used in the formulation

3. Thin layer chromatography (TLC)

Thin Layer Chromatography (TLC) is a simple, rapid and reliable chromatographic technique employed for the qualitative analysis and standardization of herbal extracts. In this study, TLC was performed to identify the presence of phytoconstituents in the extract by separating its components using a suitable mobile phase. The developed chromatographic plates were observed and the retention factor (R_f) values obtained for the extract were compared with those of the standard compound. TLC analysis aids in confirming the identity, purity and chemical profile of the extract and helps in detecting adulteration or variation in composition. The similarity of R_f values between the extract and the standard indicates the presence of active constituents and ensures the consistency and authenticity of the extract used for formulation.

Formulation of anti-Diabetic Chocolate

Steps carried out in the preparation of antidiabetic chocolate were as follows.

1. Melting of Cocoa Butter:

The cocoa butter (28%) was introduced into heat resistant bowl placed over a boiling water bath and allowed to melt completely

2. Incorporation of Additional Ingredients:

Subsequently, cocoa powder (37%) and *Mangifera* extract (5%) were gradually added and thoroughly mixed into the melted cocoa butter.

3. Sweetening and Stabilizing:

After a brief period, monk fruit (28%) was incorporated as a sweetener, followed by the addition of a stabilizer (2%).

4. Moulding and Setting:

The resulting mixture was then poured into silicone moulds and placed in a refrigerator to set for 24 hours.

Table 3: Formulation Table:

Sr.No	Ingredients	Quantity	Use
1	Standardized Mango leaves extract	5%	Anti-Diabetic
2	Cocoa Powder	37%	Flavoring Agent
3	Cocoa Butter	28%	Base
4	Monk Fruit & Erythritol	28%	Taste Masking
5	Stabilizer	2%	Texture Stabilizing

Blooming Test:

Blooming test is an important quality evaluation parameter used in chocolate formulations to assess surface stability and aesthetic acceptability during storage. Blooming refers to the appearance of a dull, whitish or grayish layer on the surface of chocolate which occurs due to improper processing or storage conditions. Although bloom does not make the chocolate unsafe for consumption, it adversely affects its appearance, texture and consumer acceptance. The blooming test helps in determining the physical stability of chocolate and its resistance to environmental factors such as temperature fluctuations and humidity.

There are two major types of blooming observed in chocolate: fat bloom and sugar bloom. Fat bloom occurs due to the migration and recrystallization of cocoa butter fats on the surface, usually caused by improper tempering or temperature variations. Sugar bloom results from exposure of chocolate to moisture, leading to dissolution and recrystallization of sugars on the surface.

The absence of blooming indicates good formulation stability, proper tempering and suitable storage conditions, thereby ensuring product quality and shelf stability.

Stability Testing:

Stability testing is an essential evaluation parameter carried out to determine the ability of the formulated chocolate to maintain its physical, chemical, and organoleptic properties during storage over a period of time. It helps in assessing the effect of environmental factors such as temperature, humidity and light on product quality.

In the present study, stability testing was performed by storing the formulation at low and room temperature conditions and observing any changes in appearance, texture, melting behavior, blooming, and overall physical stability. Stability testing is significant as it ensures product safety, efficacy, and shelf-life by confirming that the formulation remains stable without undergoing undesirable changes such as fat or sugar blooming, texture softening, or phase separation. The

stability of the chocolate formulation under different storage conditions indicates proper formulation design and suitability for long-term storage.

MTT Assay:

MTT assay is a colorimetric in-vitro method used to evaluate cell viability, cytotoxicity, and proliferative potential of test samples by measuring mitochondrial metabolic activity which was used to assess the effect of M. extract on INS-1 832/3 cell lines and Metformin (100 μ M/ml) was used as a standard control in the study. [3,7,8,9]

The assay is based on the ability of viable cells to reduce the yellow tetrazolium salt (MTT) into insoluble purple formazan crystals through the action of mitochondrial lactate dehydrogenase enzyme. The intensity of the purple color formed is directly proportional to the number of metabolically active cells and is measured spectrophotometrically at 570 nm.

RESULTS AND DISCUSSION

Table 4: Physical Evaluation of Chocolate

Sr. No	Parameters	Observation
1	Colour	Dark Brown
2	Texture	Smooth & Glossy
3	Mouth Feel	Soft
4	Taste Of Chocolate	Sweet
5	Hardness	11.48
6	Melting Point	31.1°C
7	Blooming Test	No Bloom
8	Physical Stability	Stable at low & room temperature

Table 5: Phytochemical Screening

Phytoconstituent	Test	Observation	Inference
Alkaloids	Mayer's test Few drops of mayers reagent were added to 1 ml of extract.	Yellow precipitate was formed.	Alkaloids are present.
Glycosides	Keller killani test A solution of 0.5ml, containing glacial acetic acid and 2-3 drops of ferric chloride, was mixed with 2ml of extract. Later, 1ml of concentrated H ₂ SO ₄ , was added along the walls of the test tube.	Deep blue color observed at the junction of two liquids.	Glycosides are present.
Carbohydrates	Molisch test Few drops of alcoholic alpha - naphthol solution were added to 2 ml of extract. Later, few drops of concentrated H ₂ SO ₄ were added along the walls of test tube.	At the junction of two liquids, a violet ring appeared.	Carbohydrates are present.

Saponins	Foam test 5ml of extract was shaken vigorously, then it was left to rest for five minutes.	Foam formation	Saponins are present.
Flavonoids	Shinoda test 10 drops of dilute HCL and a piece of magnesium were added to 1ml of extract.	Deep pink color was observed	Flavonoids are present.
Tannins	Ferric chloride test Add a few drops of 5%FeCL ₃ solution to the extract.	Blue- black color was observed	Tannins are present.

Standardization

UV Analysis of extract

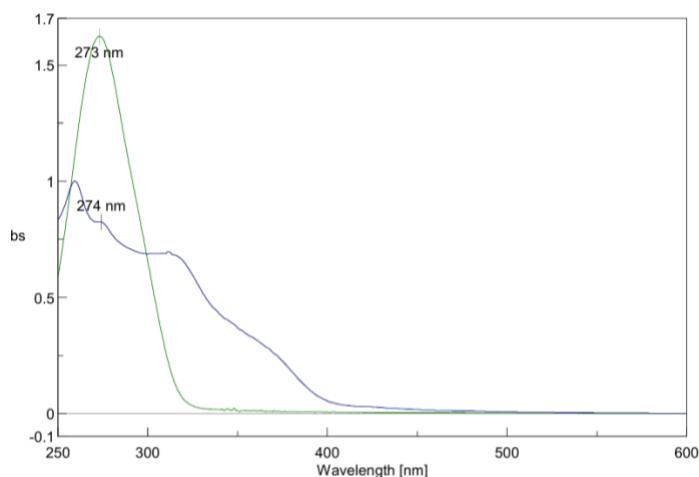


Image 1: UV of M. extract

Thin Layer Chromatography (TLC):

Drug	Solvent	Observed Rf Value	Standard Rf Value
Mango Leaves	Toluene: Ethyl acetate: Glacial acetic acid: Formic acid (4.5:2:2:0.5)	0.38	0.35-0.40

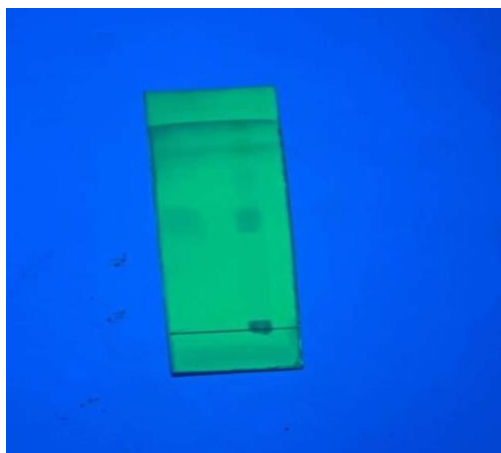


Image 2: TLC of M. extract

MTT Cytotoxicity Study Results:

In the present study, the *Mangifera indica* extract (M. extract) was evaluated using the MTT assay, and the results indicated that the extract was non-cytotoxic at a concentration of 50 $\mu\text{g/ml}$.

Key Implications:

The observed increase in cell viability suggests a supportive effect on cell proliferation. These findings highlight the safety of the extract at the tested concentration and provide preliminary evidence of its potential therapeutic relevance, particularly in the context of antidiabetic activity.

Diabetes is associated with loss or dysfunction of pancreatic β -cells, the extract supports β -cell survival or proliferation, it may help: Maintain insulin-secreting cell mass, Improve insulin availability. Thus, mild proliferation suggests regenerative or protective potential

However, further mechanistic studies are required to confirm its role in glucose metabolism and insulin secretion.

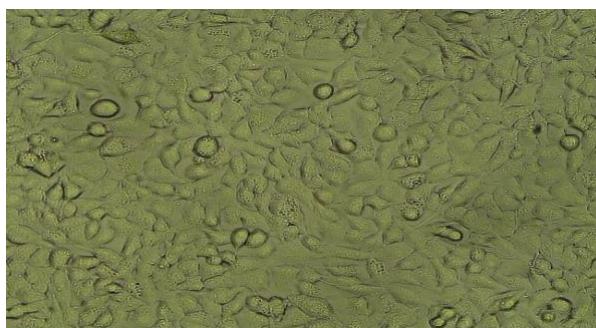


Image 3: Metformin 100uM

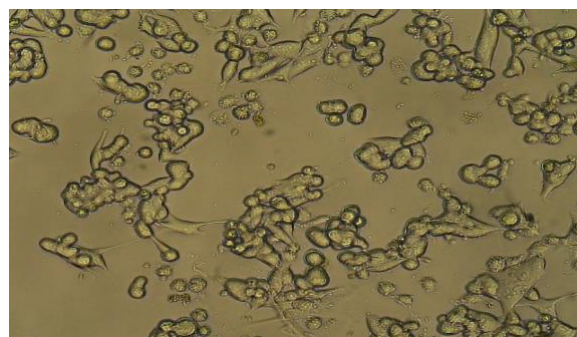


Image 4: Standardized Extract 100ug

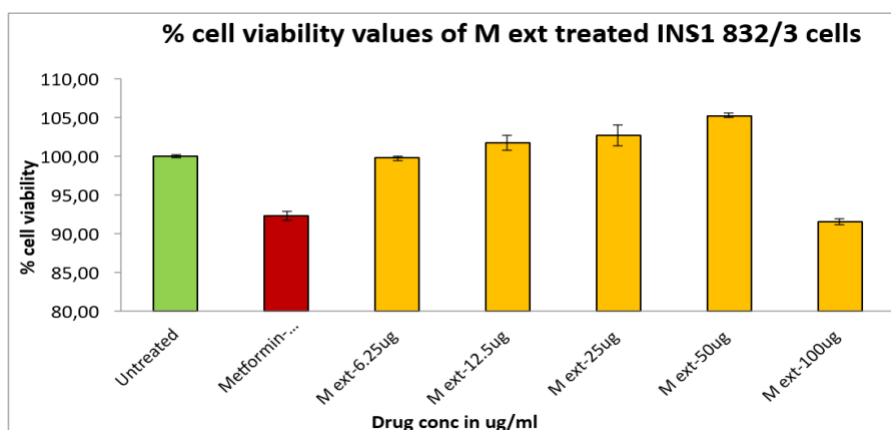


Image 5: % Cell Viability of M. Ext treated INS1 832/3 cells

SUMMARY:

The formulated antidiabetic chocolate was successfully developed using an optimized combination of *Mangifera indica* extract, cocoa powder, cocoa butter, monk fruit, and erythritol. The chocolate exhibited desirable physical characteristics, including an appealing appearance, smooth texture, consistent odor, and satisfactory taste, effectively masking the bitterness of the medicinal

components. The blooming test and physical stability evaluation confirmed the product's integrity over time, demonstrating no significant changes in texture or appearance under standard storage conditions.

The MTT assay conducted on INS-1 832/3 pancreatic beta-cell lines demonstrated the formulation's non-cytotoxic nature, with cell viability comparable to that of the standard drug, Metformin. The results indicated that the medicated chocolate does not exert harmful effects on pancreatic cells, supporting its potential as a safe nutraceutical intervention for diabetes management.

Overall, the study successfully formulated and evaluated an antidiabetic chocolate that not only retained the sensory attributes of conventional chocolate but also offered potential health benefits. The results suggest that the developed product is safe for consumption and may contribute to blood glucose regulation, making it a promising dietary supplement for individuals with diabetes or those seeking preventive measures. Further in vivo studies and clinical trials are recommended to validate its long-term efficacy and therapeutic potential.

CONCLUSION:

Our study represents a novel approach to diabetic-friendly confectionery, combining the therapeutic benefits of standardized *Mangifera* extract. Designed to provide a guilt-free indulgence, it offers potential glycemic control support without compromising taste. To ensure the quality and efficacy of the formulation, TLC was performed for standardization, confirming the presence of key bioactive compounds. Additionally, UV spectroscopic analysis was conducted to assess the purity and consistency of the extract. By integrating functional ingredients with established antidiabetic properties, it addresses the rising demand for nutraceutical-based solutions in diabetes management. Its formulation reflects a balance between efficacy and palatability, making it a promising alternative to conventional chocolates. Further research and clinical validation can strengthen its positioning as a supportive dietary choice for individuals with diabetes

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