

FORMULATION AND EVALUATION OF ANTIDIABETIC HERBAL CHOCOLATE USING *AEGLE MARMELOS* LEAVES

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ABSTRACT

The study was concluded the active constituents present in *Aegle marmelos* leaves extract exhibit the superior inhibition against diabetic activity. The chocolate formulation results shown in the specific limits for the respective evaluation, parameters of color, texture and chemical composition variation melting point, physical stability. The alkaloids present in the leaves are well extracted through 95% ethanol. During separation, it was observed under UV light 366nm the alkaloids are separated successfully by thin layer chromatography technique.

KEYWORDS: Aegle marmelos, Antidiabetic activity, Alkaloids, Thin layer chromatography.

INTRODUCTION

DIABETES MELLITUS

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion, insulin action, or both.¹⁻⁴ Insulin deficiency in turn leads to chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism.^[1] As the disease progresses tissue or vascular damage ensues leading to severe diabetic complications such as retinopathy, neuropathy, nephropathy, cardiovascular complications and ulceration. Thus, diabetes covers a wide range of heterogeneous diseases.^[2] Diabetes is the most common endocrine disorder and by the year 2010, it is estimated that more than 200 million people worldwide will have DM and 300 million will subsequently have the disease by 2025. The diagnostic criteria and the classification of diabetes was first put forward by the World Health Organization (WHO) in 1965 then by the National Diabetes Data Group (NDDG) in 1979, and this was followed by

simplified recommendations by the WHO in 1980.^[3] These WHO recommendations were modified slightly in 1985. The latest recommendations have been published by the American Diabetes Association (ADA) in 1997 and by the WHO in 1999. Both groups agree on the recommendations and criteria.^[4]

According to the ADA recommendation changes in 1997, the fasting glucose concentration should be used in routine screening for diabetes as well as epidemiological studies; the threshold for fasting glucose was changed from 7.8 mmol/L (140 mg/dl) to 7.0 mmol/L (126 mg/dl); however the 2-h glucose criterion remains as = 11.1 mmol/L (200 mg/dL).^[5] For the diagnosis of diabetes, at least one criteria must apply:

- Symptoms of diabetes (polyurea, polydipsia, unexplained weight loss, etc) as well as casual plasma glucose concentration = 11.1 mmol/L (200 mg/dL).
- Fasting plasma glucose = 7.0 mmol/L (126 mg/dL), with no caloric intake for at least 8 h. 2-h plasma glucose = 11.1 mmol/L (200 mg/dL) during an oral glucose tolerance test (OGTT), with the glucose load containing 75 g anhydrous glucose in water.

The WHO diagnosis and classification of diabetes mellitus (1999) are identical to those of ADA, a fasting glucose = 7.0 mmol/L (126 mg/dl) and /or a 2-h glucose = 11.1 mmol/L (200 mg/dL).^[6] The report states that diagnosis should not be based on a single glucose determination but requires confirmatory symptoms or blood/plasma determination. Ideally, therefore, both the 2-h and fasting value should be used. These recommendations contrast with those of ADA Expert Committee which gives primacy to the 'fasting plasma glucose'.^[7] The WHO classification includes both clinical stages (normoglycaemia, impaired glucose tolerance/impaired fasting glucose (IGT/IFG), diabetes) and aetiological types of diabetes mellitus, identical to the ADA except that WHO group includes classification formerly known as gestational impaired glucose tolerance (GIGT) and GDM: fasting glucose = 7.0 mmol/L (126 mg/dL) and/or 2-h glucose = 7.8 mmol/L (140 mg/dL) after a 75-g OGTT.^[8]

Diabetes mellitus may be categorized into several types but the two major types are type 1 and type 2. On the basis of aetiology, the term type 1 and type 2 were widely used to describe IDDM and NIDDM, respectively; other specific types of diabetes and gestational diabetes are given in Table 1. The term juvenile-onset diabetes has sometimes been used for IDDM and maturity-onset for NIDDM.^[9]

TYPES OF DIABETES

Type 1 is present in patients who have little or no endogenous insulin secretory capacity and who therefore require insulin therapy for survival. The two main forms of clinical type 1 diabetes are type 1a (about 90% of type 1 cases in Europe) which is thought to be due to immunological destruction of pancreatic β cells resulting in insulin deficiency; and type 1b (idiopathic, about 10% of type 1 diabetes), in which there is no evidence of autoimmunity.^[10]

Type 1a is characterized by the presence of islet cell antibody (ICA), anti- glutamic acid decarboxylate (anti-GAD), IA-2 or insulin antibodies that identify the autoimmune process with β -cell destruction. Autoimmune diseases such as Grave's disease, Hashimoto's thyroiditis and Addison's disease may be associated with type 1 diabetes mellitus.^[11]

There is no known etiological basis for type 1b diabetes mellitus. Some of these patients have permanent insulinopaenia and are prone to ketoacidosis, but have no evidence of autoimmunity. This form is more prevalent among individuals of African and Asian Origin.^[13]

Type 2 diabetes is the commonest form of diabetes and is characterized by disorders of insulin secretion and insulin resistance. In Western countries the disease affects up to 7% of the population. Globally, it affects 5-7% of the world's population. This prevalence is underestimated because many cases, perhaps 50% in some population, remain undiagnosed. The prevalence of type 2 diabetes varies considerably throughout the world, ranging from <1% in certain population of the developing countries for example rural Melanesians in Papua New Guinea, and rural Chinese, to over 50% in the Pima Indians of Arizona. There is a higher incidence of type 2 diabetes in urban than in rural areas. Its incidence is associated with population whose lifestyle has changed from traditional patterns to a modern "Westernized" model.^[14] The classical example include the Pima Indians, Chinese who moved to Mauritius and Japanese who emigrated to Hawaii. Traditionally, type 2 diabetes is common in individuals over the age of 40. It is often associated with obesity, decreased physical activity and heredity. Recent data from several countries show that type 2 diabetes is increasingly becoming a problem among adolescents and even children. In some countries, childhood diabetes type 2 is more common than type 1. The disease is usually controlled through dietary therapy, exercise and hypoglycaemic agents.^[15]

SYMPTOMS

Symptoms are similar in both types of diabetes but they vary in their intensity. Symptoms develop more rapidly in type 1 diabetes and more typical. The symptoms include polyurea, polydipsia, polyphagia, weight loss, fatigue, cramps, constipation, blurred vision, and candidiasis. Long standing type 1 DM patients are susceptible to microvascular complications; and macrovascular disease (coronary artery, heart, and peripheral vascular diseases). Symptoms in type 2 DM are similar but insidious in onset. Most cases are diagnosed because of complications or incidentally. Type 2 DM carries a high risk of large vessel atherosclerosis commonly associated with hypertension, hyperlipidaemia and obesity. Most patients with type 2 diabetes die from cardiovascular complications and end stage renal disease. Geographical differences exists in both the magnitude of these problems and their relative contributions to overall morbidity and mortality.

PREVENTION

Insulin replacement therapy is the mainstay of treatment in patient with type 1 diabetes while type 2 diabetes should be regarded as a potentially preventable disease. A study done in Australia Aborigines demonstrated marked improvement in carbohydrate and lipid metabolism in patients with type 2 DM who reverted to a traditional lifestyle. An important large- scale prospective study in China, examined the effects of diet and exercise upon the rate of progression of IGT to diabetes; both the measures, alone or together reduced the progression of the disease by 40% after 6 years. Similar studies done in Sweden also demonstrate the effectiveness of life-style changes in preventing diabetes. More recently, the Finnish Diabetes Prevention Study showed that lifestyle intervention reduced by 58% the risk of subjects with IGT progressing to type 2 diabetes.

ANTI-DIABETIC ACTIVITY OF *AEGLE MARMELLOS*

Diabetes mellitus (DM) is the most common endocrine disorder that affects more than 100 million people worldwide (6% of the population). It is caused by the deficiency or ineffective production of insulin by pancreas which results in increase or decrease in concentrations of glucose in the blood. The effect of *Aegle marmelos* fruit extract in streptozotocin induced diabetes, a histopathological study was evaluated for its antidiabetic property. This study was designed to elucidate the protective effect of an aqueous extract of *Aegle marmelos* fruits on the histopathology of the

pancreas in streptozotocin induced diabetic rats.

All the extracts of *Aegle marmelos* proved to be active against diabetic rabbits, however, among the various extracts, methanolic extract of leaves showed maximum anti diabetic effect. Methanolic extract (120 mg/kg.p.o.) was administered to alloxan induced diabetic rats and on 12th day sugar levels was found to be reduced by 54%.

The aqueous extract of *A. marmelos* leaves lowers blood sugar in streptozotocin-induced diabetes rat model. It boosts insulin secretion by partial regeneration from the β -cells of pancreatic islets. The effects seen in the fruit extract treated mice were better when compared with animals treated with glibenclamide. The present study's in-vitro assay demonstrated a potent antidiabetic effect from lectin extract, as measured by glucose uptake in yeast cells. A fruit lectin extract with an IC₅₀ of 3.36 μ g/ml had greater efficiency than the usual medication metformin at increasing glucose uptake by yeast cells. This study found that *A. marmelos* fruit extract had hypoglycemic activity, which could be attributed to its antioxidant activity and high content of active constituents. As a result, the various parts of *A. marmelos* plant could be beneficial as a portion of healthy food and in developing antidiabetic drugs. The active components in the leaf and callus materials reduce blood sugar levels in STZ-diabetic rabbits, and *A. marmelos* callus powder methanol extract is as powerful as the leaf extract in treating diabetes. This study indicates the aqueous seed extract of *A. marmelos* reduces the blood glucose level in normal as well as in severely diabetic rats and improves glucose tolerance in sub and mild diabetic animals and is referred to standard as tolbutamide. The alcoholic extract of *A. marmelos* leaves significantly inhibited the enzymes α -amylase and α -glycosidase with IC₅₀ values of 46.21 and 42.07 μ g/ml, respectively. *A. marmelos* significantly reduced ROS levels that were elevated due to high glucose and enhanced glucose consumption in HepG2 cells ($p < 0.05$).

PLANT PROFILE



Fig. No.1: *Aegle marmelos* Tree.

TAXONOMICAL CLASSIFICATION

Kingdom - Plantae

Subkingdom - Tracheobionta

Division - Manoliophyta

Super division - Spermatophyta

Class - Magnoliopsida

Family - Rutaceae

Subclass - Rosidae

Subfamily - Aurantioideae

Genus - Aegle

Order - Spindales

Species - Bael patra, Bael

Aegle marmelos is commonly known as Bael, bilva golden apple, Indian quince and stone apple.^[16] *A. marmelos* (Bael tree) belongs to the citrus family Rutaceae.^[17] It is the most significant underutilized medicinal, Indigenous fruit crop of India. This plant is of high economic value and is known in India since 800 B.C. as per historical reports. In 1629 A.D., the Chinese Buddhist pilgrim, Hiuen Tsiang also noticed the presence of the Bael tree during his visit to India.^[18]

As per Hindu culture, the plant is considered as a sacred tree that is grown by the sides of Hindu temples as the plant is dedicated to Lord Shiva. The trifoliolate leaves of the plant are used in the prayers of Lord Shiva and Parvati, hence the plant is also known by the name of Shivaduma. It is also believed that Lord Shiva resides under the Bael tree. Besides this, the plant carries great medicinal value and its medicinal description is also mentioned in the Vedas (Yajurveda), Puranas and has also been portrayed in the paintings of Ajanta Caves.^[19]

It also acts as a Climate Purifier that absorbs the poisonous gases from the atmosphere and making them inactive or neutral. The main active phytochemical constituents isolated from the fruit part of the plant include marmelosin (helps in curing stomach diseases), psoralen, luvangetin, tannins and marmin.^[20] The present study gives the complete profile of *aegle marmelos* belongs to family Rutaceae, commonly known as a bael in India. A number of biologically active compounds (Alkaloids, Terpenoids, Vitamins, Coumarins, Tannins, Carbohydrates, Flavonoids, Fatty Acids, and Essential Oils) isolated from various parts of *aegle marmelos*, which belongs to various chemical groups.

The ripened fruit is aromatic, cool, alternative, laxative and nutritive. It is beneficial in usual constipation, chronic dysentery and dyspepsia when consumed in fresh form. It also treats flatulent colic in patients suffering from chronic gastrointestinal discharge. Ripened fruit marmalade is beneficial in preventing cholera epidemics. The fruit pulp converted into powder form after drying is used as febrifuge, antiscorbutic, stimulant and antipyretic (Patkar, 2012). Unripen fruit in powdered form is crucial in countering intestinal parasite *Entamoeba histolytica* and *Ascaris lumbricoides*. Brijesh (2009), examined the decoction of unripe fruit to be an astringent which has beneficial effect in diarrhea and chronic dysentery. Bael extract is found to significantly lower blood urea, reduction in lipid peroxidation, cholesterol whereas on the other hand increase the levels of super dioxide dismutase, catalase, glutathione peroxidase and glutathione level in serum along with liver in experimental animals having diabetes (Sharma, 2007).^[21]

Various parts of this plant such as leaves, roots, seed, bark and fruit, possess anti-microfilarial, antifungal, immune modulatory, anti-proliferative, wound healing, antifungal, analgesic, anti inflammatory, antipyretic, hypo glycaemic, anti dyslipidemic, antifertility, and insecticidal activity. Various phyto pharmacological evaluations have been reported in this writing for the important potential of the *Aegle marmelos*.^[22]

Indian Medicinal plants are considered a vast source of several pharmacologically active principles and compounds, which are commonly used in home remedies against multiple ailments.^[23] Bael (*Aegle marmelos* (L.) Corr.) is another Indian medicinal plant; which has enormous traditional values against various diseases and many bioactive compounds have been isolated from this plant.^[24]

LEAVES OF AEGLE MARMELOS

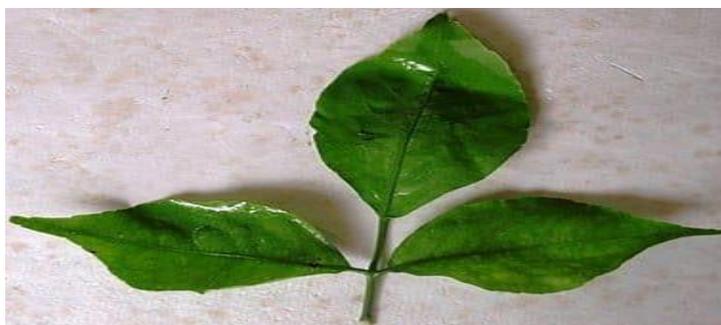


Fig.No.2: Leaves of Aegle marmelos.

The leaves of *Aegle marmelos*, commonly known as the Bael tree, are a sight to behold. These trifoliate leaves are a deep green color, with a smooth, glossy texture that invites touch. Each leaf is made up of three leaflets, attached to a central stalk. The leaflets are elliptical in shape, with a pointed tip and a rounded base. The edges of the leaflets are entire, meaning they are smooth and not serrated.^[25]

The bael leaves are relatively large, measuring between 4-6 inches (10-15 cm) in length. The leaflets are arranged in an alternate pattern along the stem, giving the tree a lush, full appearance. One of the most distinctive features of the leaves of *Aegle marmelos* is their aroma. When crushed or bruised, the leaves emit a strong, pungent scent that is often used in traditional medicine.^[26]

Aegle marmelos leaves are also highly valued for their medicinal properties. They are said to have anti-inflammatory, antipyretic, and antimicrobial properties, making them a popular ingredient in Ayurvedic medicine. In addition to their medicinal properties, the leaves of *Aegle marmelos* are also used in traditional rituals and ceremonies. In Hinduism, the leaves are considered sacred and are often used in puja (worship) ceremonies.^[27]

These leaves are also a popular ingredient in traditional cooking. They are often used to make teas, infusions, and decoctions, and are said to have a number of health benefits. In terms of their growth habits, the leaves of *Aegle marmelos* are relatively slow-growing. They are evergreen, meaning they remain on the tree year-round, and are relatively drought-tolerant.^[28]

Every leaf of bael is also relatively pest- and disease-free. They are occasionally susceptible to pests like aphids and whiteflies, but these can be easily controlled with organic pest control methods. In terms of their cultivation, the leaves of *Aegle marmelos* prefer well-drained soil and full sun to partial shade. They are relatively adaptable to different soil types and can thrive in a variety of environments.^[29]

The leaves of *Aegle marmelos* are also highly ornamental, making them a popular choice for landscaping and gardening. They add a touch of tropical elegance to any setting, and are relatively low-maintenance. In addition to their ornamental

value, the leaves of *Aegle marmelos* are also highly symbolic. In Hinduism, the leaves are considered a symbol of good fortune and prosperity.^[30]

The leaves of *Aegle marmelos* are also highly revered in traditional medicine. They are said to have a number of health benefits, including reducing inflammation, lowering fever, and fighting off infection. In terms of their chemical composition, the leaves of *Aegle marmelos* contain a number of bioactive compounds, including alkaloids, glycosides, and terpenoids. These compounds are responsible for the leaves' medicinal properties.^[31]

Bael leaves are also richly valued for their antioxidant properties. They contain a number of antioxidants, including flavonoids and phenolic acids, which help to protect the body against free radicals. Overall, the leaves of *Aegle marmelos* are a truly remarkable sight. With their deep green color, smooth texture, and distinctive aroma, they are a popular choice for landscaping, gardening, and traditional medicine.^[32]

DRUG PROFILE

Its leaves are trifoliate, having round base and pointed tip. Young leaves are light green and matured leaves are dark green in colour.

The leaves of *A. marmelos* are alternate, trifoliate, aromatic, deciduous borne as single or compound and comprises of 3 to 5 oval, pointed shallowly, thin toothed leaflets with length 4-10 cm and 2-5 cm in width, terminal one have long petiole while the lateral one is without a petiole. Leaves are composed of 3 to 5 leaflets. Leaf petiole is long and glabrous. Mature leaves possess a disagreeable odor when bruised.

The phytoconstituents present in leaves of *aegle marmelos* are Skimmianine, Aegeline, Lupeol, Cineol, Citral, Citronella, Cuminaldehyde, Eugenol, Marmesinine.

Traditional Uses

Aegle marmelos leaves have been used in traditional medicine for centuries, particularly in Ayurveda and Unani systems. They are used to treat various ailments, such as:

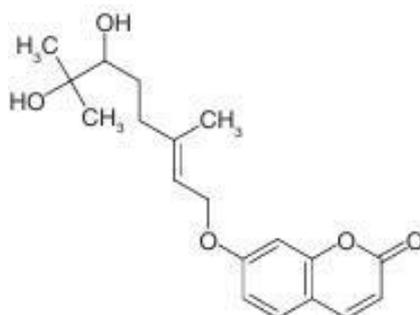
- Fever
- Infections
- Inflammatory conditions
- Respiratory problems
- Skin diseases

Phytochemical Constituents

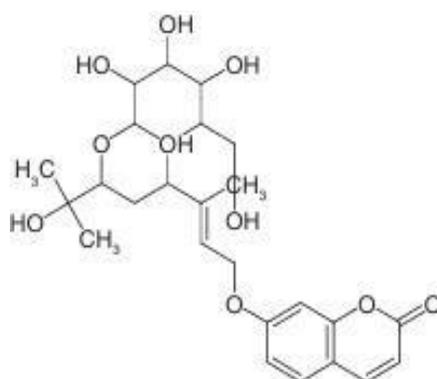
Aegle marmelos leaves contain a variety of phytochemicals, including:

- Alkaloids (e.g., aegeline, marmeline)
- Flavonoids (e.g., quercetin, kaempferol)
- Glycosides
- Phenolic acids
- Tannins

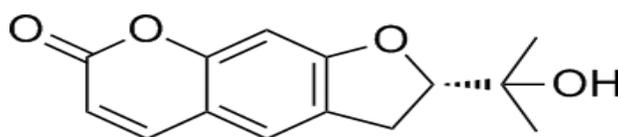
The leaves contain many constituents like alkaloids, aegeline, alkaloid coumarine, and marmine, sterol sitosterol, and essential oils d-limonene. The structures of few known alkaloids present in leaves are:



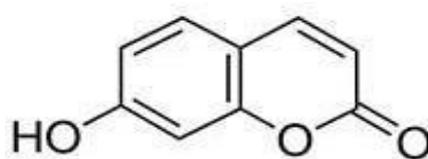
Aegeline



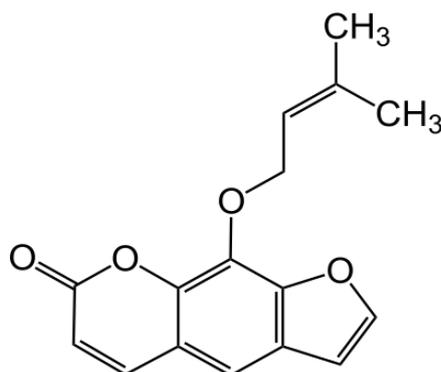
Marmerin



Marmesine



Umbeliferin



Marmelosin

Pharmacological Activities

Aegle marmelos leaves have been reported to exhibit various pharmacological activities, including:

- Anti-inflammatory
- Antimicrobial
- Antioxidant
- Antipyretic
- Anti-diabetic
- Immunomodulatory

Dosage Forms and Preparations:

Aegle marmelos leaves are available in various dosage forms, including:

- Leaf extract
- Leaf powder
- Leaf decoction
- Leaf infusion

Therapeutic Uses

Aegle marmelos leaves are used to treat various conditions, including:

- Fever and respiratory infections
- Skin and wound healing
- Diabetes and metabolic disorders

Safety and Toxicity

Aegle marmelos leaves are generally considered safe for consumption. However, high doses or prolonged use may cause:

- Gastrointestinal upset
- Allergic reactions
- Interactions with other medications

Contraindications

Aegle marmelos leaves are contraindicated in:

- Pregnancy and lactation (due to lack of studies)
- Patients with known allergies to the plant

Interactions

Aegle marmelos leaves may interact with:

- Diabetes medications (due to its hypoglycemic effects)
- Blood thinners (due to its antiplatelet effects)
- Other medications with similar mechanisms of action

AIM AND PRESENT STUDY

The aim and present study was to formulate medicated chocolate containing medicated containing herbal ingredients to preventing diabetes. One such endeavor is the formulation and evaluation of an anti-diabetic herbal chocolate blend infused with the therapeutic property of *Aegle marmelos* leaves powder along with complementary flowers and functionalization of chocolate, sucrose, coffee, cocoa butter, cardamom, powder sodium benzoate.

The consumption of chocolate is enjoyed by all ages of people and also health concerns such as obesity, high blood pressure mainly diabetes.

The prepared chocolate formulation were evaluated for parameter such as hardness, pH, general appearance, color, texture, chemical composition, variation, melting, physical stability.

Most of these properties are believed to be due to presence of bioactive alkaloids in bael. The study was carried out to develop an easy method of extraction and separation of alkaloids from dried bael leaves. Alkaloids from the dried leaves of bael were extracted in 95% ethanol and separated with by thin layer chromatography.

FORMULATION OF ANTI-DIABETIC CHOCOLATE

Materials and Equipment

- Mixing Bowls:** Used for blending and mixing the ingredients to prepare the chocolate formulation.
- Measuring Instruments:** Weighing balance for accurately measuring ingredients.
- Blender or Grinder:** Used to grind or blend ingredients such as guava leaves or *Aegle Marmelos* leaves (Bael) into powder form.
- Heating Apparatus:** Water bath for melting the dark chocolate.
- Stirring Rods or Spatulas:** Used for thorough mixing and homogenization of the formulation.
- Refrigerator:** Used to freeze the chocolate formulation.
- Chocolate Mould:** Used to give proper shape to the liquid chocolate before it sets.

Excipients Profile

Table- 1.

Excipients	Rich components	Properties
Dark Chocolate	Polyphenols, flavonols	Anti-oxidants
Cocoa butter	Fatty acid, minerals	Solidifying agent
Coffee	Chlorogenic acid, caffeine	Flavor enhancer
Sodium Benzoate	Benzoic acid, sodium	Preservatives
Cardamom Powder	Essential oils	Control insulin secretion

EXPERIMENTAL WORK

Table- 2.

S. No.	INGREDIENTS	QUANTITY TAKEN			CATEGORY
		F1	F2	F3	
1.	<i>Aegle Marmelos</i> leaves powder	2.5	2.5	2.5	Anti-diabetic Agent
2.	Dark chocolate	50	50	50	Antioxidant
3.	Cocoa butter	2.5	2.6	2.7	Solidifying agent
4.	Sucralose	2.5	2.5	2.5	Sweetening Agent
5.	Cardamom powder	01	01	01	Flavouring Agent
6.	Coffee	01	01	01	Flavouring Agent
7..	Sodium Benzoate	0.05	0.05	0.05	Preservative

METHODOLOGY

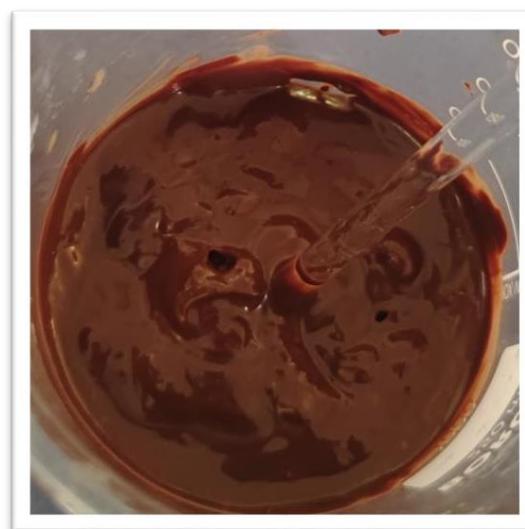
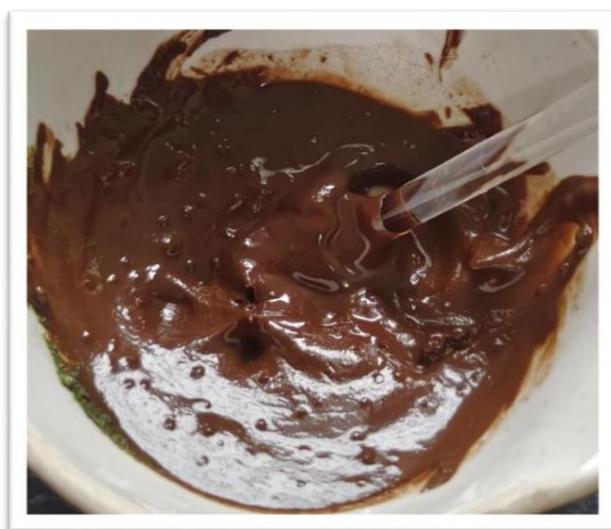
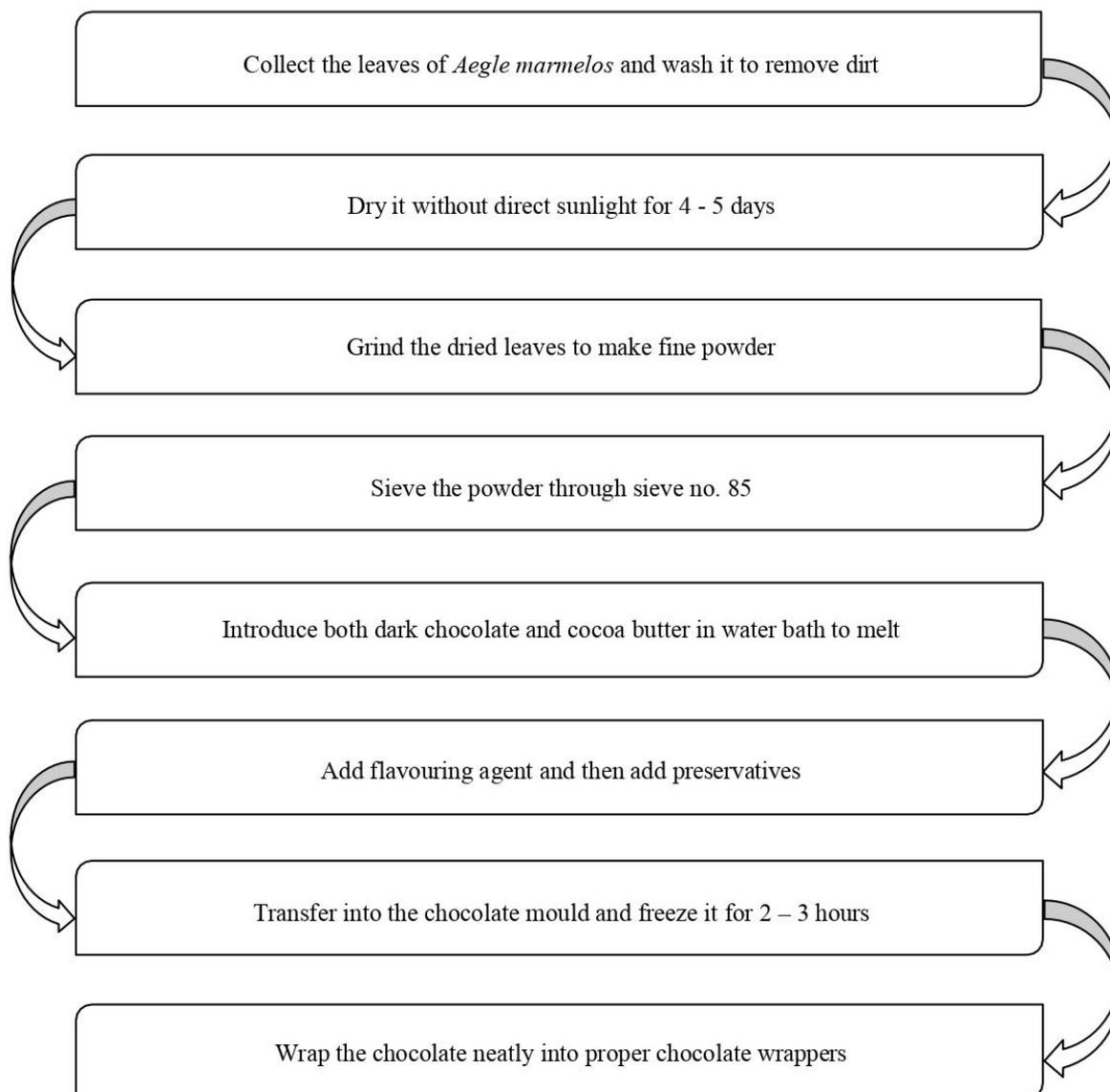




Fig No. 5: Chocolate Formulation.

RESULT AND DISCUSSION

The evaluation parameters for chocolate:

S. No	Test	F1	F2	F3
1.	Color	Dark brown	Dark brown	Dark brown
2.	Taste	Sweet	Sweet	Sweet
3.	Texture	Smooth	Smooth	Smooth
4.	pH	6.8	6.9	7.0
5.	Aroma test	Sweet and floral	Sweet and floral	Sweet and floral
6.	Stability	Stable	Stable	Stable
7.	Melting point	60 ^o C	61 ^o C	62 ^o C
8.	Consistency	Solid	Solid	Solid
9.	Environmental impact assessment	Biodegradable	Biodegradable	Biodegradable

EVALUATION TEST

1. PHYSICAL APPEARANCE

- a.) **Color:** The chocolate formulations F1, F2, F3 exhibits brown indicating uniformity in the chocolate preparation.
- b.) **Texture:** All formulations showed ideal texture – smooth glossy and even, proper blending and homogenization of ingredients.
- c.) **Taste of the chocolate:** Sweet, aromatic and slightly tangy with undertones of marmalade.
- d.) **Mouth Feel:** Smooth, creamy and melt in your mouth.
- e.) **Hardness:** Hardness test of chocolate formulations being tested using Digital Portable Hardness tester around 20 N/nm²
- f.) **pH:** The pH of chocolate formulations was determined using pH meter within a close range.

pH level: 6.8 to 7.0 (slightly acidic to neutral)

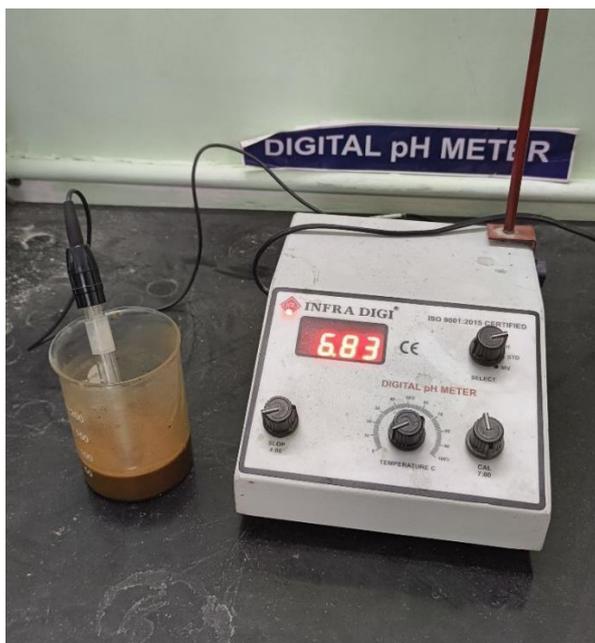


Fig no. 4: Digital pH Meter.

g.) **Environment impact assessment:** The chocolates were found to be biodegradable indicating their ecofriendly nature and minimal environmental impact.

h.) **Aroma test:** The chocolate exhibited a sweet and floral aroma enhancing the sensory experience for consumers.

2. CHEMICAL TEST

a.) **Test for Carbohydrate (Fehling's Test):** A solution containing equal quantities of Fehling's solution A and B was added and heated. The formation of a brick-red precipitate indicates the presence of carbohydrates.

b.) **Test for Protein (Biuret Test/General Test):** Chocolate formulation was mixed with 4% NaOH and a few drops of 1% copper sulphate solution. The formation of a violet color indicates the presence of protein.

c.) **Test for Amino Acids:** The test solution was heated, and 5% Ninhydrin solution was added. Boil for 10 minutes. The formation of a purple color indicates the presence of amino acids.



Fig. No. 5: Chemical test.

3. MELTING POINT

The residual heat of the chocolate will melt it. When the microwaving of chocolate, it is fully melted and use an instant read thermometer to check the chocolate's temperature while melting.

4. BLOOM TEST

The chocolate was heated to 40°C for 30 minutes and then cooled to 20°C. Fat bloom is caused by the recrystallization of fat, this will cause the chocolate to lose its gloss and soft white layer will appear. Storage at a constant temperature will delay the appearance of fat bloom.



Fig. No. 6: Observation of chocolate from bloom test.

5. PHYSICAL STABILITY

To check physical stability, samples of chocolate were kept in closed containers for 1 month at 28°C. After 1 month, test samples of chocolate were observed for physical appearance and drug degradation.

THIN LAYER CHROMATOGRAPHY

Powder of 40 gm of dried leaves was added to 150 ml of 95% ethanol, heated at 65°C and kept overnight at room temperature. The extract was then filtered and again 150 ml of 95% ethanol was added and procedure was repeated one more time for extraction of alkaloids from the leaves. The combined extract was allowed to dry. A greenish viscous extract was obtained.

Separation method

Analytical and preparative thin layer chromatography was performed on silica gel plates or glass plates coated with silica gel G 60) with chloroform as solvent system. Chromatograms were visualized under ultra violet light.

Ethanol extract obtained from bael leaves was carried out to thin layer chromatography in order to establish the purity and composition of materials. The purified components of ethanol extract of bael leaves were identified observed under ultraviolet light. It was shown in Fig no. The Rf values of separated components of bael leaves extract were measured. The Rf value of components was shown in table no.

$$\text{Rf value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}}$$

Distance travelled by compound	Rf value of each compound
0.4	0.33
0.7	0.069
1.0	0.101



Spots of TLC plate

CONCLUSION

The study was concluded the active constituents present in *Aegle marmelos* leaves extract exhibit the superior inhibition against diabetic activity. The chocolate formulation results shown in the specific limits for the respective evaluation, parameters of color, texture and chemical composition variation melting point, physical stability.

Among the formulation tested batch F1 emerged as the optimized batch and it has ideal properties.

The alkaloids present in the leaves are well extracted through 95% ethanol. During separation, it was observed under UV light 366nm the alkaloids are separated successfully by thin layer chromatography technique.

This extraction and separation method will be useful further to study more medicinal properties of the alkaloids that are present in the extract of Bael leaves.

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