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FORMULATION AND ASSESSMENT FOR DIABETIC FOOT ULCER FROM MUSA PARADISIACA (L.) EXTRACT

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ABSTRACT

Diabetic foot ulcer is a common skin disease on the foot due to neuropathic and vascular complications shown in type 2 Diabetic mellitus patient. Appropriate foot care is vitally important in the treatment of foot lesion. *Musa paradisiaca* L is a popular Indian medicinal plant belong to the Musaceae family, exhibits various pharmacological properties and can be utilized in the treatment of diabetic foot ulcers. The study aimed to formulate and evaluate a powder formulation of *Musa paradisiaca* L using the Soxhlation extraction method, targeting its application in the wound healing treatment of diabetic foot ulcers (DFU). Musa leaves extract was prepared and collected through Soxhlet extraction and the methanolic extract was analyzed using the GC-MS method to identify its chemical constituents. Various formulations were prepared using the crude extract and appropriate excipients and the selected formulations were subsequently evaluated. The powder formulation was tested for physical parameters, which shows excellent consistency and flow property. Significant wound healing properties were obtained from in vitro assays analysis such as, antioxidant, anti-inflammatory and antimicrobial activity. Based on the background of all studies conducted, the powder formulation has proven to be effective for diabetic foot ulcers (DFU). Besides its efficacy, this formulation is also easily applicable and economically beneficial for patients.

KEYWORDS: DFU, *Musa Paradisiaca* L, antioxidant, anti-inflammatory and antimicrobial activity.

1. INTRODUCTION

It has been increasingly apparent in recent years that medicinal plants play a crucial role in maintaining health and managing illness. The primary cause of this is the usage of artificial pharmacological molecules, which have detrimental side effects that are comparably rare in medications derived from plants to their increased availability, strong safety margin, and hence lower cost. Medicinal plants are widely accessible, particularly in tropical regions. There are four main ways that plants are useful in modern medicine: first, they can be used to produce direct therapeutic agents; second, they can be used as a source of raw materials to create more complex semi-synthetic chemical compounds; third, the chemical structures derived from plant sources can be used as models for new synthetic compounds; and last, they can be used as taxonomic markers to help find new compounds. In allopathic medicine, herbal preparation plays a major role. A number of pharmaceuticals currently in use such as codeine, morphine, aspirin, vinblastine, vincristine, pilocarpine, cocaine, atropine, emetine, and ephedrine, have their origins in medicinal plants.[1]

Bananas and plantains are herbaceous monocotyledonous plants belonging to the genus Musa, Musaceae, which has a widespread distribution around the world. Various parts of banana plant are commonly used in traditional medicines. *Musa paradisiaca* Linn. is a popular Indian medicinal plant belonging to the Musaceae family. *Musa paradisiaca* Linn., known as Kadali in sanskrit is a highly valued medicinal plant widely used in Indian traditional system of medicine for curing various ailments. This plant commonly known as plantain or banana is highly eating nutritious fruit over the world. *M. paradisiaca* is still largely unexplored source for the development of new drugs. A wide range of phytochemical constituents have been isolated from this plant. It has long been used in traditional ayurvedic Indian medicine for various diseases. This plant is pharmacologically studied for analgesic activity, antidepressant activity, anticonvulsant activity, CNS depressant activity, antidiarrheal activity, antiulcerative activity, antimicrobial activity, antidiabetic activity, antioxidant activity, antilipidemic activity, antihypertensive activity, antiatherosclerosis activity, cytotoxic activity, thrombolytic activity, antimalarial activity, mutagenic activity, hepatoprotective activity, hair growth promoting activity and wound healing activity and many other activities. Bananas are considered a significant nutritional source of energy. A proximate analysis of *M. paradisiaca* revealed the presence of numerous nutrients, including lipids, carbohydrates, dietary fibre, protein, and minerals like potassium, magnesium, phosphorus, calcium, sodium, zinc, and iron. Additionally, water-soluble vitamins like thiamine (vitamin B1), riboflavin

(vitamin B2), niacin (vitamin B3), ascorbic acid (vitamin C), and folic acid were found. Several chemical substances, such as eicosanoic acid, methyl ester, hexadecanoic acid, 9,12-Octadecadienoic acid, tetracosanoic acid, and Phytol, have been isolated from different portions of the plant. Additionally, the GC-MS analysis of *M. paradisiaca* revealed the presence of vitamin E, octadecenamide, β-sitosterol, and stigmasterol as major phytochemicals in the fruit peel extract and phytol, octadecatrienoic acid, hexadecanoic acid, and octadecadienoic acid as major components in the leaf extract; all of these constituting compounds were reported to have antioxidant activities.[2,3]

According to the GC-MS results, the main constituents of the leaf of *Musa paradisiaca* are eicosanoic acid, methyl ester, heptadecanoic acid, 9-octadecenoic acid, hexadecanoic acid, phytol, docosanoic acid, tetracosanoic acid, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, 2-Propenoic acid, 3-(4-hydroxy-3-methoxyphenyl), and their properties include Arachidic acid, also referred to as eicosanoic acid, which increases inflammation. A diterpene called phytol has a range of biological properties, such as anti-microbial, immune-modulating, antioxidant, anti-inflammatory and anti-cancer properties. Hexadecanoic acid has some biological activities, including hypocholesterolaemia and antioxidants. The anti-inflammatory compound, 9,12,15-Octadecatrienoic acid was another ingredient. A fatty acid with anti-diabetic properties is methyl tetracosanoate. These beneficial effects observed in *Musa paradisiaca* leaves and the presence of various major compound indicate potential anti-diabetic properties and various other biological activity is carried out.[4,5]

1.1. DIABETIC FOOT ULCER

Diabetic foot ulcer is the most common complication in type 2 DM patient which is not well controlled. It is usually the result of poor glycaemic control, underlying neuropathy, peripheral vascular disease or poor foot care. It is also one of the common causes for osteomyelitis of the foot and amputation of lower extremities. The ulcer are usually in the area of foot which encounters repetitive trauma and pressure sensation. Staphylococcus is the most common infective organism. Local debridement and appropriate foot wear and foot care are vitally important in the early treatment of foot lesion.[6]

TYPES OF DIABETIC FOOT ULCER

1. Neuropathic diabetic foot ulcer
2. Ischemic diabetic foot ulcer
3. Neuro-ischemic diabetic foot ulcer

RISK FACTORS

- Increase with age
- Duration of diabetics
- Neuropathy
- Trauma
- Peripheral vascular disease
- Abnormal foot pressures
- Hyperglycaemia[6,7]

1.2. TREATMENT AND MANAGEMENT

The DFU treatment include debridement of the wound, management of the infection, revascularisation procedures when indicator and offloading of the ulcer. Other methods have also being suggested to be beneficial as add on therapies such as hyperbarric oxygen therapy,use of advanced wound care product and negative pressure wound therapy.

1) Debridement

2) Offloading

3) Dressing

1.2.1. DEBRIDEMENT

Debridement should be carried out in all chronic wounds to remove surface debris and necrotic tissues. It improves healing by promoting the production of granulation tissue and can be achieved surgically, enzymatically, biologically and through autolysis.

Surgical debridement is effective in removing hyperkeratosis and dead tissue. Enzymatic debridement is indicated for ischemic ulcer. Recent report suggest that biological debridement method is also effective in the elimination of drug resistant pathogen such as methicillin resistant staphylococcus aureus from wound surface.

Autolytic debridement involves the use of dressings that create a moist wound environment so that host defence mechanism can clear devitalized tissue using the body's enzymes. Autolysis is enhanced using proper dressings, such as hydrocolloids, hydrogel and film. Autolysis is highly selective, avoiding damage to the surrounding skin.

1.2.2. OFFLOADING

Off-loading of the ulcer area is extremely important for the healing of plantar ulcers. In addition, any existing foot deformities may increase the possibility of ulceration.
Especially in the presence of diabetic peripheral neuropathy and inadequate off-loading.
Therapeutic shoes, custom insoles, and the use of felted foam are alternative methods to off-load wounds located on the forefoot, and can reduce pressure at the site of ulceration by 4–50%.

1.2.3. DRESSINGS

Ulcers heal more quickly and are often less complicated by infection when in a moist environment. The ideal dressing should be free from contaminants and able to remove excess exudates and toxic components, maintain a moist environment at the wound dressing interface, be impermeable to microorganisms, allow gaseous exchange, and, finally, should be easily removed and cost-effective.[8]

1.2.4. OTHER THERAPIES

1. Hyperbaric oxygen

It is the one of the adjunct therapies which is used from decade to treat complex DFU, in chronic wound affected tissue become hypoxic, hence oxygen plays a big role in chronic wound healing. In HBOT patient is kept in a chamber with 100% breathing oxygen and increase atmospheric pressure greater than sea level for better clinical outcome.

2. Glycemic control

Most foot ulcer have their origin in inadequate control of blood sugar, which results in development of lower limb neuropathy. There is no excellent evidence that improved control of diabetes can markedly reduce the incidence of neuropathy.

3. Tissue engineering

Despite adequate blood flow and good wound care, some neuropathic DFU fail to heal. Impairment of the normal cellular functions involving growth factors and fibroblasts necessary for wound healing has been postulated to account for the failure to close theses wounds.[9,10]

1.3. TOPICAL FORMULATION FOR WOUND HEALING

Commonly used topical formulation for wound healing are discussed below.

Table 1: Different types of topical formulation for wound healing.

TYPES	FUNCTIONS	ADVANTAGES	DISADVANTAGE
Films	maintain moist environment in wound area impermeable and prevent bacterial growth provide barrier against external environment	easy to inspect because of its transparent nature high patient compliance stabilises the wound	potential risks of damaging wound bed while removing film dressing because of its adhesive nature. Accumulated fluid might damage newly differentiated keratinocytes No absorptive capacity
Foam	potential to absorb variable amounts of wound drainage Powerful adsorbent Balance environment moist	Greater absorbance Moisture vapour permeability	Cannot be used in dry wound Requires regular dressing[11]
Hydro colloids	They form gel when contact with the wound exudate Provide moist environment Reduce the pH and lower the bacterial growth.	Biodegradable Biocompatible Good for deeper wounds It's a type of interactive dressing	They over promote the growth of granulation tissue Not suitable for neuropathic ulcer Typically, secondary dressing
Hydrogel	Rehydrate wound bed and provide moist. promoting tissue regeneration through granulation and re-epithelialization	its flexibility and adjustable qualities used to treat dry chronic wounds Its soft elastic nature Nonirritating	Difficult to handle Low mechanical strength Not beneficial for high level exudate wounds.
Wound fillers	Moisten the wound area Autolytic debridement	Biocompatible Biodegradable non-toxic. Easy application and removal	May adhere to wounds[12,13]

1.4. DRY POWDER

A mixture of dry, finely separated pharmaceuticals and/or chemicals is called a pharmaceutical powder. These are pill dose forms that are solid which have both internal and external usage. They can be found in both crystalline and amorphous forms. The powder particle size has a significant impact on the dosage forms chemical, biological and physical characteristics. The dissolution, absorption and therapeutic effectiveness of medications are all correlated with the size of the powder particles.

In terms of handling, identification, administration they are easy for patients to use and effective. Bulk supply of some solids is packaged and provided. Both fine powder and granules are available for the bulk forms intended for internal consumption. Insufflations,

snuffs, dusting powders and tooth powders are examples of bulk powders intended for external usage.[14]

The powders are generally used in the following forms:

a. Based on the composition

- Simple powders: Simple powder contain only one ingredient either in crystalline or amorphous form. It may include inert substance as fillers and diluents. Example, Aspirin powder.
- Compound powders: It contain two or more than two substances mixed and divided into desired number of individual doses. Example, Aspirin Paracetamol and caffeine Powder.

b. Based on the intended use

- Topical powders: applied to the skin or mucous membranes for local effects, such as antiseptics, astringents, or absorbent powders.
- Oral powders: Designed for ingestion and may be administered directly or mixed with liquids or food for oral consumption. Example, Sensodent-k
- Inhalation powders: Administered through inhalation devices for respiratory condition such as asthma or chronic obstructive pulmonary disease (COPD).
- Injectable powders: Intended for reconstitution with a suitable solvent to form a solution or suspension for parental administration.

c. Based on the preparation method

- Bulk powders: Prepared by mixing the active ingredients and excipients in large quantities, which are then divided into individual doses.
- Divided powders: Prepared by dividing a bulk powder mixture into individual doses using precise measurements.

d. Based on the particle size

- Fine powders: Have particles that are smaller than 180 micrometres in diameter and are often used for inhalation or as topical preparation.
- Coarse powder: Have particle size that are larger than 180 micrometres in diameter and are usually intended for oral administration after being mixed with liquids and foods.

e. Based on the specific characteristics

- Ophthalmic powders: Powders intended for use in the eyes, often formulated as sterile ophthalmic solutions or suspension after reconstitution.
- Effervescent powders: Contain ingredients that release gas when dissolved in water, often used for oral administration to improve
  palatability or aid in drug delivery. Example, Eno Fruit Salt, Cetro soda.
- Dusting powders: Extremely fine powders used for dusting purpose
  such as antifungal powders for treating skin infection. Example, Neosprin, Myoderm.

Advantage of Powder

1. Powders are usually more stable than liquids because chemical reactions take place more rapidly in the atmospheric conditions when the drug is in liquid dosage form than powder.
2. Incompatibility is less in case of powders than liquid.
3. Faster onset of action, smaller particle size of powders produces more rapid dissolution in the body fluids than other solid dosage forms.
4. The rapid dissolution increases the blood concentration in shorter time there by
5. Powders are easier to carry.
6. They are more economical when compared to other dosage forms because they
   do not require any special technique or machinery.
7. Children and old person s who cannot swallow solid dosage forms can easily ingest powders which can be dispersed in water or any other liquid and may administered.[14]

2. REVIEW OF LITERATURE

1. Raquel de Oliveira vilhena *et al.*, (2018) conducted a study on Antidiabetic potential of *Musa* spp. inflorescence: a systematic review. This review assessed Musa spp.'s suitability as a natural diabetes mellitus treatment option. There was no discernible difference between the treated and control groups in the one trial that assessed the antidiabetic impact in humans. Comparing the plant extracts to different pharmaceutical medications, the animal-induced diabetic models demonstrated an equal glycaemic level reduction that was thought to be dose dependant. They found that plant extract was able to correct defects in the pancreatic islet brought on by high insulin levels.[15]

2. Mariana M. Fachi *et al.*, (2018) The study indicates the antidiabetic effect of the musa species. Numerous biological actions have been demonstrated, including antibacterial, anti-inflammatory, anti-diabetic, antioxidant, and antihyperglycemic effects. There are several chemical constituents present in the Musa species including anthocyanins, phenolic acids

flavanones and terpenoids etc. Compounds from these classes are recognized to have anti-diabetic properties. Additionally, they assist in lowering blood glucose levels and raising insulin sensitivity, which reduce complications for diabetes patients. According to the investigation they concluded that pharmacological medications linked to the diabetic action and plant extract have similar effects.[16]

3. Sharma, Gadiya and Dhanawat *et al.*, (2018) focused cosmetic formulation, used to decorate their bodies for improvement of appearance. Now days, cosmetics are considered as essential components in life. They not only, attract the people towards it but also impart psychological effects. It has gained popularity in the last 3-4 decades and its use has been increased exponentially both-in males and females. The most popular cosmetics are hair dyes, powders and creams. In case of powders various types of powders are used as body powder, face powders, compacts medicated powders (which are used preventing microbial growth on the surface of the skin), deodorant powders and foot powders for treatment purposes. The study conducted to screen properties, formulation, preparation, and evaluation of cosmetics.[17]

4. Amutha K *et al.*, (2016) investigates the antibacterial, and wound healing properties of the methanolic stem extract of *Musa paradisiaca* Linn. Phytochemical analysis revealed rich content of glucosides, tannins, alkaloids, saponins, flavonoids, and phenols. The extract exhibited significant antibacterial activity against Pseudomonas aeruginosa and Staphylococcus aureus. Wound healing experiments on Wistar albino rats, involving burn wounds treated with the extract, demonstrated superior healing activity compared to the control group. The study concludes that the methanolic extract of *Musa paradisiaca* Linn. holds potential for wound healing applications.[18]

5. Lakshmi, Agarwal and Mahdi *et al.*, (2015) focused on natural product remedies. There are many infectious diseases have been treated with herbal, almost 70% modern medicine derived from natural products. *M. paradisiaca* is still largely unexplored source for the development of new drugs. The plant extract and its active constituents have been used in the treatment of various human ailments. The plant extracts were found to increase hydroxyproline, hexuronic acid, hexosamine and superoxide dismutase as well the wound breaking strength and reduced glutathione level. They also decreased the wound area, scar area and lipid peroxidation. The review of literature summarized chemical constituents and pharmacological activities of *Musa paradisiaca linn.*[19]

6. Naikwade P.V *et al*., (2014) conducted investigations to assess the antibacterial potential of *Musa paradisiaca* leaves. Using the agar diffusion method, the ethanol extracts of *Musa paradisiaca* demonstrated a wide range of antibacterial activity against microorganisms such as Escherichia coli and Staphylococcus aureus. The *Musa paradisiaca* L. ethanol extract is made with the aid of the Soxhlet apparatus. The leaf extract of *Musa paradisiaca* L. is prepared using solvents such as petroleum ether, chloroform, and ethanol. They found that more potent of the two is the ethanolic extract. Because the leaf extract of *Musa paradisiaca* L. appears to have a broad spectrum of antibacterial action, it may find utility in the formulation of antiseptics and disinfectants.[20]

7. Vijai Lakshmi *et al*., (2014) studied the antidiabetic potential of various parts of *M. paradisiacal* L. due to concerns over harmful side effects of synthetic drugs. Ethanolic extracts from leaves and fruit peels show promising antidiabetic activity, although isolated compounds from these fractions do not. Further research is needed to identify active molecules for the development of an effective antidiabetic drug.[21]

8. Alexiadou Doupis *et al*., (2012) conducted studies on cause and pathogenetic mechanism of diabetic foot ulcer and to focus management of this condition. When foot affected by ulceration associated with neuropathy or peripheral arterial disease of the lower limb in a patient with diabetes. Treatment of these include debridement of wound, management of any infection, off-loading of ulcer. Other methods have been suggested to be beneficial as add on therapies such as hyperbaric oxygen therapy, negative pressure therapy and advanced wound care products.[22]

3. AIM AND OBJECTIVE

The aim of study was to formulate and evaluate *Musa paradisiaca* by soxhlation method, with an objective of treatment of wound healing of diabetic foot ulcer. The study was carried against Staphylococcus aureus to find out the effective formulation for *Musa paradisiaca* powder with respect to that of Neosporin antibiotic powder by comparison using topical application.

3.1. OBJECTIVE

- To carry out extraction of *Musa paradisiaca* by soxhlation using solvent methanol for the treatment of diabetic foot ulcer.

- Formulation of powder with the excipient's talc, Micro Crystalline Cellulose, Sodium benzoate which having the properties like mucoadhesive, preservative, flow property respectively.
- Determine the effective concentration of the formulation for wound healing.
- To evaluate its biological activity like antidiabetic, antimicrobial and anti-inflammatory of the formulation.

4. MATERIAL USED

4.1 PLANT PROFILE

4.1.1. SCIENTIFIC CLASSIFICATION

Kingdom : Plantae
Division : Magnoliophyta
Class : Liliopsida
Subclass : Zingiberidae
Order : Zingiberales
Family : Musaceae
Genus : Musa L.
Species : M. paradisiaca L.

4.1.2. SYNONYMS

Sanskrit :Vana laxmi, Kadali
English : Plantain or Banana
Hindi : Kela
Telugu : Kadalamu, Ariti
Tamil : Kadali
Malayali : Vazha[23, 24]

4.1.3. ORGIN AND CULTIVATION

It is a perennial herb that often grows in tropical and subtropical regions, reaching heights of 10 to 40 feet (looking like trees). Approximately three hundred types of bananas are cultivated worldwide, with the bulk of them occurring in the tropical regions of Asia, Indo-Malaysia, and Australia. Tamil Nadu, Andhra Pradesh, Bihar, Madhya Pradesh, West Bengal, Maharashtra, and Gujarat are the states in India where it is most prevalent.[41]

4.1.4. BIOLOGICAL SOURCE

Musa paradisiaca L. is evergreen tropical monoherbacious plant belongs to family Musaceae, commonly known as vana laxmi, kadali, banana, kadalamu, valei, vala, bali hannu and plantain.

4.1.5. BOTANICAL DESCRIPTION

Musa paradisiaca Linn. often referred to as plantain is one of the tallest herbaceous flower producing plant. Plant is up to 9 m (10-40 feet) long with pseudo stem, a crown of large elongated oval deep-green leaves that grows through hollow stem with a prominent midrib, each plant produces a single inflorescence like drooping spike and large bracts opening in succession, ovate, 15-20 cm long, concave, dark red colour and in somewhat fleshy. Fruits are oblong, fleshy, 5-7cm long in wild form and longer in the cultivated varieties. It contains various chemical constituents such as starch, protein, fat, dietary fibre. It is source of vitamins and minerals such as calcium, potassium and phosphorus along with alkaloids, glycosides, saponins, and steroids.[25]

4.1.6. CHEMICAL CONSTITUENTS

The presence of bioactive compounds like apigenin glycosides, myricetin glycoside, myricetin-3- O-rutinoside, naringenin glycosides, kaempferol-3-O-rutinoside, dopamine, N-acetyl serotonin and rutin, has been reported in different species of Musa.[26]

USE: Antimicrobial, Antidiabetic, Antiulcer, Antibacterial, Antioxidant, Mutagenic activity, Antihypertensive, Wound healing, Thrombolytic activity.

4.2. EXCIPIENT PROFILE

4.2.1. Talc

Synonym

Magnesium Silicate, soapstone

Source

It is obtained from natural sources and may contain small amounts of aluminium silicate which belongs to the general mineral family of the Layered Silicates.

Use

- Talc is used as a glidant for improved powder flow and as a lubricant in tablet formulation.

- Talc is also used extensively in the cosmetic industry in face, body, and foot powders, as well as in aerosol formulations. [27, 28]

4.2.2. Micro Crystalline Cellulose (MCC)

Synonym

CP-MCC, Avicel PH 101

Source

It is cellulose product obtained from raw cotton of Cochlospermum planchonii which belongs to family Bixaceae.

Use

- MCC works as a disintegrant and lubricant and has a high dilution potential in direct compression formulations.
- MCC is used as a diluent in tablets prepared by wet granulation as well as a filler for capsules and spheres.[29]

4.2.3. Sodium Benzoate

Synonym

Sodium salt of benzoic acid

Source

It's an odourless, crystalline powder made by combining benzoic acid and sodium hydroxide.

Use

- Sodium benzoate is commonly used as a preservative in cosmetics and personal care items, such as hair products, baby wipes, toothpaste, and mouthwash.
- Sodium benzoate may be used as a stabilizer in photo processing and to improve the strength of some types of plastic. [30, 31]

5. METHODOLOGY

5.1 COLLECTION AND DRYING

The study conducted on leaves of *Musa paradisiaca* Linn. The sample obtained from cultivated local farmland Thrissur, Kerala during march 2024. Identified and authenticated by Dr. M Bheemalingappa, scientist-B, forest botany department, KSCSTE-Kerala Forest research institute, Peechi. The leaves were separated from banana plant (*Musa paradisiaca*).

Leaves are large, oblong petioles long channeled, bright glossy green. They cut into small pieces. After a thorough cleaning, they were spread out on filter paper. Dried in shade at room temperature for 20 days, powdered with mechanical grinder. Stored for further use.[32, 33]

Figure 1: Process of Drying.

5.2. SOXHLET EXTRACTION

In order to identify the phytochemicals, *Musa paradisiaca* L. (AB) is extracted using methanol. Soxhlet equipment is used for extraction. A piece of laboratory equipment created by Franz von Soxhlet in 1879 is known as a Soxhlet apparatus. Its original purpose was to extract a lipid from solid matter. However, a Soxhlet extractor is not limited to the extraction of lipids. Typically, a Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent.[34]

Chemical Used

1. Methanol

Procedure

- Weigh accurately 150gm powdered dried leaves of Musa paradisiaca are placed inside the thimble made from thick filter paper, which is loaded in main Soxhlet extractor.

- Soxhlet extractor placed onto around bottom flask containing extraction solvent methanol 1000ml.
- The Soxhlet is then equipped with a condenser. The solvent is heated to reflux
- The solvent vapour enters the chamber containing the thimble of powdered leaf after ascending a distillation arm.
- The chamber containing the dry powder slowly fills with warm water.
- The Soxhlet chamber automatically empties itself using a syphon side arm when it is nearly full, allowing the solvent to return to the distillation flask.
- After many cycles the desired compound is concentrated in the round bottom flask.
- After extraction solvent is removed from Soxhlet apparatus, where solvent contain the extracted compound.
- Once the solvent has been extracted, the solid portion that is not soluble is left in the sample and often discarded.[35]

5.3. GC-MS ANALYSIS

This study uses GC-MS analysis for exploring phytochemical present in the alcoholic extract GC-MS begins with the gas chromatography, where the sample is volatized. This will effectively vapourizes the sample (the gas phase) and separates its various components using a capillary column packed with a stationary (solid) phase. The compounds are propelled by an inert carrier gas such as argon, helium or nitrogen. As the components become separated, they elute from the column at different times, which is generally referred to as their retention times. Once the components leave the GC column, they are ionized by the mass spectrometer using electron or chemical ionization sources. Ionized molecules are then accelerated through the instrument's mass analyser, which quite often is a quadrupole or ion trap. It is here that ions are separated based on their different mass-to-charge(m/z) ratios. The final steps of the process involve ion detection and analysis, with compound peaks appearing as a function of their m/z ratios. Peak heights, meanwhile, are proportional to the quantity of the corresponding compound. A complex sample will produce several different peaks, and the final readout will be a mass spectrum. Using computer libraries of mass spectra for different compounds, researchers can identify and quantify unknown compounds and analytes.[36]

6. FORMULATION OF POWDER

Objective: To formulate a formulation with the drug extract which contain specific quantity of talc.

Formula

Table 2: Different Formulations.

INGREDIENTS	F1		F2		F3	
	WT (gm)	QTY IN %	WT (gm)	QTY IN %	WT (gm)	QTY IN %
Drug extract	5g	25	5g	25	2g	10
MCC	8g	40	4g	20	2g	10
Talc	6g	30	10g	50	15.9g	79.5
Sodium benzoate	1g	5	1g	5	0.1g	0.5
Total	20g	100	20g	100	20g	100

Preparation of powders

Procedure

Weigh precisely particular gram of ingredients using various formulas. Then, pass through sieve no 66 in descending order of their weight. After weighing the necessary quantity of crude extract, fill the petridish with the weighed materials. Blend well to form a powder. Proceed through the Sieve no 66 after blending. If there is any excess moisture, put in tray dryer until constant weight obtained.

Figure 2: Powder Formulation

7. EVALUATION OF POWDER

Organoleptic characters

- COLOUR

Examine the sample's colour visually. Observe the colour

- ODOUR EXAMINATION

Perform an odour evaluation of the powder sample. Open the container or remove the lid to expose the sample and take a sniff. Note the aroma, intensity and unusual smells present.

- PARTICLE SIZE

The size of the particle is determined by using micrometrics.

- PHYSICAL APPEARANCE

The physical appearance of the prepared formulation was evaluated. Using a spatula or clean hands to examine the texture and smoothness.[37,38]

Physical characters

- ANGLE OF REPOSE

determined by substituting the values of the base radius ‘r’ and height of the pile ‘h’ in the given equation given below,

Tan θ = h/r

Where,

θ = Angle of repose,

h = Height of pile,

r = Radius of base

- SOLUBILITY

Solubility of the *Musa paradisiaca* leaf powder determined in various solvents

- NON-IRRITANCY TEST

Applied to the skin, observe Angle of repose is used to measure flow properties. Improper flow of powder is due to frictional forces are qualified by angle of repose. Angle of repose was any irritation.

Biological Activity

- ANTIDIABETIC ACTIVITY

ALPHA AMYLASE ASSAY

Principle

One of the therapeutic targets currently introduced in the management of type 2 DM is inhibition of α-glucosidase and α-amylase to decrease the reabsorption of glucose in the intestine. The alpha-amylase (α-1,4-glucan-4-glucanohydrolases) is a prominent secretory product of the pancreas and salivary gland responsible for the initial step in the hydrolysis of complex carbohydrate to a mixture of oligosaccharides and disaccharides in the intestinal

mucosa. These sugars are further digested to monosaccharide by the action of alpha-glucosidase. There is, an urgent need to search for complementary and alternative therapies with minimal side effects that can serve as adjunct to the management of DM. Thus, the anti-diabetic action was determined by the suppression of alpha amylase.[39]

Reagents Required

- DNSA colour reagent

(1g 3,5 – dinitrosalicylic acid, 30 g sodium potassium tarterate and 20µl of sodium hydroxide to get final volume of 100 ml in distilled water)

- Starch solution

(1g starch in 0.02 M sodium phosphate buffer containing 0.0067 M of sodium chloride in 100 ml)

- Sodium phosphate buffer

Procedure:

Initially 1mg of powder weighed and diluted with methanol. 120µl of each formula, 480µl of distilled water, and 1.2ml of starch solution were combined. An enzyme solution in 600µl was added to the test tube containing different formulas to start the reaction, which was then kept at room temperature for three minutes. After 3 minutes, 600µl of the mixture were taken and combined with 300µl of DNSA colour reagent. For 15 minutes, test tubes were maintained in the water bath between 85 and 90°C. Following that, the sample was allowed to cool in room temperature and add 2.7 ml of distilled water in each test tube. The absorbance was measured by UV-visible spectroscopy at 540 nm. Plant extract was substituted with 120µl of solvent to make the control. Formula used to calculate the inhibition percentage.

$$\text{Inhibition percentage} = \frac{(\text{control}_{540} - \text{sample}_{540})}{(\text{Control}_{540})} \times 100$$

- IN VITRO ANTI-INFLAMMATORY STUDY

EGG ALBUMIN DENATURATION ASSAY

Principle

The objective of the egg albumin denaturation experiment is to find out if certain substances or agents can prevent or hinder the denatured state of egg albumin. The process by which a protein undergoes structural modification and loses its biological activity is known as

denaturation. Using egg albumin as a model protein, denaturation is achieved by subjecting it to high or low temperatures, changes in pH, or other denaturing agents. Denaturation alters the initial structure of egg albumin, resulting in changes to its physical properties and loss of its functional activity. The egg albumin denaturation assay assesses a substance's ability to prevent or reduce egg albumin denaturation in order to determine whether or not it has anti-inflammatory properties. Based on the theory that anti-inflammatory drugs could stabilize protein structures and prevent denaturation—which is often associated with inflammation and tissue damage—the egg albumin denaturation assay was developed. Therefore, substances or treatments that in this test considerably reduce the denaturation of egg albumin may have anti-inflammatory qualities. Calculate using the formula.

$$\text{Inhibition \%} = \frac{\text{Control}_{(680)} - \text{Sample}_{(680)}}{\text{Control}_{(680)}} \times 100$$

Material required:

- Egg albumin solution

(Preparation of 1% egg albumin solution: To properly make egg-albumin solution with a fresh hen's egg, fracture the egg gently, then add 1 mL of the translucent section to 100 mL of W/V distilled water and stir well. Egg albumin is the name of the transparent part of the egg. When creating the solution, the water needs to be cold.)

- Phosphate buffered saline
- DMSO

Procedure

10ml of DMSO reagent was added to 1 mg of each formulation (F1, F2, and F3). Take 1ml of the prepared solutions, then add distilled water to make 10ml. From the above solution 2ml of sample or standard (Ibuprufen), 2.5ml of phosphate buffered saline, 0.5ml of egg albumin, and incubate for 5 minutes at 37ºC. After that, the reaction mixtures were heated in a water bath set at 70ºC for 15 minutes. UV-visible spectroscopy was used to quantify absorbance at 280nm after cooling. Other reagents were added without the sample or standard to create the control. Compare the sample with standard and observe the result.[40]

- ANTIOXIDANT ACTIVITY

DPPH SCAVENGING ASSAY

Principle

The basic principle behind the DPPH method was the presence of antioxidant chemicals that provided DPPH with H+, converting purple DPPH free radicals into non-radical molecules that were either pale yellow in colour or completely lost. Antioxidants are substances that possess the ability to neutralise or eliminate free radicals, hence preventing oxidative damage to bodily cells. Antioxidants themselves have the purpose of minimizing the occurrence of free radical-induced skin damage, loss of sensory quality, and skin nutrition.

$$\text{Inhibition \%} = \frac{\text{Ab (control)} - \text{Ab (sample)}}{\text{Ab (control)}} \times 100$$

Materials Used

- Methanol
- DPPH

Procedure

weigh 1mg of powder towards it add 3ml of DPPH solution and make up to 10ml with methanol. Prepare the three formula F1, F2, F3 as per mentioned above. Prepare the standard by adding 3ml DPPH in ascorbic acid make up to 10ml using methanol. Observe the colour change from purple to pale yellow. Compare the sample with standard. After that, take absorbance at 517nm in UV visible spectroscopy.[41]

- ANTIMICROBIAL STUDY

Principle

The extract contain compound which shows antimicrobial activity that inhibits the growth of bacteria like Staphylococcus and Escherichia coli, hence accelerate the healing process of wounds.[42]

Procedure

The MHA plate was evenly covered with the inoculum suspension using a sterile swab, and the inoculum were then given 5 minutes to dry. After loading 70 microliters of extract into a 6mm well, the mixture was given 5 minutes to diffuse. A laminar airflow chamber with aseptic condition was used for the entire process. The plates were incubated for 24hrs at

37 ºC for the growth of microorganism. The inhibition zone developed around the disc at the conclusion of the incubation were measured in millimetres.[43]

- STERILITY TESTING

The test for sterility is done by detecting the presence of viable form of bacteria, fungi and yeast in or on pharmacopeial preparations. The test must be carried out under strict aseptic condition in order to avoid accidental contamination of the product during the test. All glass apparatus required for the test must be sterile.

Procedure

The method involves the filtration of the sample under test through a membrane filter having normal porosity of 0.45 µm, and a diameter of approximately 47 mm. After the filtration, the membrane is removed aseptically from the metallic holder and divided into two halves. The first half is transferred into 100 ml of culture media meant for fungi and incubated at 20° to 25°C for not less than seven days. The another half is transferred into 100 ml of fluid thioglycollate medium and incubated at 30° to 35°C for not less than 7 days. Observe the growth in the media.[44]

8. RESULTS

ORGANOLEPTIC CHARACTERS

Table 3: Organoleptic characters.

SL NO.	CHARACTERISTICS	OBSERVATION
1.	Colour	Cream colour
2.	Odour	odourless
3.	Particle size	0.25µm
4.	Texture	Smooth, fine powder

PHYSICAL CHARACTERS

Solubility of Musa paradisiaca in various solvents

Table 4: Solubility.

SL NO.	SOLVENT	OBSERVATION
1.	Ethanol	Soluble
2.	Methanol	Fully soluble
3.	Water	Soluble

Flow properties

Table 5: Flow property.

BATCH	ANGLE OF	TAPPED	BULK	CARR'S

	REPOSE	DENSITY	DENSITY	INDEX
F1	25.56	0.465	0.420	9.67
F2	21.31	0.555	0.5	9.9
F3	23.75	0.571	0.518	9.2

Observation: Based on the value obtained F3 formulation shows excellent flow property.

NON-IRRITANCY TEST

The applied powder shows no irritancy on the skin.

GC-MS ANALYSIS

GC-MS chromatogram of methanolic extract was obtained as follows.

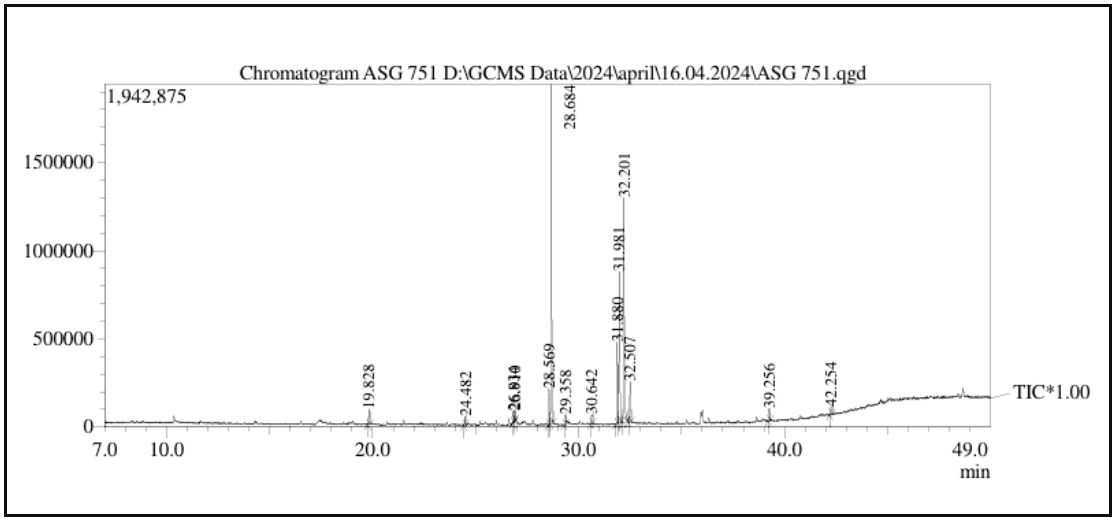

Fig 3: GC-MS chromatogram of methanolic extract of *Musa Paradisiaca* Linn.

Table 6: Peak Report TIC.

Peak	R. Time	Area %	Height %	Name	Base m/z
1	19.828	1.46	1.58	EICOSANOIC ACID, METHYL ESTER	74.05
2	24.482	0.82	0.89	HEPTADECANOIC ACID,METHYL ESTER	74.05
3	26.834	1.13	1.16	2-Propanoic acid,3-(4-hydroxy-3-methoxyphenyl)-,methyl ester	208.07
4	26.910	0.79	0.98	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	82.10
5	28.569	3.56	3.76	METHYL (7E)-7-HEXADECENOATE#	55.05
6	28.684	31.84	35.54	HEXADECANOIC ACID, METHYL ESTER	74.05
7	29.358	1.21	1.04	9-OCTADECENOIC ACID(Z)-	57.05
8	30.642	0.89	0.98	Hexadeconic acid, 15- methyl-,methyl ester	74.05
9	31.880	8.02	8.56	9,12-Octadecadienoic acid, methyl ester	67.05

10	31.981	17.60	15.76	9,12,15-OCTADECATRIENOIC ACID, METHYL ESTER,(Z,Z,Z)-	79.05
11	32.201	26.48	23.47	Phytol	71.05
12	32.507	4.08	4.24	Methyl stearate	74.00
13	39.256	1.26	1.26	DOCOSANOIC ACID, METHYL ESTER	74.00
14	42.254	0.86	0.78	TETRACOSANOIC ACID, METHYL ESTER	74.00
		100	100.07		

BIOLOGICAL ACTIVITY

Invitro assay associated with the wound healing are performed with F3 formulation such as the anti-inflammatory, antioxidant and antimicrobial activity.

1. Antidiabetic Activity – Alpha Amylase Inhibitory Assay.

Table 7: percentage alpha-amylase inhibition.

BATCH	ABSORBANCE	INHIBITION %
Control	0.87	
F3	0.494	43.2
STANDARD	0.38	53.32

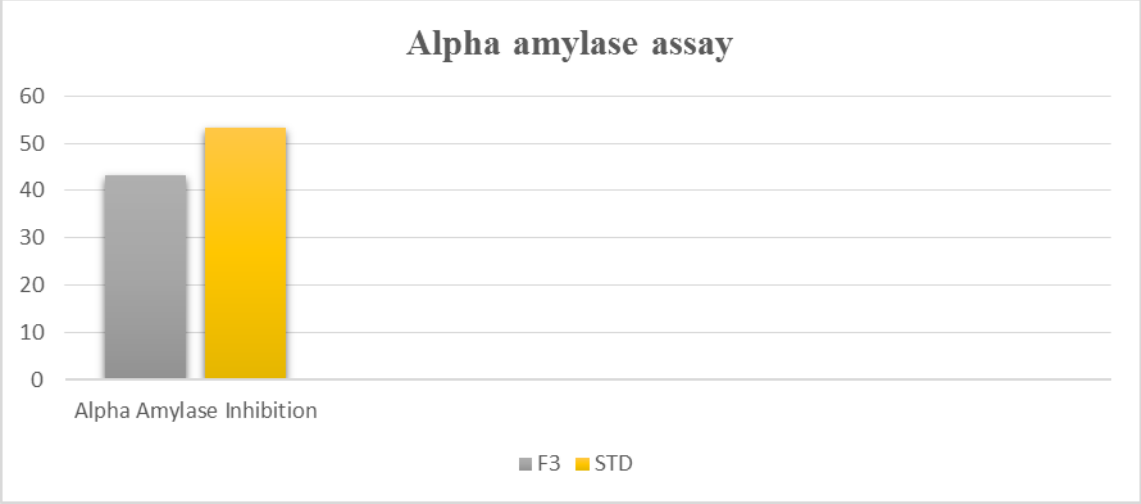

Figure 4: Comparison of percentage alpha amylase inhibition of sample and standard.

2. Anti-inflammatory Activity – Egg Albumin Denaturation Assay

Table 8: Percentage of egg albumin denaturation.

BATCH	ABSORBANCE	INHIBITION %
Control	0.78	
F3	0.154	80.25
Standard	0.112	85.64

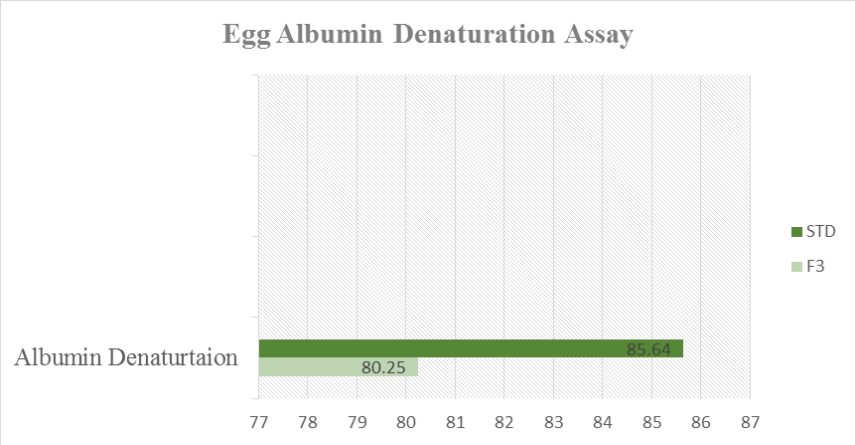

Figure 5: Comparison percentage of egg albumin denaturation of sample and standard.

3. Antioxidant activity-DPPH Scavenging Assay

Table 9: Percentage inhibition of DPPH.

BATCH	ABSORBANCE	INHIBITION %
Control	0.953	
F3	0.054	94.33
Standard	0.008	99.16

Figure 6: Antioxidant Activity.

OBSERVATION

In various formulas, the colour shift from purple to pale yellow were observed. While the F1, F2, F3, and STD formulations have antioxidant properties, the control sample is in purple colour and exhibits no antioxidant activity. The F3 formulation has a higher inhibitory percentage than the others. Consequently, it possesses more antioxidant properties.

4. Antimicrobial study

Observation

Using selected formula, the powder's antimicrobial properties against Staphylococcus aureus were studied. The figure shows the zone of inhibition. The results obtained indicate that *Musa paradisiaca* possesses antimicrobial properties. The presence of bioactive substances with antimicrobial qualities, such as phenolic compounds, flavonoids, alkaloids, and terpenoids, may be responsible for these effects.

Figure 7: Antimicrobial Activity.

9. CONCLUSION

Musa paradisiaca is a popular Indian medicinal plant, various parts of this plant are commonly used in traditional medicine. But *Musa paradisiaca* is still largely unexplored source for the development of new drug. The present study has been undertaken to explore the diabetic foot ulcer activity of *Musa paradisiaca* leaves by formulating powder dosage form. Diabetic foot ulcer are the most common complication in type 2 DM patient. It is usually the result of poor glycaemic control, underlying neuropathy, peripheral vascular disease or poor foot care. Effective management of chronic wounds presents major clinical burdens to the patients, healthcare providers and the healthcare system around the globe.

Phytochemical screening initiated with methanolic extraction by soxhlation and phytochemical investigation through GC-MS analysis. Where 14 constituents identified. Among the 14 constituents Tetracosanoic acid, methyl ester identified as anti-diabetic activity. Other constituents mainly have anti-oxidant and anti-inflammatory properties, they are potentially active. The inflammatory phase is the first and essential stage in the wound healing process. Antioxidants reduce oxidative stress in wound and accelerate wound healing. In-vivo screening for anti-diabetic and anti-oxidant activity of methanolic extract provided satisfactory result in comparison with standard. Anti-diabetic activity of methanolic extract

by alpha amylase inhibition method exhibited significant activity in compared with the standard acarbose. The DPPH scavenging assay was used to measure the antioxidant activity, the results show a colour change from purple to pale yellow, indicating that the formula has antioxidant activity. By using egg albumin assay, anti-inflammatory activity of the formula exhibited significant comparison with standard ibuprofen drug. A visible zone of inhibition was observed when staphylococcus microorganisms are used in an in vitro study of wound healing activity.

Based on the background of all studies conducted, the powder formulation has proven to be effective for diabetic foot ulcers (DFU). Besides its efficacy, this formulation is also easily applicable and economically beneficial for patients.

10. DISCUSSION

The most frequent uncontrolled consequence in patients with type 2 diabetes mellitus is diabetic foot ulcers. It is typically caused by inadequate foot care, peripheral vascular disease, underlying neuropathy, or poor glycaemic control. The most commonly occurring Infectious organism is Staphylococcus. For the early treatment of the foot lesions, local debridement, proper footwear, and foot care are essential. There is a higher chance of wound advancement following the development of a DFU, which could ultimately result in amputation. For modifying the wound environment, wound dressings can assist by cutting-edge products which will maximise the healing outcomes. Biosynthetic and tissue engineering have made it possible to build skin substitutes that offer innovative, efficient, and temporary wound covering. Advanced treatments consist of laser therapy, ultrasound therapy and hyperbaric oxygen therapy.

Traditionally, *Musa paradisiaca* has been used to treat dyspepsia, diabetes mellitus, rectal cancer and diarrhoea. It has a strong chance of reducing blood pressure, hyperlipidemia, stroke risk, and re-establishing regular bowel movements. Additionally, it may help prevent Alzheimer's disease, preserve renal function, boost immunity, facilitate weight loss, diminish bleeding associated with menorrhagia, and reduce the risk of acquiring specific cancers.

By creating a powder dosage form, the current study aims to investigate the diabetes and wound-healing properties of *Musa paradisiaca* leaves. For the comprehensive analysis, GC-MS analysis was carried out and found that the ethanolic extract of *Musa paradisiaca* was shown to contain tetracosanoic acid, which has anti-diabetic properties.

Natural therapies are gaining popularity since they frequently have fewer side effects and a higher patient tolerance rate.

The study witnesses to the anti-diabetic and wound-healing properties of the powder formulation that include *Musa paradisiaca* extract.

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