

PREPARATION AND EVALUATION OF HERBAL BASED NANO GEL FOR WOUND HEALING

Submitted in partial fulfilment of the requirements for the award of
Master of Technology degree in Biotechnology

By

J. HARIPRIYA

(42810003)



**DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF BIO AND CHEMICAL ENGINEERING**

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

CATEGORY -1 UNIVERSITY BY UGC

Accredited with Grade "A++" by NAAC | 12B Status by UGC | Approved by AICTE

JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI - 600 119

APRIL-2024



SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited "A++" Grade by NAAC | 12B Status by UGC | Approved by AICTE

www.sathyabama.ac.in

DEPARTMENT OF BIOTECHNOLOGY

BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **HARIPRIYA.J (42810003)** who carried out Project Report entitled "**PREPARATION AND EVALUATION OF HERBAL BASED NANO GEL FOR WOUND HEALING**" under our supervision from November 2023 to April 2024.

Internal Guide

External Guide

Dr. V. RAMESH KUMAR M. Phil., Ph.D.

(Associate Professor)

Dr.T. RAJASEKAR Ph.D.

(Assistant professor- Research)

Head of the Department

Dr. V. RAMESH KUMAR M. Phil., Ph.D.

Submitted for Viva-voce Examination held on 20.04.2024

Internal Examiner

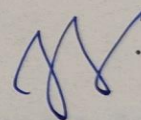
External Examiner

DECLARATION

I, **J. HARIPRIYA (42810003)**, hereby declare that the project Report entitled entitled "**PREPARATION AND EVALUATION OF HERBAL BASED NANO GEL FOR WOUND HEALING** " done by me under the guidance of Internal guide **Dr. V. RAMESH KUMAR M. Phil., Ph.D.**, Head of the Department, Department of Biotechnology, Sathyabama Institute of Science and Technology, Chennai, and External guide **Dr. T. RAJASEKAR Ph.D. (Assistant Professor- Research)** Centre for Drug Discovery and Development ,Sathyabama Institute of Science and Technology, Jeppiaar Nagar, Rajiv Gandhi Road, Chennai-600119 is submitted in partial fulfilment of the requirements for the award of Master of Technology degree in Biotechnology.

Date: 20.04.2024

Place: Chennai



Signature of the candidate

ACKNOWLEDGEMENT

I am pleased to acknowledge my sincere thanks to Board of Management of SATHYABAMA for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey my thanks to **Dr. V. RAMESH KUMAR M. Phil., Ph. D**, Head of the Department, Dept. of Biotechnology for providing me necessary support and details at the right time during the progressive reviews.

I would like to express my sincere and deep sense of gratitude to my Project Internal Guide **Dr. V. RAMESH KUMAR M. Phil., Ph. D** and External Guide **Dr. T. RAJASEKAR Ph.D.** (Assistant Professor- Research) Centre for Drug Discovery and Development, Sathyabama Research Park, Sathyabama Institute of Science and Technology Jeppiaar Nagar, Rajiv Gandhi Road, Chennai-600119 for valuable guidance, suggestions, and constant encouragement paved way for successful completion of my project work.

I sincere thanks to **Ms. Vaishnavi; Ms. Blessy cleatus** and **Mrs. Ananthi** from Centre for Drug Discovery and Development, Sathyabama Research Park, Sathyabama Institute of Science and Technology, Jeppiaar Nagar, Rajiv Gandhi Road, Chennai-600119. I wish to express my thanks to all Teaching and Non-teaching staff members of the Department of Biotechnology who were helpful in many ways for the successful completion of the project.

ABSTRACT

At present, diabetes affects over 425 million people worldwide. Foot ulcer is a wound that occurs approximately 15% patient with diabetes and commonly located on the bottom of the foot. Wound healing remains a critical medical challenge, necessitating the development of innovative therapies. In this project, my aim is to develop an herbal based healing Nano gel for this foot ulcer. *Ocimum Sanctum* and *Carica Papaya* responsible for anti-bacterial and anti-oxidant activity. Synthesis of silver nanoparticles was carried out using the both extracts. Reduction of Ag^+ ions in solution were monitored using UV–visible absorption spectroscopy, and the corresponding surface plasmon resonance of AgNPs at 460 nm. The solution changed color from green to reddish-brown upon bio reduction of silver nitrate in *Ocimum Sanctum* and *Carica Papaya* extract. The Nano gel product was characterized by UV-vis spectroscopy, X-ray diffraction, and transmission Electron Microscopy, Invitro scratch assay techniques. This study aimed to formulate and evaluate an herbal-based Nano gel for its efficacy in promoting wound healing. The results showed that the plant has strong antibacterial action against *Escherichia coli*, *Staphylococcus aureus* 13, *Staphylococcus aureus* 23. Finally, *Ocimum Sanctum* (Tulsi) and *Carica Papaya* (Papaya) showed that extract can be used to efficiently, quickly, and simply create silver nanoparticles with strong antibacterial qualities. the XRD pattern that the crystalline quality of the silver nanoparticles produced by Combination 1:4 extract and TEM micrographs shows clear particle size of AgNPs is 10 nm. At the maximum treatment concentration of Combination 1:4 extract, the percentage vitality of (3T3-L1) cells was observed to be 98.70% and the minimum treatment concentration was observed to be 56.40% in the invitro scratch assay. The Nano gel was prepared using a combination of potent medicinal herbs known for their wound healing properties.

Keywords: Anti-bacterial; Anti-oxidant and Wound healing

LIST OF CONTENTS

S.NO	CONTENTS	PG.NO
	LIST OF FIGURES	iv
	LIST OF TABLES	vi
	LIST OF ABBREVIATIONS	vii
1	INTRODUCTION	1
	1.1 DIABESTES MELLITUS	1
	1.2 DIABETIC FOOT ULCER	2
	1.3 USE OF MEDICINAL HERBS IN TRADITIONAL MEDICINE FOR DIABETIC FOOT ULCER	3
	1.3.1 <i>Ocimum Sanctum</i> (TULSI)	4
	1.3.2 <i>Carica Papaya</i> (PAPAYA)	5
	1.4 SILVER NANOPARTICLE (AgNPs)	6
	1.4.1 BENEFITS	7
	1.4.2 ADVANTAGES	7
	1.4.3 APPLICATION	8
	1.5 GEL FORMATION	8
2	REVIEW OF LITERATURE	9
	2.1 DIABETIC FOOT ULCER	9
	2.1.1 RISK FACTORS OF DFU	9
	2.1.2 SIGN & SYMPTOMS	10
	2.1.3 CAUSES	10
	2.2 FACTORS AFFECTING WOUND HEALING	10
	2.3 <i>OCIMUM SANCTUM</i>	12
	2.3.1 <i>OCIMUM SANCTUM</i> EXTRACT	13
	2.4 <i>CARICA PAPAYA</i>	14

2.4.1	CARICA PAPAYA EXTRACT	15
2.5	SILVER NANOPARTICLE (AgNPs)	16
2.5.1	OCIMUM SANCTUM EXTRACT ALONG WITH NANOPARTICLE (NPs)	17
2.5.2	CARICA PAPAYA EXTRACT ALONG WITH NANOPARTICLE (NPs)	18
3	AIM & OBJECTIVE	20
4	METHODOLOGY	21
4.1	COLLECTION OF PLANT AND FRUIT MATERIAL	21
4.2	PREPARATION OF PLANT AND FRUIT EXTRACTS	21
4.2.1	EXTRACTION PROCESS	21
4.2.2	DILUTION OF EXTRACTION	22
4.3	SYNTHESIS OF SILVER NANOPARTICLES WITH PLANT AND FRUIT EXTRACTS	22
4.4	QUALITATIVE ANALYSIS	23
4.5	ANTIBACTERIAL ACTIVITY	23
4.6	GEL FORMATION	24
4.6.1	GUARGUM	24
4.6.2	PREPARATION OF GUARGUM	24
4.7	INVITRO SCRATCH ASSAY	25
4.8	CHARACTERIZATION	25
4.8.1	UV-VIS SPECTROSCOPY	25
4.8.2	XRD ANALYSIS	26
4.8.3	TEM ANALYSIS	26
4.8.4	FTIR ANALYSIS	26
5	RESULTS & DISCUSSION	27
5.1	COLLECTION OF PLANT AND FRUIT MATERIAL	27
5.2	PREPARATION OF PLANT AND FRUIT EXTRACTS	27

	5.2.1 EXTRACTION PROCESS	28
	5.3 SYNTHESIS OF SILVER NANOPARTICLES WITH PLANT AND FRUIT EXTRACTS.	28
	5.4 QUALITATIVE ANALYSIS	29
	5.5 ANTIBACTERIAL ACTIVITY	30
	5.6 GEL FORMATION	37
	5.7 CHARACTERIZATION	37
	5.7.1 UV-VIS SPECTROSCOPY	37
	5.7.2 XRD ANALYSIS	40
	5.7.3 FTIR ANALYSIS	41
	5.7.4 TEM ANALYSIS	42
	5.7.5 INVITRO SCRATCH ASSAY	42
	DISCUSSION	46
6	SUMMARY & CONCLUSION	48
7	REFERENCE	50
	ANNEXURE-I	57
	ANNEXURE-II	61
	ANNEXURE-III	63

LIST OF FIGURES

S.NO	FIGURES	PG.NO
1.1	Diabetes mellitus complications	2
1.2	Diabetic foot ulcer	3
5:1	Fresh leaves and fruit of <i>ocimum sanctum</i> (Tulsi) and <i>carica papaya</i> (Papaya)	27
5:2	Dried powder of <i>ocimum sanctum</i> (Tulsi) and <i>carica papaya</i> (Papaya)	27
5:3	Crude extract of <i>ocimum sanctum</i> (Tulsi) and <i>carica papaya</i> (Papaya)	28
5:4	Synthesis of Silver nanoparticle	28
5.5	Phytochemical screening of <i>ocimum sanctum</i>	29
5.6	Phytochemical screening of <i>carica papaya</i>	29
5.7	Antimicrobial activity assay of silver nanoparticles along with individual extract and combination extract (A (Tulsi)-1:4; 2:3;2.5:2,3:2,5:1; B (Papaya)-2:3 & C(Combination)-1:4) for 10:10 dilution	31
5.8	Antimicrobial activity assay of silver nanoparticles along with individual extract and combination extract (A (Tulsi)-1:4; 2:3;2.5:2,3:2; B (Papaya)-2.5:2,3:2 & C(Combination)-1:4; 2:3;2.5:2) for 1:9 dilution	32
5.9	Antimicrobial activity assay of silver nanoparticles along with individual extract and combination extract (A (Tulsi)-1:4; & C(Combination)-1:4) for 1:9 dilution	36
5.10	Nano gel formation	37
5.11	UV-VIS spectra of silver nanoparticles along with individual extract & combination extract for the dilution of 10:10	38
5.12	UV-VIS spectra of silver nanoparticles along with individual extract & combination extract for the dilution of 1:9	39

5.13	XRD Analysis	40
5.14	FTIR Analysis	41
5.15	TEM Analysis	42
5.16	Percentage of cell line viability of combination 1:4 extract	45
5.17	Images of invitro scratch assay	45

LIST OF TABLES

S.no	Tables	Pg.no
5.1	Phytochemical screening of extracts	30
5.2	10:10 Dilution of zone of inhibition	32
5.3	1:9 Dilution of Zone of inhibition	34
5.4	1:4 ratio of combination of zone of inhibition	36
5.5	XRD Analysis	40
5.6	Percentage of cell line viability for combination 1:4 extract	44

LIST OF ABBREVIATIONS

DFU	Diabetic foot ulcers
FTIR	Fourier-Transform infrared spectroscopy
ROS	Reactive oxygen species
TEM	Transmission electron Microscopy
XRD	X-RAY Diffraction unit
NPs	Nanoparticles
AgNPs	Silver Nanoparticles

CHAPTER-1

INTRODUCTION

1.1 DIABETES MELLITUS

Diabetes mellitus is a condition that impairs the body's capacity to produce or utilize insulin. The hormone insulin, which is generated by the pancreas, aids in the movement of blood sugar, or glucose, from the circulation into the cells where it may be broken down and utilized as energy. Insulin is essential to human survival. A person with diabetes has abnormal blood glucose levels. Serious short- and long-term effects, such as heart disease, amputations, and brain damage (American Diabetes Association, 2007).

Globally, diabetes mellitus affects over 422 million people and is thought to be the cause of 2 million deaths annually (GBD 2019 Diabetes in the Americas Collaborators, 2022). Insulin secretion may be reduced, insulin activity may be impaired, or both may be the cause of the hyperglycaemic condition that characterizes diabetes, a metabolic syndrome. Globally, diabetes affected at least 171 million people in 2000, according to the World Health Organization, and because the disease is becoming more and more common, it is predicted that by 2030, the number will have doubled. The current global diabetes mellitus rate is 382 million, which accounts for 8.9% of the total population (International Diabetes Federation, 2013).

In poor countries as opposed to industrialized ones, diabetes prevalence is higher. About 61.2 million Indians suffer from diabetes, and by 2030, that number is predicted to rise to 101.2 million (Whiting *et al.*, 2011). Diabetes patients' dysregulated metabolism is associated with secondary pathophysiological effects on multiple organ systems, which places a significant strain on the healthcare system. Diabetic problems are linked to numerous complex issues (International Diabetes Federation, 2012). These include retinopathy, neuropathy, atherosclerosis, nephropathy and foot ulcers (Grunfeld, 1991).

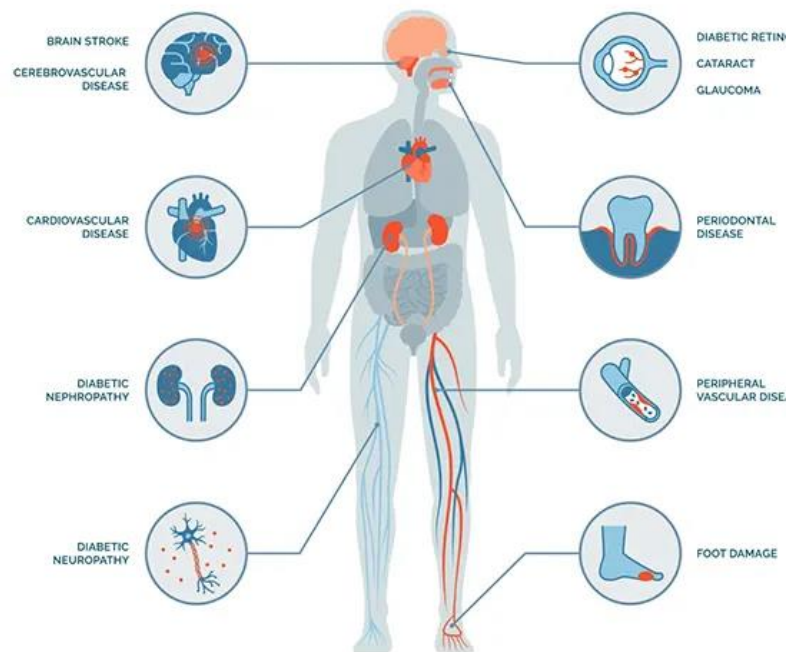


Fig 1.1: Diabetes mellitus complications

1.2 DIABETIC FOOT ULCER

One of the most frequent side effects of uncontrolled diabetes mellitus is diabetic foot ulcers (DFU). It frequently results from peripheral vascular disease, inadequate foot care, underlying neuropathy, or poor glycaemic management. It is also a frequent cause of lower limb amputations and foot osteomyelitis. These ulcers typically occur in foot regions that experience pressure and repetitive damage (Singer *et al.*, 2018). 20% to 40% of health care resources are used to treat diabetic foot infections, indicating that complications from foot ulcers are the primary cause of hospitalization and amputation in individuals with diabetes. They also result in large health care expenses. What the World Health Organization refers to as "ulceration of the foot associated with the neuropathy and different grades of ischemia and infection" is diabetic foot infection (Tuttolomondo *et al.*, 2015). Foot conditions include ulcers, infections, and illnesses that result in hospitalization for patients with diabetes mellitus.

At some point throughout their diabetic treatment, 15 to 20 percent of the 16 million Americans who are projected to have diabetes mellitus will require hospitalization due to foot syndrome. Unfortunately, a significant number of these individuals may need to have their feet and ankles amputated due to peripheral ischemia or serious infections. One of the risk factors for ulcers and amputations is neuropathy. Millions of dollars are spent on direct medical costs due to diabetic foot problems, which also result in extended hospital stays and periods of disability. The ulceration, damage, and deformity of the diabetic foot are the most typical lesions (Frykberg, 2012).

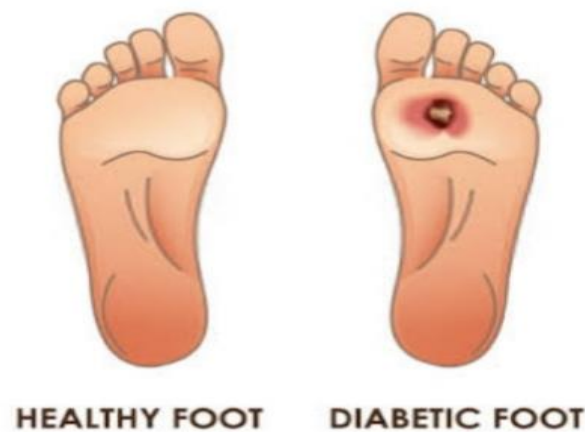


Fig 1.2: Diabetic foot ulcer

1.3 USE OF MEDICINAL HERBS IN TRADITIONAL MEDICINE FOR DIABETIC FOOT ULCERS

Traditionally, medical plants have been used to treat inflammation, burns, ulcers, and surgical wounds for over 5,000 years. This includes the Egyptians and indigenous peoples of Africa, Asia, the Americas, and Rome. Numerous naturally occurring bioactive substances found in them aid in accelerating the healing process of wounds and promote tissue regeneration at the wound site. By supplying various essential elements (vitamins, proteins, and minerals) needed at different phases of wound regeneration and proliferation, the herbs also speed up tissue repair.

It has also been discovered that herbal remedies are more affordable and safer than allopathic ones. The potential use of herbs and their mechanisms provide alternative treatment for DFUs (Radhakrishna *et al.*,2014).

1.3.1 *Ocimum Sanctum (Tulsi)*

Ocimum tenuiflorum, commonly known as *Ocimum sanctum*, Tulsi, or Holy Basil, has been referred to as the "Queen of plants" and the "mother medicine of nature." Tulsi aqueous extracts are used to cure a variety of illnesses, including as heart disease, inflammation, stomachaches, headaches, malaria, and common colds. It has been suggested that Tulsi oil, which is extracted from the leaves and inflorescence, has many beneficial qualities. These include anti-inflammatory and stress-relieving effects; anti-asthmatic, hypoglycemic, hepatoprotective, hypotensive, hypolipidemic, and immunomodulatory effects (Pattanayak *et al.*,2010).

I. Antimicrobial activity

Tulsi contains antibacterial properties that work against a variety of infections. It can be used as a mouthwash, to treat wounds, and to preserve food. Mosquitoes can be killed using tulsi, which also has antibacterial, antiviral, antifungal, antiprotozoal, and antimalarial properties

In addition to having antioxidants, it also functions as an anti-inflammatory, anti-cataract, and radiation protector. It also benefits the liver, nerves, and heart, acts as an anti-cancer agent, fortifies the immune system, central nervous system, and memory, and treats thyroid and asthma. It also resolves problems related to infertility (Yamani *et al.*,2016).

ii.Benefits

- Tulsi's combination of antiviral, antifungal, antibacterial, and antifungal qualities have led to its usage in treating wounds and infections.
- Additionally, it contains anti-inflammatory qualities that aid in lowering swelling and hastening the healing of wounds.

- Tulsi helps hasten the healing of wounds and strengthen the tissue that breaks them. Tulsi has also been demonstrated to have anti-ulcer and ulcer-healing properties.

iii.Application

Tulsi is utilized in animal husbandry, wound healing, food preservation, the preservation of herbal raw materials, mouthwash, water purification, and traveler health.

1.3.2 *Carica papaya* (papaya)

Carica papaya is known by several distinct names, including tapaya, paw paw, and kepay. It has the digestive enzyme papain, which is also used to heal ulcers and certain microbial illnesses. At greater dosages, papain is particularly efficient against gram-negative bacteria. Benzyl isothiocyanate, a compound with bactericidal, bacteriostatic, and fungicidal activities, is present in papaya fruit extract. Numerous scientific investigations have been conducted to identify and evaluate the bioactive components found in papaya leaves. It may have antibacterial, antiviral, hypoglycemic, and anticancer effects since they include alkaloids, saponin, tannin and glycosides, according to the photochemical analysis (Nugroho *et al.*,2017).

I. Antimicrobial activity

Gram-positive and gram-negative organism growth is inhibited by the antibacterial activity found in *carica papaya* fruit. The behavior that was seen was unaffected by the fruit's mature state. Proteolytic enzymes of papaya, papain and chymopapain are helpful decrease in the inflammation. The antibacterial properties of *carica papaya* may be helpful in the treatment of chronic skin ulcers, aiding in their healing process. Strong therapeutic qualities including antibacterial, antiviral, anticancer, hypoglycemic, and anti-inflammatory action are present in papaya leaf extract (Singh *et al.*,2020).

ii.Benefits

- Plant-based antioxidants and beneficial enzymes abound in papaya. These ingredients have the ability to cleanse the skin's surface of impurities such as tissues, dead and damaged cells, and debris.
- Papaya has been used as a natural remedy for scars, burns, and other skin disorders. Papaya enzymes exfoliate dead skin cells and remove pollutants from the skin.
- Direct application of papaya pulp to the affected skin region can help you to treat skin conditions such as eczema and psoriasis. It can also help to alleviate itching and redness.

iii.Application

The papaya tree is a fruit tree with significant economic value and a wealth of different nutrients and antioxidants that are good for human health. Papaya is a strong source of enzymes like papain and chymopapain and contains vitamins A and C. These two enzymes are employed in the food and pharmaceutical industries.

1.4 SILVER NANOPARTICLE (AGNPS)

Since metal nanoparticles are thought to be able to bridge the gap between bulk and atomic dimensions, they are of tremendous scientific interest. High reactivity to nanoparticles is provided by their high surface area, adjustable particle size, and shape. The noble metal nanoparticles Ag, Au, Pd, and Pt have been examined the most in the literature that is currently available. Silver is one of the reported noble metal nanoparticles that has been studied in great detail and is of particular interest to the biological and medical sciences. Because of their non-toxicity to humans, silver nanoparticles are a preferred choice for their antibacterial, antiviral, anticancer, antifungal, and anti-inflammatory properties due to their chemical stability and catalytic impact. Research on silver nanoparticles has been conducted in many different areas, including metallic inks, catalysis, sensing, and medicine (Chen X et al, 2010).

The most common four oxidation states of silver are Ag⁰, Ag¹⁺, Ag²⁺, and Ag³⁺. Among these, Ag³⁺ exhibits instability in aquatic settings. The acute toxicity of silver in the environment is contingent upon the presence of free silver ions. According to research (WHO, 2002), the amounts of Ag⁺ ions are insufficient to be harmful. Many consumer goods, including food containers, cosmetics, medical equipment, textiles, and cosmetics, integrate silver nanoparticles into their composition (Risks, 2002). Ag nanoparticles can be produced by a variety of techniques, such as chemical, physical, and biological ones. The chemical synthesis approach, which uses a range of organic and inorganic reducing agents, is one of the most well-reported ways. The use of ecologically friendly green measures to prevent dangerous and harmful items from entering the environment is becoming more and more popular (Reidy *et al.*, 2013).

1.4.1 Benefits

- Localized and systemic inflammation are both suppressed by silver nanoparticles. Wound healing involves the inflammatory response in a significant way. Different inflammatory mediators are released to modify the wound-healing process.
- AgNPs has antibacterial and anti-inflammatory qualities that aid in the healing of chronic wounds, AgNPs can also be utilized as an intrinsic therapeutic strategy.

1.4.2 Advantages

Silver nanoparticles have been proven to exhibit

- excellent electrical conductivity,
- chemical stability,
- catalytic and
- antimicrobial properties, as well as
- cytotoxic effects on cancer cells.

1.43 Application

Silver nanoparticles' antibacterial properties have been utilized to limit the growth of bacteria in a range of settings, such as dentistry, surgery, the treatment of burns and wounds, and biomedical equipment. It is commonly known that bacteria are extremely poisonous to silver ions and compounds based on silver.

1.5 GEL FORMATION

- Gel is a significant advancement in treatment for diabetic neuropathic foot ulcers that have a sufficient blood supply. Studies using randomized controlled trials have demonstrated its efficacy in speeding up the healing process.
- Materials for dressing wounds serve as a barrier to keep infections out while the wound heals. These substances need to be biocompatible.

CHAPTER-2

REVIEW OF LITERATURE

2.1 DIABETIC FOOT ULCER

Evaluating the frequency of diabetic foot ulcers and risk variables among individuals with diabetes. 198 patients with diabetes participated in a quantitative cross-sectional study that took place at Tikur Anbessa Specialized Hospital between April 1 and May 1, 2017. The selection of respondents was done by ordinary random sampling. In the research area, diabetes patients had a prevalence of DFU of 20.7%. Factors that were substantially linked with DFU were occupation (AOR = 8.16; 95% CI: 1.2–9.5) and place of residence (AOR = 5.97; 95% CI: 1.47–24.3). This study's DFU prevalence among diabetes individuals was 20.7%. The occupation and residential location were linked to DFU. Patient education regarding foot hygiene could lower the frequency of DFU (Yimam *et al.*,2021).

About 15% of people with diabetes may eventually get DFU, and between 14% and 24% of them will need to have their ulcerated foot amputated because of problems connected to bone infections or other ulcer-related issues. The three pathologic mechanisms that underlie DFU are neuropathy, vascular insufficiency, and subsequent infection brought on by foot trauma. Reducing morbidity, amputations, and death from DFU is made possible by a combination of cutting-edge techniques such as stem cell treatment and conventional local and invasive care. The pathogenesis, preventative measures, and final management of DFU are the main topics of our review of the recent research in this work (Raja *et al.*,2023).

2.1.1 Risk factors of DFU

Risk factors for foot ulcers in patients with diabetes include:

- Amputation
- History of a foot ulcer
- Foot deformity
- Peripheral vascular disease

2.1.2 Sign & Symptoms

- Swelling, discoloration, and warmth on every side of the wound
- Pain and firmness
- Callused or thickened skin around the ulcer
- Fever and chills in stages of foot ulcers

2.1.3 Causes

- Poor circulation of blood
- High blood sugar level
- Nerve damage
- Irritated or wounded feet

2.2 FACTORS AFFECTING WOUND HEALING

After a physical, chemical, thermal, microbial, or immunological insult, a wound results in a disruption of the cellular, anatomical, and functional integrity of the skin. This is followed by a disruption of the structure and function of the normal tissue beneath the wound. The fundamental reaction to wound healing is a connective tissue restoration process that is comprised of four overlapping phases: hemostasis, inflammation, proliferation, and remodelling. To facilitate the repair process, various cells, growth factors, and cytokines must be coordinated.

The process of wound healing involves the interplay of numerous cellular and biochemical processes that work together to restore the structural and functional integrity of wounded tissues while also enhancing their strength.

Factors that affect wound healing are discussed below-

I. Location of wound

- wound infections frequently result in poor wound healing, the location of the wound plays a significant role in the healing process.
- A number of bacteria, including *Staphylococcus aureus* and *Pseudomonas aeruginosa*, can cause wound infections. Studies have indicated that *S. aureus* is the primary pathogen linked to diabetic foot infections.

ii. Immune state

- Patients with diabetes experience several immune system dysfunctions. Polymorphonuclear leukocyte activity has been shown to be diminished, especially in the presence of acidosis.
- Leukocyte adherence, chemotaxis, and phagocytosis may all be adversely affected in diabetic states, which may lead to a delay in wound healing.
- Diabetes patients' wounds are more prone to infection because their antioxidant systems, which are involved in bactericidal activity, may be compromised.

iii. Age

- The process of wound healing appears to be correlated with an individual's age. Age appears to be a factor in the healing of wounds.
- This may be because older individuals have less fibroblast activity and growth, while older individuals who have been damaged also have decreased collagen synthesis and wound contraction.

iv. State of disease

- In the early stages of diabetic foot infections, *S. aureus* and *beta-hemolytic streptococci* are treated as pathogens.
- According to these findings, diabetic women get bacterial infections more frequently than non-diabetic women.
- Patients with diabetes appear to be more vulnerable to wound infections. According to this analysis, diabetic patients had a greater incidence of wound infection (11%) than did patients in general.

v. Reactive oxygen species (ROS)

A high ROS concentration may cause significant tissue damage, which may then result in neoplastic transformation and impede the healing process by damaging cells, DNA, proteins, and lipids (Oluwafemi & Oguntibeju, 2019).

2.3 OCIMUM SANCTUM (TULSI)

- **Family & location**

Ocimum sanctum L., a member of the Labiatae family, is an herb that is mainly native to tropical and subtropical Asia. It is frequently referred to as "holy basil" in English and Tulsi in nearly all Indian languages. It is currently grown and naturalized in various tropical regions

- **Special name**

Tulsi, or *Ocimum sanctum* L., is well known as the "Queen of herbs" and the "Elixir of life."

- **Active Components**

Major constituents include cineol, anethol, estragol, thymol, citral, camphor, linolen, ocimene, pinene, methyl chavicol, and eugenol (Srivastava et al, 2023)

- **Morphology**

The erect, heavily branched sub-shrub *Ocimum sanctum* L., also known as Tulsi, grows to a height of 30 to 60 cm. It has hairy stems and simple, opposite, intensely scented green or purple leaves. Petioles are present on oval, up to 5 cm long leaves that are typically somewhat serrated. Purple flowers grow in tight whorls of elongated racemes.

- **Therapeutic potential Activities**

Antistress, Antiviral, Antifertility, Antihelminthic, Immunomodulatory, Anti-inflammatory, Antioxidative, Antimicrobial, Anticancerous, and Antidiabetic.

- **Wound healing activity**

By improving the production of connective tissue and antioxidant status while lowering blood glucose, free radicals, and myeloperoxidase, *ocimum sanctum* appears to aid in wound healing.

2.3.1 *Ocimum Sanctum* extract

The effects of a 50% ethanol extract of *Ocimum sanctum* (OSE) on wound healing examined in diabetic rats that have concomitant wound ulcers that have been induced with streptozotocin (45 mg/kg). They came to the conclusion that studying dead space wounds in diabetic patients and observing various histological, physiologic, and metabolic data demonstrated the ability of *Ocimum Sanctum*'s 50% ethanol to promote wound healing. Reduced blood sugar, tissue damage caused by free radicals, encouraging effects on antioxidant status, quicker collagen deposition, and other formations appeared to be the cause of the healing effects (Manish Kumar Gautam & Raj Kumar Goel, 2013).

A green synthesis of titanium dioxide (TiO₂) nanoparticles (NPs) mediated developed by *Ocimum sanctum* leaf extract, which they then incorporated into a 2% chitosan (CS) gel for the purpose of healing diabetic wounds. The TiO₂ NPs containing CS gel demonstrated the desired thixotropic properties with pseudoplastic behaviour. TiO₂ NPs with CS gel showed remarkable wound-healing efficacy in diabetic rats as evidenced by in vivo wound healing tests and histological analyses of treated wounds. *Ocimum sanctum* aqueous leaf extract was found to be important in the creation of NPs by FTIR analysis (Ahmad *et al.*, 2022).

Medicinal potential of alcoholic basil extract in the healing of diabetic rabbit wounds. The injured control group of animals received povidone iodine treatment, while the test group received basil extract. As a result, alcoholic extract of *O. sanctum* is found to be superior to povidone iodine in preventing infections suggested by regular wound swab culture and clinical examination. Augmented wound healing is seen in the *O. sanctum* extract treated wounds evidenced by tissue architecture studies showing increased regular and compact collagen production with favourable collagen I to collagen III ratio (Francis *et al.*, 2017).

Potential of aqueous and alcoholic extracts evaluated in the healing of wounds in Wistar albino rats. Five groups of six rats each were created from the divided rodents. The other four groups received treatments consisting of two different dosages of both the alcoholic and aqueous extract of *O. sanctum*, while Group 1 received typical injured control. (Shetty *et al.*, 2008).

The effects of *Ocimum sanctum* leaf hydro-alcoholic extract evaluated on wound healing, oxidative stress, and renal epithelial cell damage. Calcium oxalate monohydrate crystals were used to harm renal epithelial cells (Vero cells), and the injured cells were subsequently treated with *Ocimum Sanctum* extract. They conclude that *Ocimum sanctum* leaf hydro-alcoholic extract has substantial cytoprotective, antioxidant, and wound-healing properties and may be useful in kidney stone prevention and therapy (Faujdar & Priyadarshini, 2021).

2.4 *CARICA PAPAYA* (PAPAYA)

- **Family & location**

The *Carica papaya*, also referred to as pawpaw, is an herbaceous succulent plant in the Caricaceae family. Although it originated in the American tropics, it is currently widely grown in various tropical regions of the world for its year-round edible melon-like fruit.

- **Special name**

Papye, pawpaw, lapaya, tapayas, and kapaya

- **Morphology**

With a single, unbranched trunk, it typically grows for five to ten years. Up to 75 cm in diameter, the palmately lobed leaves are supported by long, hollow petioles.

The blades include five to nine primary segments, and the yellowish ribs and veins are quite noticeable. On inflorescences that emerge in the leaf axils, flowers are born. The fruit is typically round or long, shaped like a melon, and can have up to 1000 seeds. When ripe, the smooth, green skin turns yellow or orange.

- **Active Components**

The compounds that may be responsible for therapeutic activity include flavonoids, alkaloids, glycosides, tannins, saponins, and glycosides (Hamed *et al*, 2022)

- **Therapeutic potential Activities**

Antibacterial, Antiviral, Antitumor, Antidiabetic, Anti-inflammatory and management of neurodegeneration

- **Wound healing activity**

Papain, a proteolytic enzyme derived from the fruit of *Carica papaya*, is a potent digestant of nonviable protein matter. At the same time, it is harmless to viable tissue. It is effective in removing dead cells, preventing burn wound infection, defibrinating wounds.

2.4.1 *Carica papaya* extract

They included were all 94 patients with diabetic foot ulcers. First, the patient's hyperglycemia was managed with surgical debridement, antibiotics, and wound care with grated papaya dressings. Every 24 hours, the dressings in this trial were replaced. Four patients (4.0%) required amputations, while six patients (7%) required additional surgical debridement. The healing process took 17 to 28 days.

The average recovery time was 21.56 days. Grated papaya dressings applied topically offer effective enzymatic wound debridement. It is less expensive, takes less time to heal, requires fewer surgical debridements, and lowers the risk of amputation (Rajaram *et al.*,2015).

Papaya dressings can be used to treat persistent ulcers in terms of cost, healing time, and need for additional surgery. There was no need for additional surgery for 120 (88.89%) patients following the initial surgical procedure and dressing. For additional debridement, surgery was performed on fifteen patients (11.11%). They concluded that Topical papaya dressing shortens healing times and reduces the need for surgery, giving patients with chronic ulcers a good and cost-effective outcome (Rabari Yash *et al.*,2016).

Effectiveness of papaya and hydrogel dressings for diabetic foot ulcers. There were 114 patients in the general surgery department of the Mayo Hospital in Lahore. Patients were randomly assigned to one of two groups: group P got papaya dressings; group H received hydrogel dressing. In contrast, Group H consisted of 28 (49.1%) female patients and 29 (50.9%) male patients.

We compared the effectiveness of these two treatment plans using the Wound Assessment Tool (PWAT). According to the final “PWAT” score, hydrogel dressing is superior to papaya dressing in terms of effectiveness for healing diabetic wounds. In treating diabetic wounds, hydrogel dressing is found to be more beneficial than papaya dressing (Rizwan *et al.*, 2023).

Enzymatic debridement using papaya and wet to dry standard saline dressing, which is what they use on a daily basis in their facility. The mean reduction in saline dressing was 13.156, with a standard deviation of 3.4558. Concluded that enzymatic debridement with papaya dressing plays a key role in the management of chronic non-healing ulcers due to its favourable and considerable ulcer healing rate, improvement, and reduction in clinical severity (Vasuki *et al.*, 2017)

Biodegradable films with gelatin and *Carica papaya* extract to aid in rat wound healing. The methanol aqueous technique (20% methanol: 80% water) was used to gather and extract *Carica Papaya* leaves. There were notable variations ($p < 0.05$) in the extract release values. In conclusion, it is advised to employ the films with high *Carica papaya* extract concentrations for wound healing applications because they produced encouraging in vitro findings (Elgadir & Aishah, 2013).

2.5 SILVER NANOPARTICLES (AGNPS)

Assessing the capacity of microfabricated silver nanoparticles (AgNPs) to cure wounds was the primary objective of our investigation. Our attention has been directed towards creating silver nanogel for the purpose of treating wounds in albino Wistar rats. In the incision, excision, and burn wound-healing model, the prepared silver nanogel showed outstanding wound-healing activity.

A concentration of 0.5 mg g^{-1} of silver nanogel demonstrated superior wound-healing in the incision wound-healing model; in contrast, concentrations of 0.1 and 1 mg g^{-1} of silver nanogel demonstrated enhanced wound-healing in the excision and burn wound-healing models, respectively (Gaikwad *et al.*, 2022).

These mechanisms are thoroughly investigated to create new treatments that maximize recovery while minimizing scarring. The healing processes are closely regulated by a number of growth hormones and cytokines released at the site of lesions. Topical therapies have been the standard approach to wound management. Because metal nanoparticles, such as those of silver, gold, and zinc, have positive effects on healing and can be used to cure and prevent secondary bacterial infections, their use in dermatology is on the rise. The present analysis begins with a quick overview of the typical wound healing approach before concentrating on the possible benefits of therapeutic wound dressing techniques enhanced by the use of silver nanoparticles (Singh *et al.*,2022).

2.5.1 *Ocimum Sanctum* extract along with Nanoparticles (NPs)

AgNPs ranging in size from 3 to 25 nm were produced by combining aqueous leaf extracts from *Ocimum basilicum* and *Ocimum sanctum*. This suggests a higher reaction rate and lower conversion energy required for the typical synthesis. When tested against the *Bacillus stearothermophilus* α -glucosidase enzyme model, the AgNPs derived from *O. sanctum* and *O. basilicum* showed an inhibitory effect at $89.31 \pm 5.32\%$ and $79.74 \pm 9.51\%$, respectively. This suggests that their respective crude extracts and the control had a lower biocatalytic potential (Ramdas *et al.*,2017).

Tulsi leaf extract (*Ocimum sanctum*) was used in the production of stable silver nanoparticles. Within eight minutes of the reaction time, it was found that *O. sanctum* leaf extract could convert silver ions into silver nanoparticles. Based on this investigation, it is possible to use *O. sanctum* leaf extract effectively to bio reduce AgNO₃ into silver nanoparticles with an average diameter of 14.31 ± 2.5 nm.

The proteins that may have served as a capping agent kept the produced silver nanoparticles stable, but more investigation is required to identify the potential biomolecule that drives the bio reduction process. Crystalline in nature, these nanoparticles have antibacterial properties (Singhal *et al.*,2011)

The effects of antibacterial activity done with aqueous extract of *Ocimum sanctum* (*O. sanctum*)-based silver nanoparticles (AgNPs). The smooth-surfaced, nearly spherical to oval or rod-shaped nanoparticles have a mean particle size of around 55 nm and a zeta potential of -2.7 mV. AgNPs from *O. sanctum* was found to be effective in suppressing the growth of *Acinetobacter baumannii* (*A. baumannii*), with a zone of inhibition of 15 mm, when tested for antibacterial activity against clinically isolated *A. baumannii* (Gautam *et al.*, 2023).

2.5.2 *Carica papaya* extract along with Nanoparticles (NPs)

Zinc oxide nanoparticles (ZnO NPs) synthesized using *Carica papaya* leaf extract. With different ZnO NP concentrations, the ZnO NPs were evaluated using the 2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. The results indicated scavenging activity with half maximum inhibitory concentrations (IC₅₀) = 130.1 and 104.9 µg/mL, respectively. The application of ZnO NPs resulted in the greatest root and plant growth, as well as seed germination, as compared to the control. Treatment with ZnO NPs boosted the activities of catalase and superoxide dismutase. This implies that during seed germination, ZnO NPs may dramatically change antioxidant metabolism (Dulta *et al.*, 2021).

Silver nanoparticles (AgNPs) synthesized by utilizing di-methyl flubendazole, a physiologically active compound that was extracted from the extract of *Carica papaya* leaves and verified by GC-MS, ¹H NMR, and ¹³C NMR analysis. Against both Gram-positive and Gram-negative bacteria, AgNPs demonstrated strong antibacterial activity. Biosynthesized AgNPs treatment of HepG2 cells resulted in a G₀/G₁ phase (52–53.37%) blockage. Finally, it shows that the utilization of di-methyl flubendazole-based silver nanoparticles demonstrated a new and economical natural antibacterial and anticancer agent (Devanesan *et al.*, 2021).

ZnO nanoparticles (NPs) Synthesized from fresh leaf and fresh stem bark aqueous extracts of *Carica papaya* were studied for their antibacterial activity against *Escherichia coli*, *Acinetobacter baumannii*, *Exiguobacterium aquaticum* and *Klebsiella pneumonia*. Compared to fresh leaf extracts, the average ZnO NPs from *Carica papaya* fresh stem bark extract with greater concentrations (100-1000 ppm) showed a stronger antibacterial action. ZnO NPs from fresh leaf extracts at lower concentrations (25–50 ppm), on the other hand, produced more appropriate results than fresh stem bark. ZnO NPs generally shown a strong antibacterial effect on the chosen microorganisms. This demonstrates that ZnO NP that has been produced can replace chemical processes (Kwaben *et al.*,2019).

Silver nanoparticles (AgNPs) from fresh leaf and fresh stem bark aqueous extracts of *Carica papaya* as the result of outcomes were obtained from AgNPs synthesized from callus when tested against the growth of fungus (*Fusarium oxysporum* and *Aspergillus niger*) and bacteria (*Xanthomonas campestris*, *Erwinia carotovera*, and *Bacillus subtilis*). With the greatest inhibitory zone of 19 mm against the bacterium *Xanthomonas campestris* and up to 14 mm against the fungus *Aspergillus niger*, the silver nanoparticles made from callus extract were the smallest and showed the most antimicrobial ability. As a result, the best substrate for reducing silver nitrate solution in a 1:20 ratio to create the finest silver nanoparticles in a biogenic manner is callus extract (Jahangir *et al.*,2023).

CHAPTER-3

AIM AND OBJECTIVE

AIM

To formulate and develop herbal based Nano gel against diabetic foot ulcer.

OBJECTIVE

- Collection and extraction compounds from the plant extracts.
- AgNPs synthesized and characterization from *Ocimum Sanctum* (Tulsi) and *Carica Papaya* (Papaya).
- Antimicrobial Assay to confirm their potential.
- Formulate the gel composition, standardize it and evaluate its physical-chemical properties.
- In-vitro studies to determine the efficacy of the Nano gel.
- Nano gel is characterized by using XRD, TEM, FTIR Analysis.

CHAPTER-4

METHODOLOGY

4.1 COLLECTION OF PLANT AND FRUIT MATERIAL

Fresh leaves of *Ocimum Sanctum* (Tulsi) and Fresh fruit of *Carica Papaya* (papaya) are collected from available market near Porur, Chennai, Tamil Nadu were used for this experiment.

4.2 PREPARATION OF PLANT AND FRUIT EXTRACTS

The collected plant and fruit material were cleaned with running tap water and rinsed in distilled water. The plant and fruit were cut into fine pieces, shade dried for 4 -6 days and grinded into fine powder by using mechanical method.

4.2.1 Extraction process:

- 1g of *Ocimum Sanctum* (Tulsi) dried powder added along with 100 ml of aqueous solution and kept in water bath for 60C for 15-20 mins after that filter the sample using Filtration method.
- 1g of *Carica Papaya* (papaya) dried powder added along with 100 ml of aqueous solution and kept in water bath for 60C for 15-20 mins after that filter the sample using Filtration method.
- Combination of 1g of *Ocimum Sanctum* (Tulsi) extracts and 1g of *Carica Papaya* (papaya) dried powder added along with 100 ml of aqueous solvent and kept in water bath for 60 C for 15-20 mins after that filter the sample using Filtration method.

4.2.2 Dilution of Process:

Preparation for 10:10 Dilution:

Individual: 10 ml of *Ocimum Sanctum* (tulsi) extract added along with 10 ml of aqueous solvent. 10 ml of *Carica Papaya* (papaya) extract added along with 10 ml of aqueous solvent.

Combination: 10 ml of Combined extracts added along with 10 ml of aqueous solvent.

Preparation for 1:9 Dilution:

Individual: 1 ml of *Ocimum Sanctum* (tulsi) extract added along with 9 ml of aqueous solvent. 1 ml of *Carica Papaya* (papaya) extract added along with 9 ml of aqueous solvent.

Combination: 1 ml of Combined extracts added along with 9 ml of aqueous solvent.

4.3 SYNTHESIS OF SILVER NANOPARTICLES WITH PLANT AND FRUIT EXTRACTS

- **Individual:** Crude extracts of individual (*Ocimum Sanctum* (tulsi) or *Carica Papaya* (papaya)) are added along with AgNO_3 solution in the ratios are 1:4, 2:3, 2.5:2, 3:2, 5:1 and kept in water bath until the colour changes from green to brown colour for Synthesis of Silver Nanoparticles. After changes of solution, analysed with UV-VIS Spectroscopy for indication of Silver Nanoparticles.
- **Combination:** Crude extracts of Combined (*Ocimum Sanctum* (tulsi) and *Carica Papaya* (papaya)) are added along with AgNO_3 solution in the ratios are 1:4, 2:3, 2.5:2, 3:2, 5:1 and kept in water bath until the colour changes from green to brown colour for Synthesis of Silver Nanoparticles. After changes of solution, analysed with UV-VIS Spectroscopy for indication of Silver Nanoparticles.

4.4 QUALITATIVE ANALYSIS

- **Test for Flavonoids:** 2ml of 2.0% NAOH mixture added along with 1ml of aqueous crude extract. concentrated yellow colour was produced, which became colourless when we added 2 drops of diluted acid to mixture. This result showed the presence of flavonoids.

- **Test for Tannin:** 0.5ml of 5% Ferric chloride added along with 1ml of aqueous crude extract and finally green colour precipitate can be obtained.
- **Test for Glycosides:** 2 ml of H₂SO₄ concentrated added along with 1ml of aqueous plant crude extract. A reddish-brown colour formed which indicated the presence of steroidal aglycone part of the glycoside.
- **Test for Steroids:** 2 ml of chloroform and concentrated H₂SO₄ were added with the 1ml aqueous plant crude extract. In the lower chloroform layer red colour appeared that indicated the presence of steroids.
- **Test for Alkaloid:** 1ml of Dragendroff solution added along with 1ml aqueous plant crude extract. An orange colour formed which indicated the presence of Alkaloids.
- **Test for Saponins:** 10 ml of distilled water was mixed with 1ml of aqueous crude plant extract in a test tube and it was mixed vigorously. The frothing was mixed vigorously and the foam appearance showed the presence of saponins (Gul *et al.*, 2017).

4.5 ANTIBACTERIAL ACTIVITY

Purpose: Antibacterial activity by using agar well diffusion method. The agar diffusion assay is one method for quantifying the ability of antibiotics to inhibit bacterial growth.

Procedure:

- Disk-diffusion method; the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface.

- Then, a hole with a diameter of 5-6 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20–100 μ L) of the antimicrobial agent or extract solution at desired concentration is introduced into the well.
- Then, agar plates are incubated under suitable conditions depending upon the test microorganism.
- The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested.

Microorganism used:

- *Escherichia coli*,
- *Staphylococcus aureus* 13,
- *Staphylococcus aureus* 23,
- *Enterococcus faecalis*,
- *Acinetobacter baumannii*.

4.6 GEL FORMATION

4.6.1 GUAR GUM

- Guar gum is a gel-forming fiber from the seed of the guar plant (*Cyamopsis tetragonal*). It's used to thicken foods and is also used as a dietary supplement.
- Guar gum acts as thickening agent and it also act against biofilm formation.

4.6.2 PREPARATION OF GUAR GUM

- Add 0.75 g of guar gum along with 50ml of synthesis of nanoparticle to form gel formation.
- After Nano gel formation is characterized by using XRD, TEM, FTIR Analysis.

4.7 INVITRO SCRATCH ASSAY

Purpose: The in-vitro scratch assay is a useful method in wound healing research to assess cell migration. In this assay, a scratch is created in a confluent cell layer by mechanically removing cells through manual scraping with a sharp-edged tool.

Procedure:

- HDF cells (4,000 cells/well) and HaCaT cells (10,000 cells/well) were seeded in 96-well plates (Corning, USA) in complete media and incubated at 37 °C and 5% CO₂ for 48 h. Scratches by wounding tool: The wounding tool and guide were sterilised with 70% ethanol and placed in the biosafety cabinet (BSC).
- The cover of the 96-well plate was replaced by the guide and the wounding tool was inserted into the slit of the guide, pushed down, and slid along the slit to create a scratch on the cell surface. The motion was repeated for wells scratched by wounding tool.
- Scratches by pipette tip: The cover of the 96-well plate was used as a straight-edged guide.
- Cells were then fixed with 2% paraformaldehyde (Thermo Fisher Scientific, USA) and stained with 0.25% Safranin O (EMD Millipore Corp, USA).
- The plate was then mounted onto the imaging template and placed on the stage of an Olympus IX71 inverted microscope. 4 × phase-contrast and brightfield images were taken which were then analysed using Image J (Chen *et al.*, 2023).

4. 8 CHARACTERIZATION OF NANO GEL FORMATION

4.8.1 UV-VIS SPECTROSCOPY

UV-Vis spectral analysis of synthesized silver nanoparticles was measured by using UV-visible spectrophotometer (UV-2450, Shimadzu, Japan) with a resolution of 1 nm to investigate the reduction of Ag⁺ to Ag⁰ by callus and leaf extracts.

The spectra were taken between 200 and 800 nm after 24 Hrs incubation of the mixtures of AgNO_3 solution and callus and leaf extracts. (Jemal *et al.*,2017).

4.8.2 XRD ANALYSIS

The synthesized silver nanoparticles were centrifuged at 10,000 rpm for 15 minutes and the pellets were redispersed in sterile double distilled and centrifuged at 10,000 rpm for 10 minutes. The purified pellets were dried at 50°C in an oven and analysed by X-ray Diffraction Unit (XRD) (Pan Analytical, X-pert pro, Netherland). X-ray diffraction (XRD) measurements of film of the biologically synthesized silver nanoparticles solution cast onto glass slides were done on an eMMA diffractometer operating at a voltage of 40 kV and current of 20 mA with Cu K(a) radiation of 1.54187 nm wavelength. The scanning as done in the region of 2 θ from 20 to 80 at 0.02 /min and the time constant was 2 s (Jemal *et al.*,2017)

4.8.3 TEM ANALYSIS

Transmission electron microscopy (TEM) technique was used to visualize the morphology of the Ag NPs. The 200 kV ultra-high-resolution transmission electron microscope (JEOL, Model No. JEM 2100 HR with EELS). TEM grids were prepared by placing a 5 μL of the AgNPs solutions on carbon-coated copper grids and drying under lamp. Additionally, addition presence of metals in the sample was analyzed by energy-dispersive spectroscopy (EDS) using INCA Energy TEM 200 with analysis software (JEOL) was used (Banerjee *et al.*,2014).

4.8.4 FTIR ANALYSIS

FTIR spectrometer was used to study the chemical composition of the synthesized silver nanoparticles. The solutions containing silver nanoparticles were centrifuged at 10,000 rpm for 15 min. The supernatants were discarded and the pellets were redispersed in sterile double distilled water and centrifuged at 10,000 rpm for 10 minutes. The purified pellets were dried at 60°C and the dried powders were subjected to FTIR spectroscopy measurement in the range 4000–400 cm^{-1} using KBr pellet method (Jemal *et al.*,2017).

CHAPTER-5

RESULTS&DISCUSSION

5.1 COLLECTION OF PLANT AND FRUIT MATERIAL

Fresh leaves of *Ocimum Sanctum* (tulsi) and Fresh fruit of *Carica Papaya* (papaya) are collected below the fig 5.1

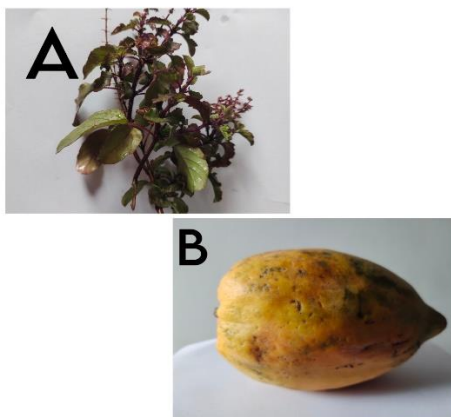


Fig 5.1: A) Fresh leaves of *Ocimum Sanctum* (tulsi) and B) Fresh fruit of *Carica Papaya* (papaya)

5.2 PREPARATION OF PLANT AND FRUIT EXTRACTS

The plant and fruit were cut into fine pieces, shade, dried and grinded into fine powder by using mechanical method below the fig 5.2.



Fig 5.2: A) Dried powder of *Ocimum Sanctum* (tulsi) and B) Dried powder of *Carica Papaya* (papaya)

5.2.1 Extraction process:

Dried powder (individual & combination) added along with 100 ml of aqueous solvent and kept in water bath for 60C for 15-20 mins after that filter the sample using Filtration method below the fig.5.3.

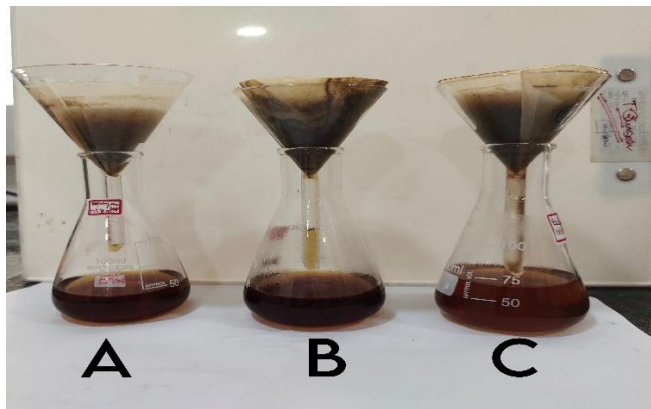


Fig 5.3: A) Crude extract of *Ocimum Sanctum* and B) Crude extract of *Carica Papaya* C) Crude extract of *Ocimum Sanctum* & *Carica Papaya*

5.3 SYNTHESIS OF SILVER NANOPARTICLES WITH PLANT AND FRUIT EXTRACTS

Crude extracts (individual & combination) are added along with AgNO_3 solution in the ratios are 1:4, 2:3, 2.5:2, 3:2, 5:1 and kept in water bath until the colour changes from green to brown colour for Synthesis of Silver Nanoparticles below the fig.5.4.



Fig 5.4: Synthesis of Silver Nanoparticles

5.4 QUALITATIVE ANALYSIS

- ***Ocimum Sanctum*:**

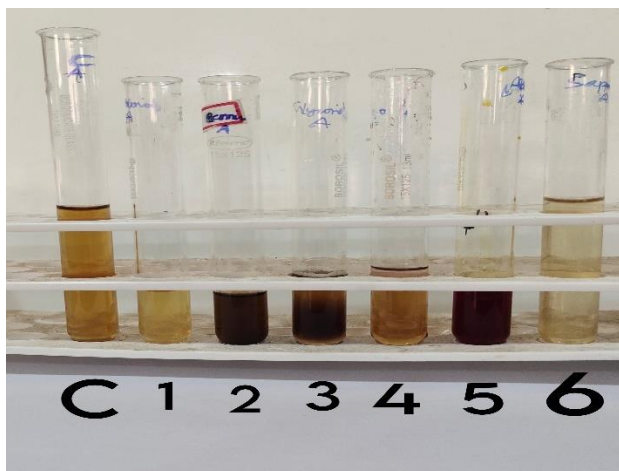


Fig 5.5: Phytochemical screening of *Ocimum Sanctum* (tulsi)

Phytochemical screening for aqueous extract of *Ocimum Sanctum* (tulsi) whereas C indicates control of *Ocimum Sanctum* (tulsi). 1) Absence of yellow colour indicates absence of flavonoids. 2) A Green colour precipitate formed indicates presence of tannin. 3) A reddish-brown colour formed indicates the presence of steroidal aglycone part of the glycoside. 4) In the lower chloroform layer red colour formed indicates the absence of steroids. 5) An orange colour formed which indicates the presence of Alkaloids. 6) A foam appearance shows the presence of saponins.

- ***Carica Papaya*:**

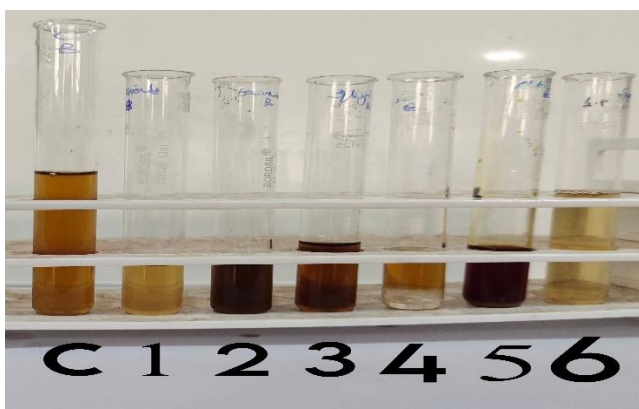


Fig 5.6: Phytochemical screening of *Carica Papaya* (papaya)

Phytochemical screening for aqueous extract of *Carica Papaya* (papaya) whereas C indicates control of *Carica Papaya* (papaya). 1) Absence of yellow colour indicates absence of flavonoids. 2) A Green colour precipitate formed indicates presence of tannin. 3) A reddish-brown colour formed indicates the absence of steroidal aglycone part of the glycoside. 4) In the lower chloroform layer red colour formed indicates the absence of steroids. 5) An orange colour formed which indicates the presence of Alkaloids. 6) A foam appearance shows the presence of saponins.

Table 5.1 refers to results for Phytochemical screening

S.no	Tests	<i>Ocimum Sanctum</i> (Tulsi)	<i>Carica Papaya</i> (Papaya)
1	Flavonoid	--	--
2	Tannin	+	+
3	Glycosides	+	+
4	Steroids	--	--
5	Alkaloids	+	+
6	Saponin	+	+

5.5 ANTIBACTERIAL ACTIVITY

- 10:10 Dilution:**

Based on the UV-VIS Spectroscopy results, Under *Ocimum Sanctum* (tulsi) are the ratios of 1:4, 2:3, 2.5:2, 3:2, 5:1; *Carica Papaya* (papaya) ratio of 2:3 and Combination are the ratios of 1:4. These are selected for Antibacterial activity. Antibacterial activity done using agar well diffusion method. Antibacterial activity done using the microorganisms are *Escherichia coli*, *Staphylococcus aureus* 23, *Enterococcus faecalis*, *Acinetobacter baumannii*. A zone of inhibition formed for 10:10 Dilution.

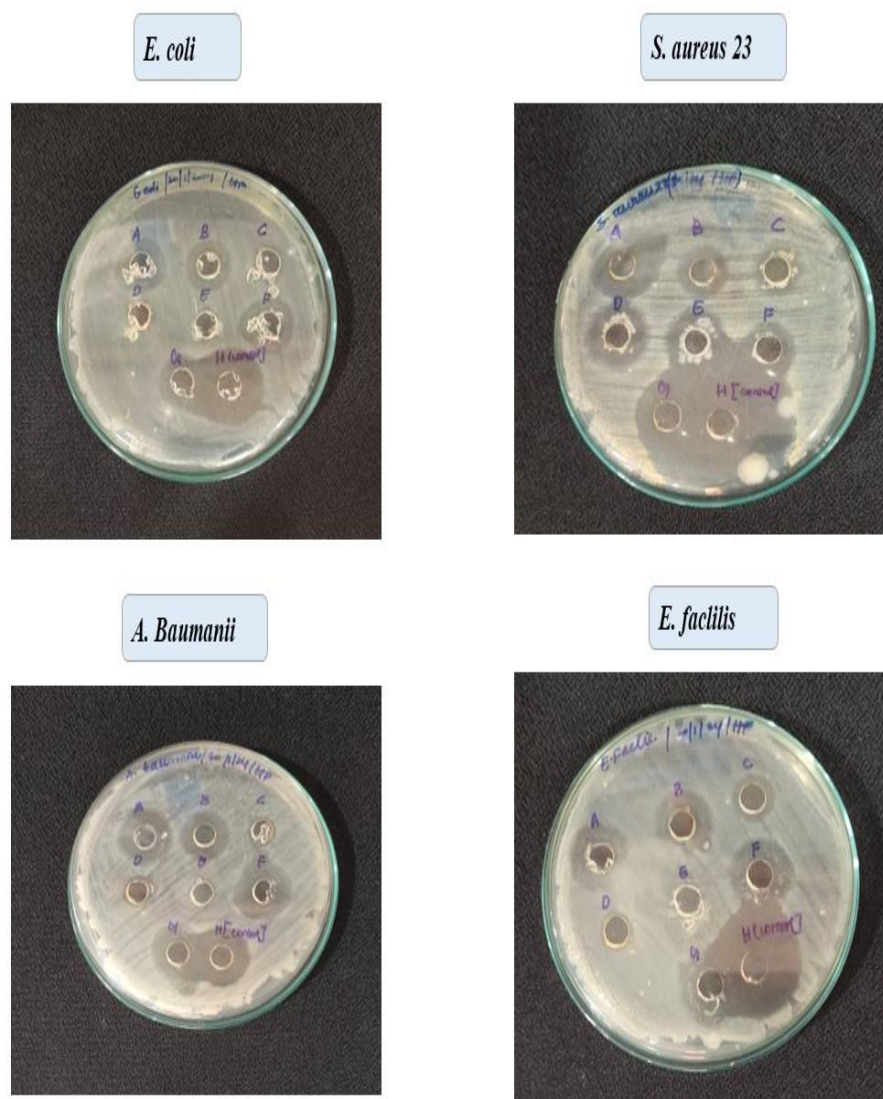


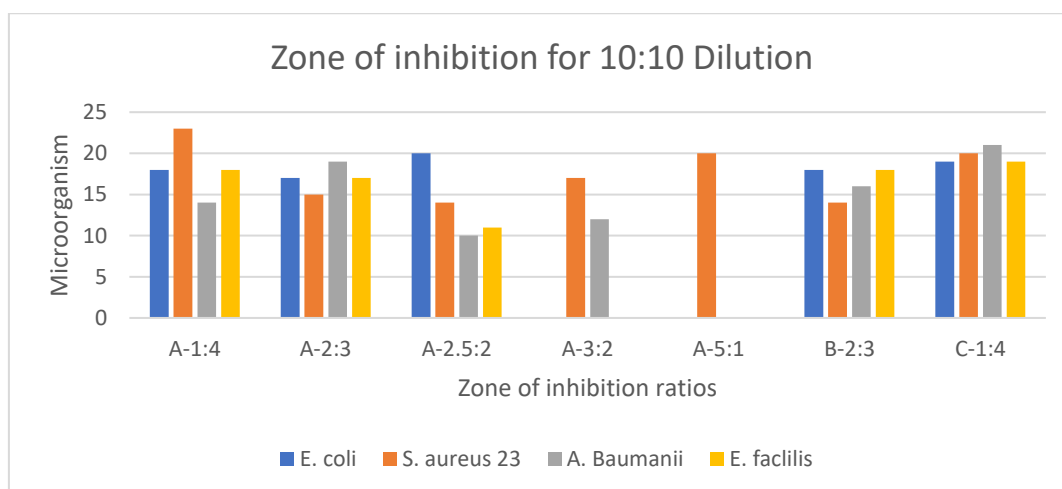
Fig 5.7: Antimicrobial activity assay of silver nanoparticles along with individual extract and Combination extract at 40 ul against different pathogens by the well diffusion method. (A) 1:4 ratio (tulsi & AgNPs) (B) 2:3 ratio (tulsi & AgNPs) (C) 2:5:2 ratio (tulsi & AgNPs) (D) 3:2 ratio (tulsi & AgNPs) (E) 5:1 ratio (tulsi & AgNPs) (F) 2:3 ratio (papaya & AgNPs) (G) 1:4 ratio (combination & AgNPs) (H) Antibiotic rifampicin at concentration $100\text{ }\mu\text{g mL}^{-1}$ was used as a control for all tested bacteria while, rifampicin at concentration 30 ul was used as a control for different pathogens.

The Zone of Inhibition formed for 10:10 Dilution represents in below the Tabular form and graphical format.

Table 5.2 refers to 10:10 Dilution of Zone of Inhibition

Microorganism	<i>Ocimum Sanctum</i>					<i>Carica Papaya</i>					Combination				
	1:4	2:3	2.5:2	3:2	5:1	1:4	2:3	2.5:2	3:2	5:1	1:4	2:3	2.5:2	3:2	5:1
<i>E. coli</i>	18 mm	17 mm	-	-	-	-	18 mm	-	-	-	19 mm	-	-	-	-
<i>S. aureus 23</i>	23 mm	15 mm	14 mm	17 mm	20 mm	-	14 mm	-	-	-	20 mm	-	-	-	-
<i>A. Baumanii</i>	14 mm	19 mm	10 mm	12 mm	-	-	16 mm	-	-	-	21 mm	-	-	-	-
<i>E. faclitis</i>	18 mm	17 mm	11 mm	-	-	-	18 mm	-	-	-	19 mm	-	-	-	-

- Graphical format form for 10:10 Dilution of Zone of Inhibition**



From the graph, whereas A indicates (tulsi & AgNPs); B indicates (papaya & AgNPs) and C indicates (combination & AgNPs). The minimum zone of inhibition shows *Carica Papaya* (papaya) ratio of 2:3. The maximum zone of inhibition shows *Ocimum Sanctum* (tulsi) are the ratios of 1:4,2:3,2.5:2,3:2,5:1 and Combination are the ratios of 1:4.

- **1:9 Dilution:**

Based on the UV-VIS Spectroscopy results, Under *Ocimum Sanctum* (tulsi) are the ratios of 1:4,2:3,2.5:2,3:2; *Carica Papaya* (papaya) ratio of 2.5:2,3:2 and Combination are the ratios of 1:4,2:3,2.5:2. These are selected for Antibacterial activity. Antibacterial activity done using agar well diffusion method. Antibacterial activity done using the microorganisms are *Escherichia coli*, *Staphylococcus aureus* 13, *Staphylococcus aureus* 23, *Acinetobacter baumannii*. A zone of inhibition formed for 1:9 Dilution.

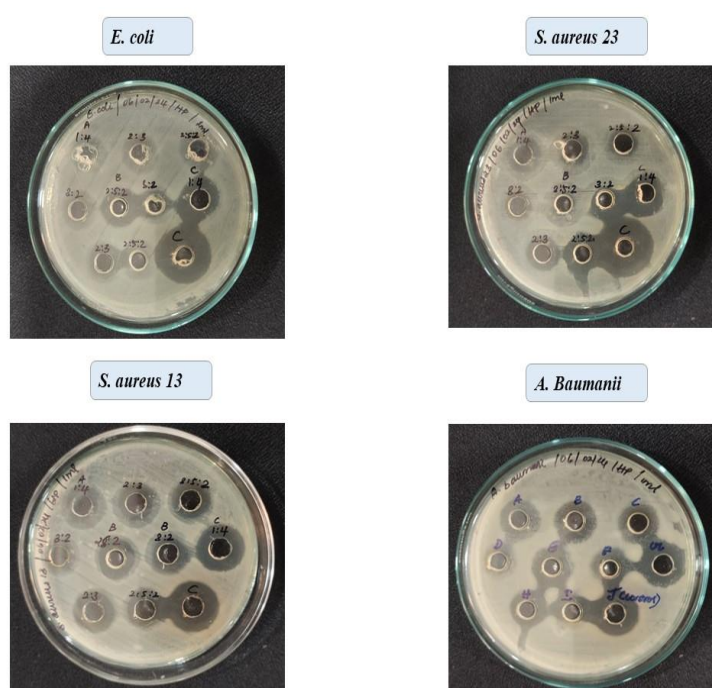


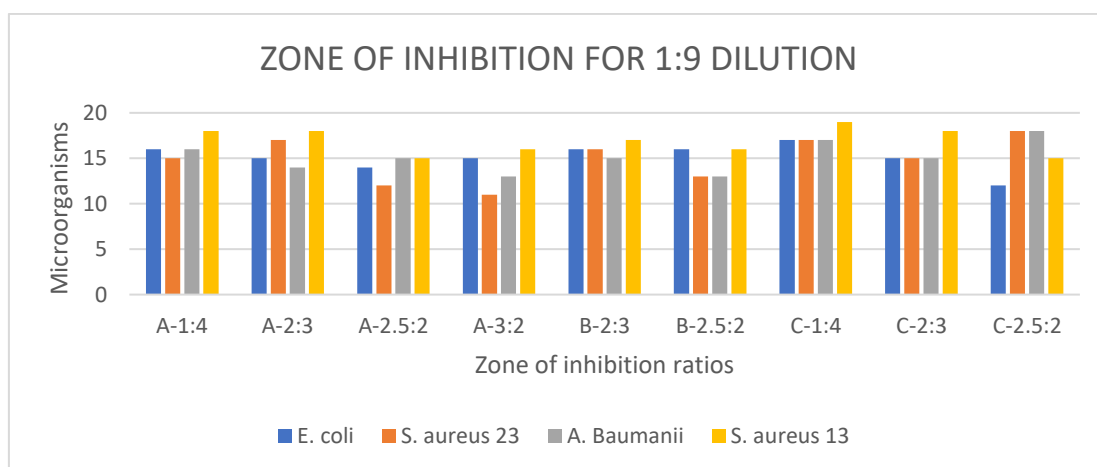
Fig 5.8: Antimicrobial activity assay of silver nanoparticles along with individual extract and Combination extract at 40 ul against different pathogens by the well diffusion method. (A) 1:4 ratio (tulsi & AgNPs) (B) 2:3 ratio (tulsi & AgNPs) (C) 2.5:2 ratio (tulsi & AgNPs) (D) 3:2 ratio (tulsi & AgNPs) (E) 2.5:2 ratio (papaya & AgNPs) F) 3:2 ratio (papaya & AgNPs) G) 1:4 ratio (combination & AgNPs) H) 2:3(combination & AgNPs) I)2.5:2(combination & AgNPs) J) Antibiotic rifampicin at concentration $100 \mu\text{g mL}^{-1}$ was used as a control for all tested bacteria while, rifampicin at concentration 30 ul was used as a control for different pathogens.

The Zone of Inhibition formed for 10:10 Dilution represents in below the Tabular form and graphical format.

Table 5.3 refers to 1:9 Dilution of Zone of Inhibition

Microorganism	<i>Ocimum Sanctum</i>					<i>Carica Papaya</i>					Combination				
Ratios	1:4	2:3	2.5:2	3:2	5:1	1:4	2:3	2.5:2	3:2	5:1	1:4	2:3	2.5:2	3:2	5:1
<i>E. coli</i>	16 m m	15 mm	14 mm	15 mm	-	-	16 m m	16 mm	-	-	17 mm	15 m m	12 mm	-	-
<i>S. aureus 23</i>	15 m m	17 mm	12 mm	11 mm	-	-	16 m m	13 mm	-	-	17 mm	15 m m	18 mm	-	-
<i>A. Baumanii</i>	16 m m	14 mm	15 mm	13 mm	-	-	15 m m	13 mm	-	-	17 mm	15 m m	18 mm	-	-
<i>S. aureus 13</i>	18 m m	18 mm	15 mm	16 mm	-	-	17 m m	16 mm	-	-	19 mm	18 m m	15 mm	-	-

- Graphical format form for 10:10 Dilution of Zone of Inhibition**



From the graph, whereas A indicates (tulsi & AgNPs); B indicates (papaya & AgNPs) and C indicates (combination & AgNPs). The minimum zone of inhibition shows *Carica Papaya* (papaya) ratio of 2.5:2,3:2. The maximum zone of inhibition shows *Ocimum Sanctum* (tulsi) are the ratios of 1:4,2:3,2.5:2,3:2 and Combination are the ratios of 1:4,2:3,2.5:2. Among the ratios, The ratio of 1:4 showed maximum zone of inhibition in *Ocimum Sanctum* (tulsi) and Combination.

Antibacterial activity done for 1:4 ratio of both Combination and *Ocimum Sanctum* (tulsi).

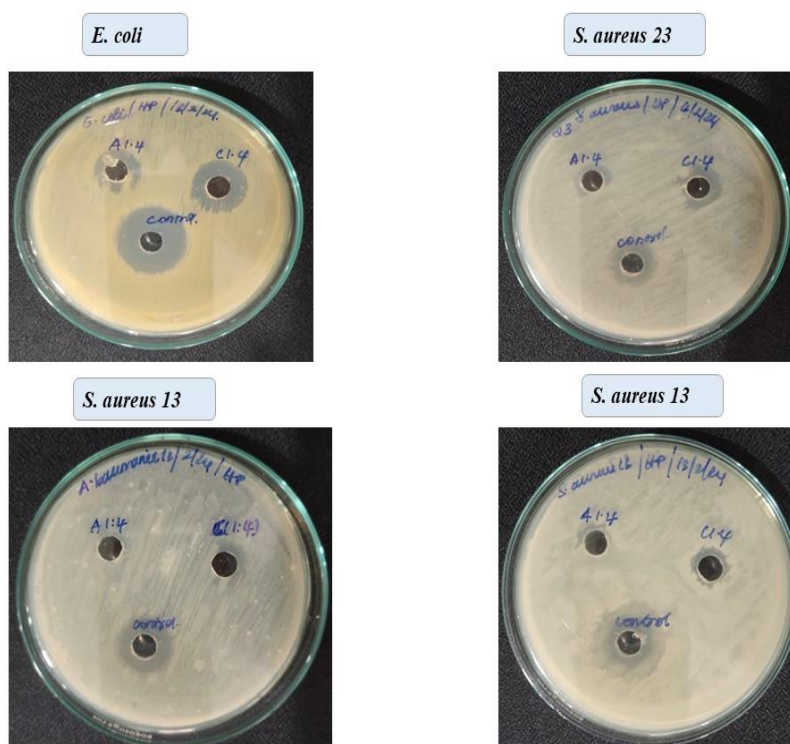


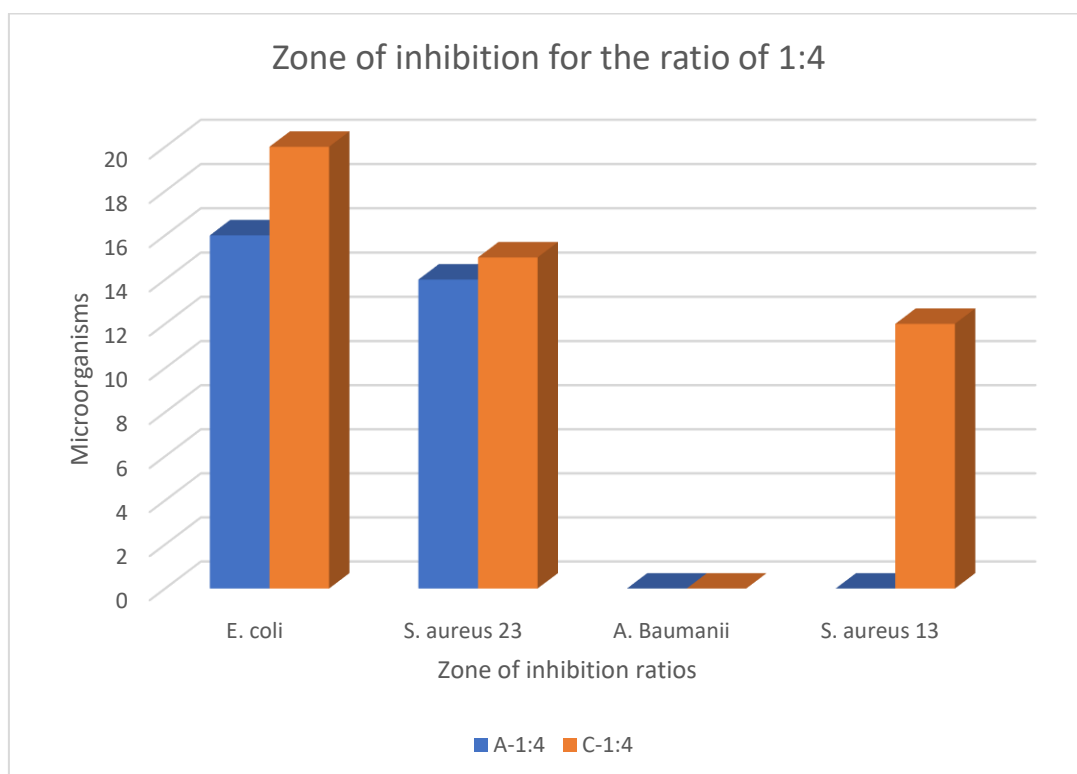
Fig 5.9: Antimicrobial activity assay of silver nanoparticles along with individual extract and Combination extract at 40 ul against different pathogens by the well diffusion method. (A) 1:4 ratio (tulsi & AgNPs) B) 1:4 ratio (combination & AgNPs) C) Antibiotic rifampicin at concentration $100 \mu\text{g mL}^{-1}$ was used as a control for all tested bacteria while, rifampicin at concentration 30 ul was used as a control for different pathogens.

The Zone of Inhibition formed for the ratio of 1:4 both *Ocimum Sanctum* (tulsi) and Combination represents in below the Tabular form and graphical format.

Table 5.4 refers to the ratio of 1:4 of Zone of Inhibition

Microorganisms	<i>Ocimum Sanctum</i> (tulsi) A (1:4)	Combination C (1:4)
<i>E. coli</i>	16mm	20mm
<i>S. aureus</i> 13	---	12mm
<i>S. aureus</i> 23	14mm	15mm
<i>A. baumannii</i>	-----	----

- Graphical format form for 10:10 Dilution of Zone of Inhibition



From the graph, whereas A indicates (tulsi & AgNPs) and C indicates (combination & AgNPs). The minimum zone of inhibition shows *Ocimum Sanctum* (tulsi) are the ratios of 1:4, The maximum zone of inhibition shows and Combination are the ratios of 1:4. Among the ratios, The ratio of 1:4 showed maximum zone of inhibition in Combination. Further, proceed with gel formation with the ratio of 1:4 combination.

5.6 GEL FORMATION

- Add guar gum along with synthesis of nanoparticle to form gel formation below the fig 2.2.
- After Nano gel formation is characterized by using XRD, TEM, FTIR Analysis.



Fig 5.10: Nano gel formation

5.7 CHARACTERIZATION

5.7.1 UV-VIS SPECTROSCOPY

- **10:10 Dilution and 1:9 dilution:**

Using UV–vis spectral analysis, the development and stability of AgNPs in an aqueous solution were examined. The solution turned brownish brown as the reaction continued, which has been linked to AgNPs' surface plasmon resonance being excited. Figure 2.2 shows UV absorption spectra from the reaction of the reduction of silver ions produced distributed nanoparticles with a broadening peak in the absorbance band at a wavelength between 200nm and 800nm. The silver nitrate solution changed from translucent white to light yellow to brown after the *Ocimum Sanctum* (tulsi); *Carica Papaya* (papaya) extract and Combination was added.

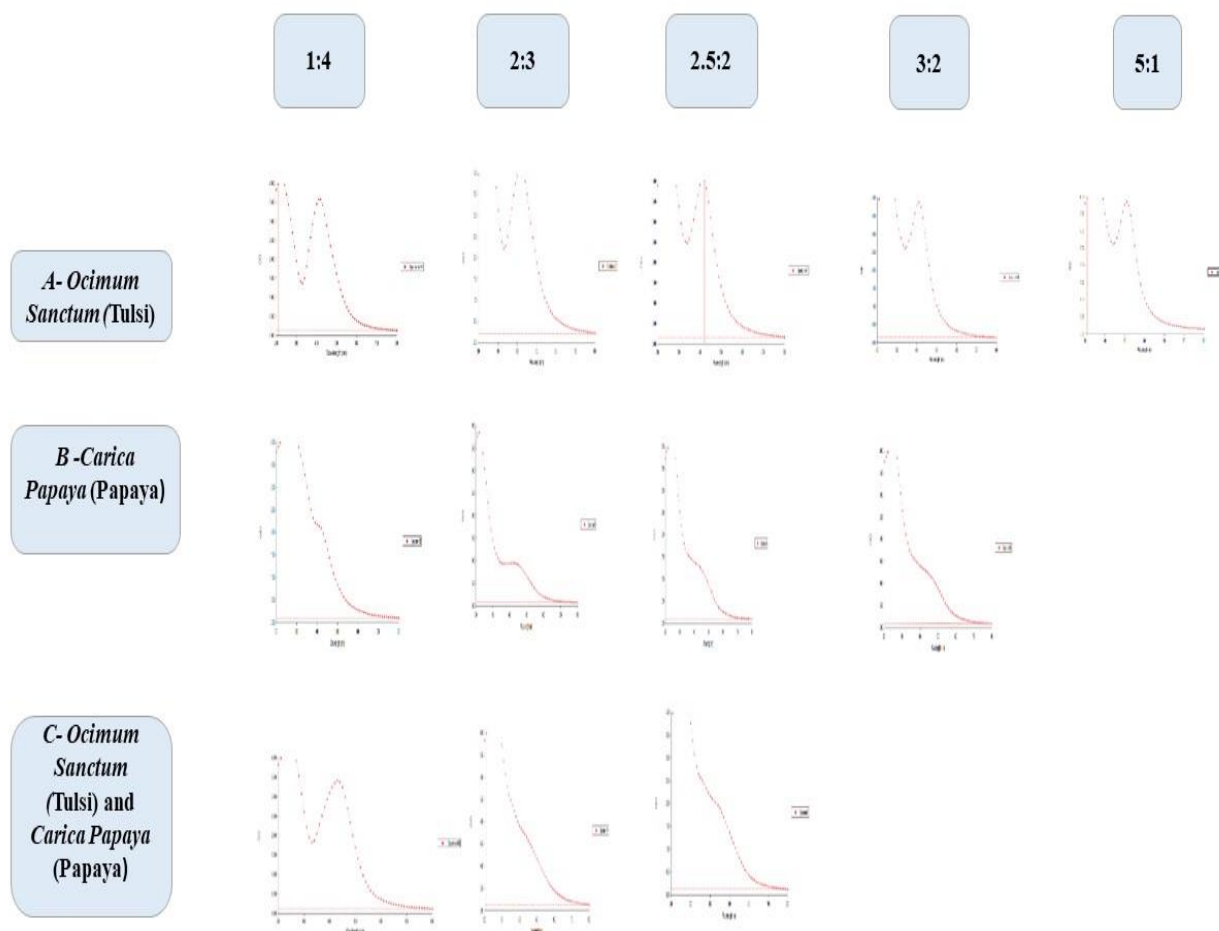


Fig 5.11: UV-vis absorption spectra of reduction of silver ions to silver nanoparticles along with individual & combination extract for the dilution of 10: 10.

The absorption spectrum of the incubated solution at different wavelengths ranging within 200–800 nm revealed whereas *Ocimum Sanctum* (tulsi) extract are the ratios of 1:4,2:3,2.5:2,3:2,5:1 is 460nm,450nm,420nm,400nm,430nm. No absorption peak was observed in UV–vis spectrum of Ag^+ solution in the *Carica Papaya* (papaya) extract. But *Carica Papaya* (papaya) extract and Combination peak observed in the ratio of 2:3 & 1:4 are 410nm and 460nm.

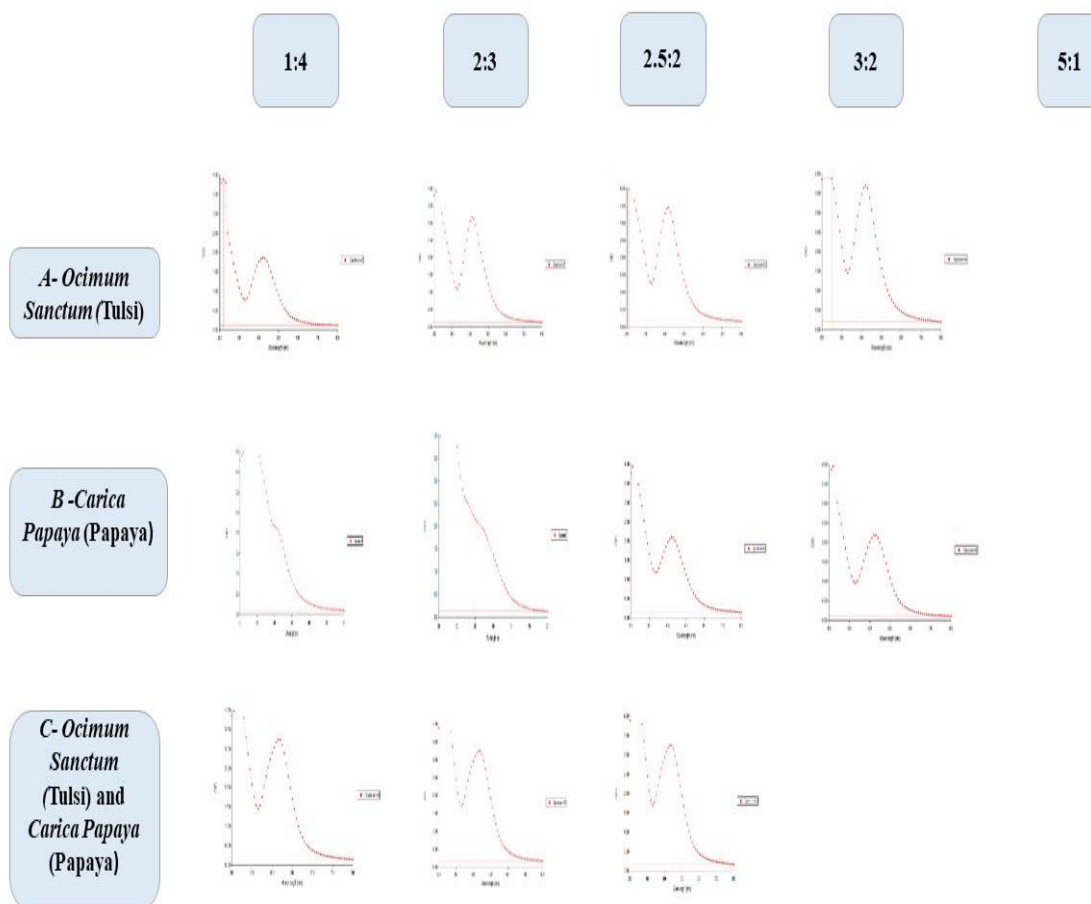


Fig 5.12: UV-vis absorption spectra of reduction of silver ions to silver nanoparticles along with individual & combination extract for the dilution of 1:9.

The absorption spectrum of the incubated solution at different wavelengths ranging within 200–800 nm revealed whereas *Ocimum Sanctum* (tulsi) extract are the ratios of 1:4,2:3,2.5:2,3:2 is 420nm,440nm,460nm,450nm. No absorption peak was observed in UV–vis spectrum of Ag^+ solution in the *Carica Papaya* (papaya) extract. But *Carica Papaya* (papaya) extract peak observed in the ratio of 2.5:2 & 3:2 is 430nm and 450nm. Combination peak observed in the ratio of 1:4, 2:3&2.5:2 is 460nm, 440nm and 460nm.

5.7.2 XRD ANALYSIS

The XRD patterns for the silver nanoparticles made using Combination 1:4 extract is displayed in Fig 5.13. With regard to the face-centered cubic structure of silver, all significant peaks are indexable. When characterizing nanoparticles, X-ray diffraction analysis can be quite helpful as it offers precise information about the crystalline size and structure of the particles. The organic content of the Combination 1:4 extract can be attributed to broad, minor peaks at lower 2θ theta values. Thus, it is evident from the XRD pattern that the crystalline quality of the silver nanoparticles produced by Combination 1:4 extract.

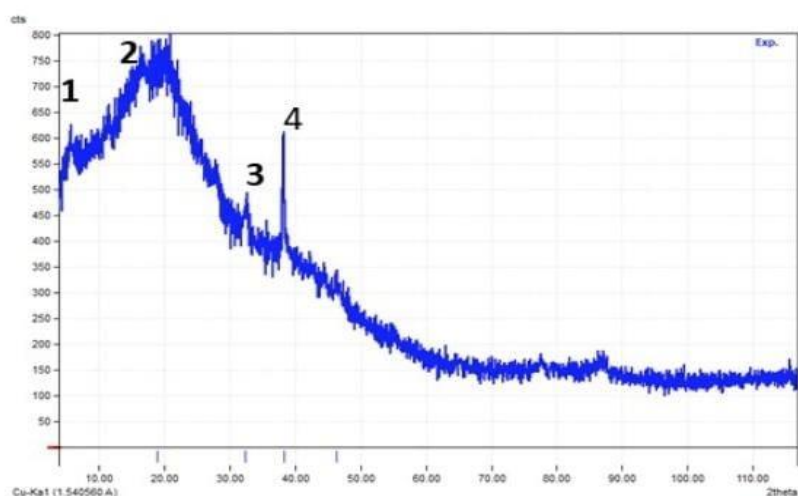


Fig 5.13: XRD spectra of AgNPs synthesized using *Ocimum Sanctum* and *Carica Papaya* extract in the ratio of Combination 1:4

Table 5.5 refers to results of XRD Analysis

S.no	2θ [°]	d [Å]
1	18.91	4.6902
2	32.40	2.7609
3	38.31	2.3477
4	46.28	1.9601

5.7.3 FTIR ANALYSIS

Numerous materials in bulk or thin films, liquids, solids, pastes, powders, fibres, and other forms can all be analysed using FTIR. When combined with appropriate standards, FTIR analysis can provide not only a qualitative (identification) study of materials but also a quantitative (quantity) analysis. The potential biomolecules responsible for encapsulating and effectively stabilizing the metal nanoparticles produced by Combination 1:4 extract was identified using FTIR studies. The kinds of functional groups engaged in the binding mechanisms are explained by shifts in peak intensity or wavenumber. The FTIR spectrum for Combination 1:4 extract at showed the intensity at 107.399, 105.665, 52.5718, 71.7365, 88.8981, 91.9094, 89.4826 & 26.9465. The effect of the functional groups of the *Ocimum Sanctum* (Tulsi) and *Carica Papaya* (Papaya) extract in the ratio of Combination 1:4 as reducing and stabilizing agents to manufacture Ag-NPs is supported by all of the intensity alterations.

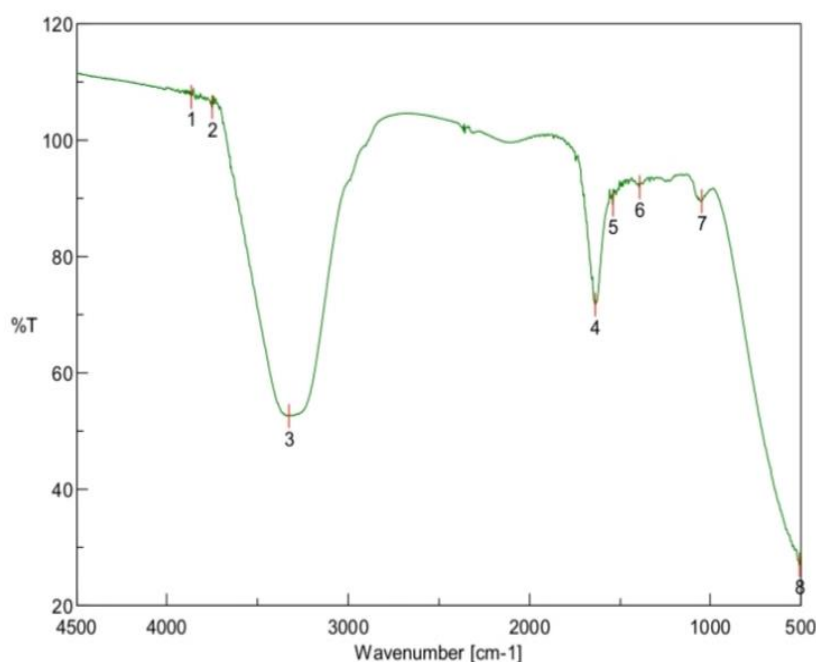


Fig 5.14: FTIR spectra of AgNPs synthesized using *Ocimum Sanctum* and *Carica Papaya* extract in the ratio of Combination 1:4

5.7.4 TEM ANALYSIS

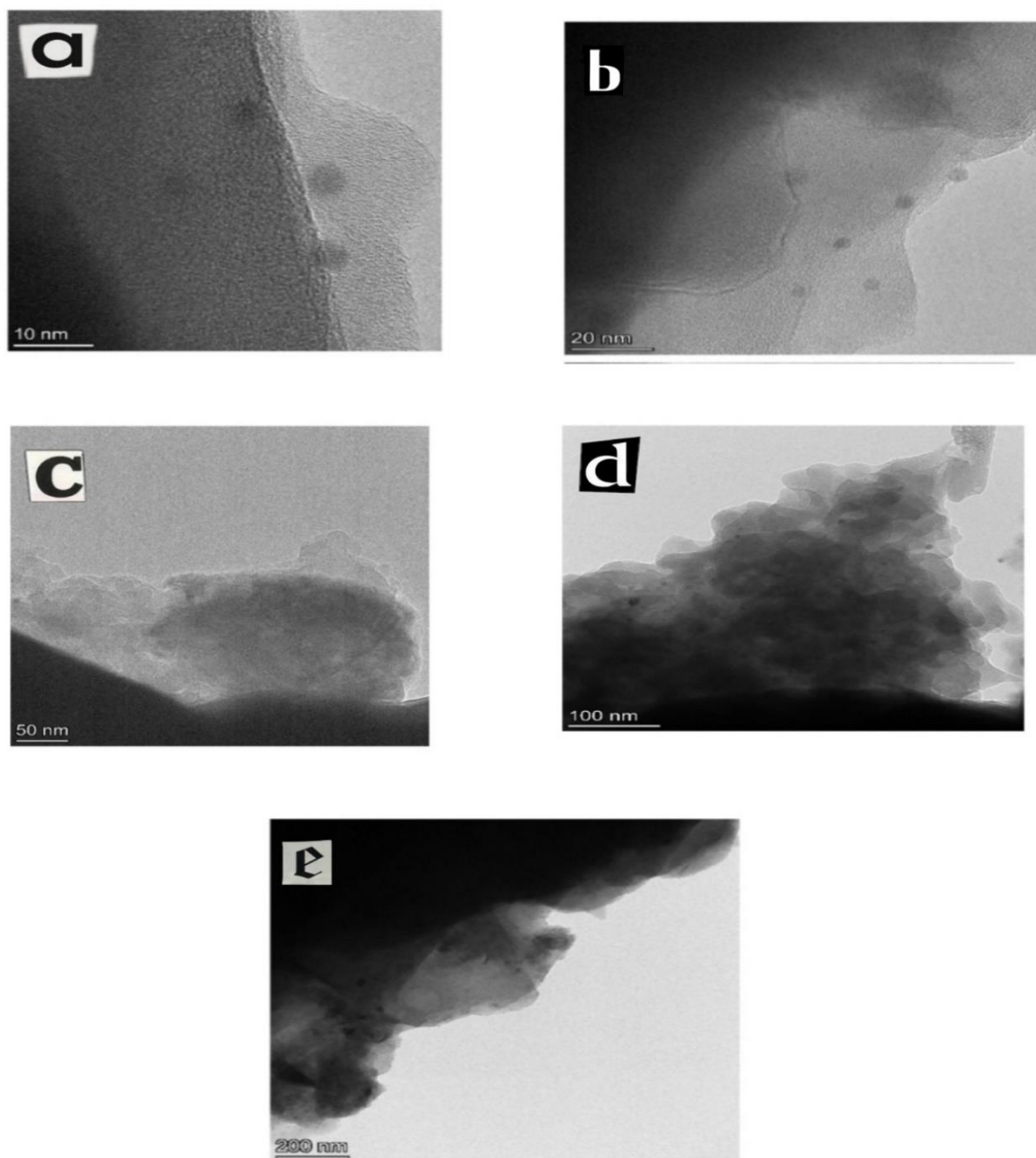


Fig 5.15 : TEM micrographs of silver nanoparticles are different magnification using *Ocimum Sanctum* (Tulsi) and *Carica Papaya* (Papaya) extract in the ratio of Combination 1:4

The nanoparticles' shape was spherical in nature. When closely examined, it becomes clear that the silver nanoparticles are encircled by a thin, pale coating of other substances, which we assume to be the organic material from the *Ocimum Sanctum* (Tulsi) and *Carica Papaya* (Papaya) extract. The resulting nanoparticles range in size from 3 to 20 nm, including a small percentage of aggregated particles.

Fig 5.15 shows TEM images of AgNPs at different magnifications using *Ocimum Sanctum* (Tulsi) and *Carica Papaya* (Papaya) extract in the ratio of Combination 1:4. Images reveal that the AgNPs are predominantly spherical in shape and are not in physical contact with each other. It is clear from the TEM micrographs that the particle size of AgNPs is 10 nm from fig.5.15-(a).

5.7.5 INVITRO SCRATCH ASSAY

The Mouse fibroblast (3T3-L1) cells is used to measure cell migration in wound healing. After being planted into a six-well plate, the cells are cultured in a CO₂ incubator until a monolayer of cells forms. Using a sterile tip, make a wound in each well. Then, load the sample at the correct concentration and place it in a CO₂ incubator. After 24 hours, the incision is checked for movement using an inverted microscope, the distance between the incisions is calculated, and photographs are captured by using inverted Phase contrast Microscope. The wound appears to be closing since the distance between the separations appears to be smaller than when it was first made. When compared to control cells, the sample's cell migration is higher, indicating that it possesses wound-healing capabilities.

In the present studies, (3T3-L1) cells were treated with 40 µg/mL of combination 1:4 extract for 24hrs. Image J software was used to calculate the wound closure distance after cell migration was recorded at 24 hours. According to the findings, 5 µg/mL of combination 1:4 extract filled up the scratch's opening by 98.701 percent in 24 hours. In the cells treated with combination 1:4 extract, 98.70% of the gap was filled in 24 hours.

- **CYTOTOXICITY STUDIES OF COMBINATION 1:4 EXTRACT**

The % cell viability was calculated using the following formula:

$$\% \text{ Cell viability} = \text{A570 of treated cells} / \text{A570 of control cells} \times 100$$

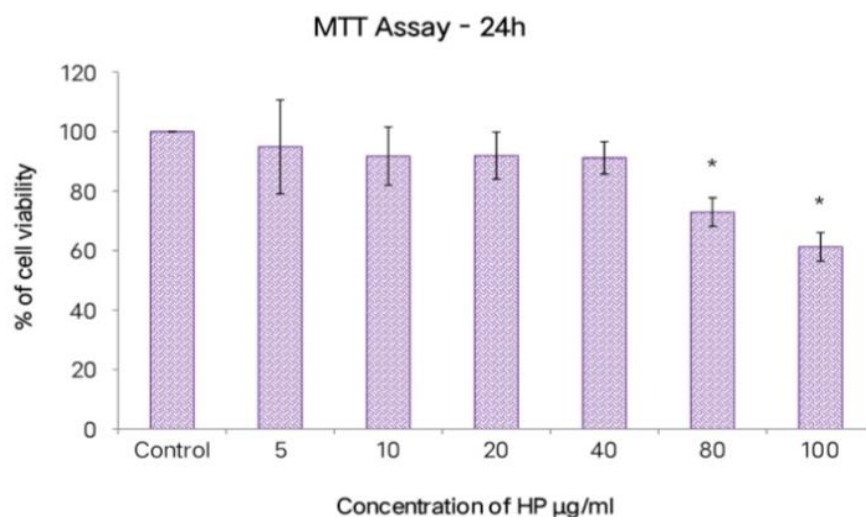
The (3T3-L1) cells is used to test the sample's cytotoxicity. The samples were serially diluted to achieve the desired concentrations are 5, 10, 20, 40, 80 & 100 and tested against the cells. The treatment and untreated cell absorbance is used to calculate the viability of the cells, which increases with decreasing concentration. The viability of the cells is approximately 80% at the highest concentration and continues to rise as the graph illustrates, indicating that the sample is not cytotoxic to the (3T3-L1) cells.

Table 5.6 refers to results for percentage of viability of the cells

S.NO	Concentration	Viability of the cells (%)
1	5 µg/mL	98.701
2	10 µg/mL	91.836
3	20 µg/mL	90.723
4	40 µg/mL	87.755
5	80 µg/mL	68.089
6	100 µg/mL	56.400

At 0 and 24 hours, the test samples' cell migration was 0.3112. Applying the above calculation, it shows that the wound closure rates for the test sample and control sample were 98.701% and 1.03%, respectively.. According to the calculation, the cell line's healing process accelerated by 98.70%. at 5 µg/mL.

At the maximum treatment concentration of Combination 1:4 extract, the percentage vitality of (3T3-L1) cells was observed to be 98.70% and the minimum treatment concentration was observed to be 56.40%. The percentage of cell viability associated with the quantities of Combination 1:4 extract utilized for treatment were tabulated in table 5.6.



The cytotoxic effects of HP on mouse fibroblast (3T3-L1) cells. Cells were treated with (5 to 100 µg/ml) for 24 h, and cell viability was evaluated by MTT assay. Data were shown as means $\pm$ SD ($n = 3$). * compared with the control group, $p < 0.05$.

Fig 5.16 : The effect of combination 1:4 extract on (3T3-L1) cell line viability was determined by MTT assay method. Each bar graph represents % viability of (3T3-L1) cells against 98.70% to 5 µg/mL concentrations combination 1:4 of extract after 24 hrs exposure.

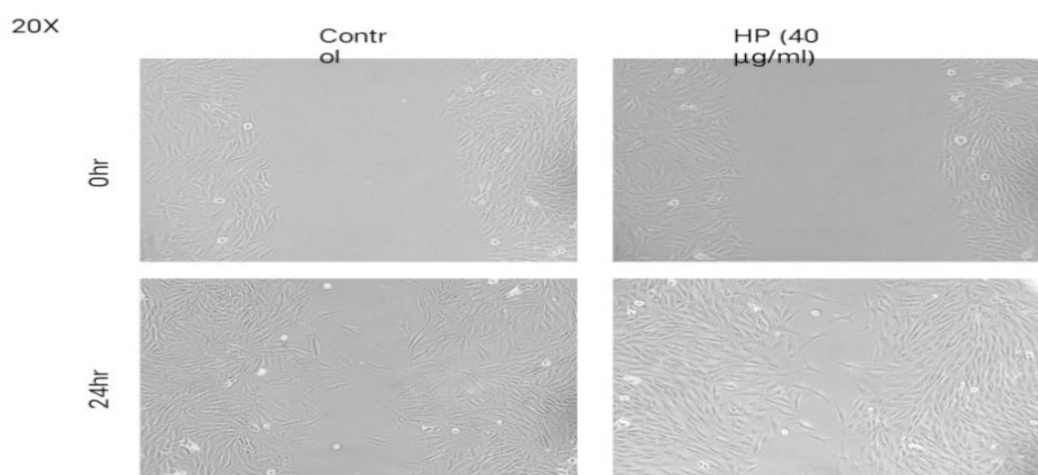


Fig 5.17: Images of (3T3-L1) cell line in inverted Phase contrast Microscopy after the exposure to combination 1:4 extract. Mouse fibroblast (3T3-L1) cells were injured and cell migration assay with and without treatment at 40 µg/mL was performed at 24 h.

DISCUSSION

For the purpose of creating AgNPs with strong antibacterial properties from plant extracts, such as leaf extract and fruit extract of *Ocimum Sanctum* (Tulsi) and *Carica Papaya* (Papaya). In the reactions with silver nitrate (AgNO_3), a frequently utilized precursor in the manufacture of silver nanoparticles, phytochemicals in the plant and fruit extract function as reducing and/or capping agents in these studies. Liquifying metallic silver nitrate in distilled water can explain the initial mechanism that occurred during the green synthesis method's production of silver nanoparticles. Silver nitrate first transforms into silver ions. When *Ocimum Sanctum* (Tulsi) and *Carica Papaya* (Papaya) was applied to AgNO_3 , the reduction process took place, resulting in the conversion of silver ions into free metallic atoms (Ag). where the distinctive transverse plasmon, which was a spherical silver nanoparticle, was similar to the absorption peaks that emerged at 460 nm as a result of the stimulation of local plasmon and the fluctuations of the transmission electrons of gold and silver nanoparticles.

Nano gel formation using guar gum is characterized by using XRD, TEM, FTIR Analysis. The pure silver nanoparticles were indicated by the angle (2θ) peak at 4.6902 as revealed by XRD analysis. Moreover, studies using X-ray diffraction demonstrated that these When *Ocimum Sanctum* (Tulsi) and *Carica Papaya* (Papaya) of the ratio combination 1:4-AgNPs (nanoparticles) were crystalline in nature. This TEM images of the combination 1:4-AgNPs (silver nanoparticles containing *Ocimum Sanctum* (Tulsi) and *Carica Papaya* (Papaya) revealed a spherical external structure and a homogeneous shape. Analysis of combination 1:4-AgNPs chemically revealed the presence of silver and other elements with silver masses. The average size of combination 1:4-AgNPs between 10 to 200 nm, which is consistent with results studies that reported combination 1:4-AgNPs to be 10 nm in size.

The absorption spectrum of the incubated solution at different wavelengths ranging within 200–800 nm revealed whereas *Ocimum Sanctum* (Tulsi) extract are the ratios of 1:4,2:3,2.5:2,3:2 is 420nm,440nm,460nm,450nm. No absorption peak was observed in UV–vis spectrum of Ag⁺ solution in the *Carica Papaya* (papaya) extract.

But *Carica Papaya* (papaya) extract peak observed in the ratio of 2.5:2 & 3:2 is 430nm and 450nm. Combination peak observed in the ratio of 1:4, 2:3&2.5:2 is 460nm, 440nm and 460nm. Antimicrobial activity assay of silver nanoparticles along with individual extract and Combination extract against *Escherichia coli*, *Staphylococcus aureus* 13, *Staphylococcus aureus* 23, *Enterococcus faecalis*, *Acinetobacter baumannii* by the well diffusion method. The minimum zone of inhibition shows *Ocimum Sanctum* (Tulsi) are the ratios of 1:4 is 16mm and 14mm against *Escherichia coli* and *Staphylococcus aureus* 23. The maximum zone of inhibition shows and Combination are the ratios of 1:4 is 20mm, 12mm and 15mm against *Escherichia coli*, *Staphylococcus aureus* 13, *Staphylococcus aureus* 23. Among the ratios, the ratio of 1:4 showed maximum zone of inhibition in Combination.

Further, proceed with Nano gel formation with the ratio of 1:4 combination. The FTIR spectrum for Combination 1:4 extract at showed the intensity at 107.399, 105.665, 52.5718, 71.7365, 88.8981, 91.9094, 89.4826 & 26.9465. The effect of the functional groups of the *Ocimum Sanctum* (Tulsi) and *Carica Papaya* (Papaya) extract in the ratio of Combination 1:4 as reducing and stabilizing agents to manufacture Ag-NPs is supported by all of the intensity alterations. At the maximum treatment concentration of Combination 1:4 extract, the percentage vitality of (3T3-L1) cells was observed to be 98.70% and the minimum treatment concentration was observed to be 56.40% in the invitro scratch assay. The prepared gel appears to have a positive impact on the healing of wounds against diabetic foot ulcer.

CHAPTER-6

SUMMARY& CONCLUSION

SUMMARY

Now days, the use of nanomaterials in wound care is expanding quickly. The current study has studied the mechanisms underlying the latest developments in nanomaterials that promote wound healing. Furthermore, light can be absorbed by AgNPs for wound healing and then converted into heat or reactive oxygen species (ROS), which kills the bacteria already present in the wounds. Moreover, AgNPs can be used to create a smart dressing for wounds infected by microorganisms, which can be used to cure these infections depending on endogenous triggers like pH, temperature, enzymes, and toxins released by the bacterium. In this study, using environmentally friendly method of producing silver nanoparticles by employing *Ocimum Sanctum* (tulsi) & *Carica Papaya* (papaya) extract for wound healing against diabetic foot ulcer. The reduction of silver nitrate with *Ocimum Sanctum* (tulsi) & *Carica Papaya* (papaya) extract is an easy, clean, effective, and room-temperature process for creating nanogels to cure diabetic foot ulcers. The extracts of *Carica papaya* (papaya) and *Ocimum Sanctum* (tulsi) have been utilized as reducing agents to create silver nanoparticles from silver nitrate. Green synthetic silver nanoparticles are verified by color shift, which was quantitatively observed at 460 nm using UV-Vis's spectroscopy. In Antibacterial activity shows that the Combination 1:4 against *Escherichia coli*, *Staphylococcus aureus* 13, *Staphylococcus aureus* 23, *Enterococcus faecalis*, *Acinetobacter baumannii*.. The structure, individual intensity of the produced nanoparticles, and bond stretch were all visible using FTIR. XRD shows broad, minor peaks at lower 2 θ theta values. Thus, it is evident from the XRD pattern that the crystalline quality of the silver nanoparticles produced by Combination 1:4 extract and TEM micrographs shows clear particle size of AgNPs is 10 nm. At the maximum treatment concentration of Combination 1:4 extract, the percentage vitality of (3T3-L1) cells was observed to be 98.70% and the minimum treatment concentration was observed to be 56.40% in the invitro scratch assay.

CONCLUSION

Extracellular AgNPs are produced by *Ocimum Sanctum* (Tulsi) & *Carica Papaya* (papaya) extract and have good stability power in the fluid. In order to combat E. Coli, which is known to be resistant to the majority of commercially available medications, the produced silver nanoparticles were used as an active substrate. With regard to bacteria that are resistant to many medications, this environmentally friendly manufacturing of silver nanoparticles may prove to be a rapid, inexpensive, and useful substitute for synthetic antibiotics. *Ocimum Sanctum* (Tulsi) and *Carica Papaya* (Papaya) was used in the green synthesis of silver nanoparticles, and the results showed that the plant has strong antibacterial action against *Escherichia coli*, *Staphylococcus aureus* 13, *Staphylococcus aureus* 23. The findings, in summary, *Ocimum Sanctum* (Tulsi) and *Carica Papaya* (Papaya) showed that extract can be used to efficiently, quickly, and simply create silver nanoparticles with strong antibacterial qualities. The prepared gel appears to have a positive impact on the healing of wounds against diabetic foot ulcer. In conclusion, the production of silver nanoparticles through biosynthesis has emerged as a highly promising option for medical applications where antibacterial action is crucial.

CHAPTER-6

REFERENCE

American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* .2007;30 Suppl. 1: S42-7.

Ahmad, M. Z., Alasiri, A., Ahmad, J., Alqahtani, A. A., Abdullah, M., Abdel-Wahab, B. A., Pathak, K., Saikia, R., Das, A., Sarma, H., & Alzahrani, S. A. Green Synthesis of Titanium Dioxide Nanoparticles Using *Ocimum sanctum* Leaf Extract: In Vitro Characterization and Its Healing Efficacy in Diabetic Wounds. *Molecules* .2022; 27(22), 7712.

Abd Elgadir.M, Aishah Adam. An in vitro evaluation of films prepared from gelatin –Carica papaya methanolic extract for wound healing, *International Journal of Drug Delivery*. 2013,233-238.

Banerjee, P., Satapathy, M., Mukhopadhyay. Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis. *Bioresour. Bioprocess*. 2014; 27(22), 7712.

Chen, X.; Zheng, Z.; Ke, X.; Jaatinen, E.; Xie, T.; Wang, D.; Guo, C.; Zhao, J.; Zhu, H. Supported Silver Nanoparticles as Photocatalysts under Ultraviolet and VisibleLight Irradiation. *Green Chemistry* .2010; 12, 414-419.

Chen, S., bin Abdul Rahim, A.A., Mok, P. An effective device to enable consistent scratches for in vitro scratch assays .2023; 23, 32.

Devanesan, S., Jayamala, M., AlSalhi, M. S., Sankaran, U., & Ranjitsingh, A. J. A. Antimicrobial and anticancer properties of Carica papaya leaves derived di-methyl flubendazole mediated silver nanoparticles. 2021; 14(5), 577–587.

Dulta, K., Ağçeli, G. K., Chauhan, P. K., Jasrotia, R., & Chauhan, P. K.. Ecofriendly Synthesis of Zinc Oxide Nanoparticles by Carica papaya Leaf Extract and Their Applications.2021; 33(2), 603–617.

Frykberg RG. Diabetic Foot Ulcers: Pathogenesis and Management. Am Fam Physician.2021; 66(9): 1655-62

Faujdar, C., & Priyadarshini, S. R. Evaluation of In-vitro Cytoprotective, Wound Healing and Antioxidant Effects of Ocimum sanctum Leaf Extract Chetna. *Current Trends in Biotechnology and Pharmacy*, 2021;15(3), 248–255.

GBD Diabetes in the Americas Collaborators. Global Burden of diabetes (GDB) and hyperglycaemia in adults in the Americas, 1990-2019: a systematic analysis for the Global Burden of Disease Study .2019;10:655-667.

Grunfeld C. Diabetic foot ulcers: etiology, treatment and prevention. Adv Intern Med .1991; 37:103–32

Gaikwad, S., Birla, S., Ingle, A. P., Gade, A., Ingle, P., Golińska, P., & Rai, M. Superior in vivo Wound-Healing Activity of Mycosynthesized Silver Nanogel on Different Wound Models in Rat. 2022; 13.

Gautam, D., Dolma, K. G., Khandelwal, B., Gupta, M., Singh, M., Mahboob, T., Teotia, A., Thota, P., Bhattacharya, J., Goyal, R. K., Oliveira, S. M. R., De Lourdes Pereira, M., Wiart, C., Wilairatana, P., Eawsakul, K., Rahmatullah, M., Saravanabhavan, S. S., & Nissapatorn, V. Green synthesis of silver nanoparticles using Ocimum sanctum Linn. and its antibacterial activity against multidrug resistant Acinetobacter baumannii. PeerJ, 11.2023;15590.

Gomathi, D., Kalaiselvi, M., Ravikumar, G., Devaki, K., & Uma, C. GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of Evolvulus alsinoides (L.) L. *Journal of Food Science and Technology*. 2013;52(2), 1212–1217.

Gul, R., Jan, S. U., Faridullah, S., Sherani, S., & Jahan, N. Preliminary Phytochemical Screening, Quantitative Analysis of Alkaloids, and Antioxidant Activity of Crude Plant Extracts from Ephedra intermedia Indigenous to Balochistan. *The Scientific World Journal*.2017; 1–7.

International Diabetes Federation. The global burden. IDF diabetes atlas. 5th ed. 2012;15590.

Jahangir, G. Z., Anjum, T., Rashid, N., Sadiq, M., Farooq, R., Akhtar, M. N., Hussain, S. F., Iftikhar, A., Saleem, M., & Shaikh, R. S. Carica papaya Crude Extracts Are an Efficient Source of Environmentally Friendly Biogenic Synthesizers of Silver Nanoparticles. *Sustainability*.2023;15(24), 16633

Jemal, K., Sandeep, B. V., & Pola, S. Synthesis, characterization, and evaluation of the antibacterial activity of Allophylus serratus Leaf and leaf derived callus extracts mediated silver nanoparticles. *Journal of Nanomaterials*. 2017;1–11.

Kwaben, D. E., Wee, B. S., Chin, S. F., Kuan, Y. K., Zaini, B. A., & Aquisman, A. E. Comparative Evaluation of Antibacterial Efficacy of Biological Synthesis of ZnO Nanoparticles Using Fresh Leaf Extract and Fresh Stem-Bark of Carica papaya. *Nano Biomedicine and Engineering*.2019;11(3).

Manish Kumar Gautam and Raj Kumar Goel. Wound Healing Effect of Ocimum sanctum Leaves Extract in Diabetic Rat. World Academy of Science, Engineering and Technology. 2013; 81.

Nimmy K Francis, Harpreet S Pawar, Nabam Peter, Gaurav Biswas, Jayjeet Majumdar, Anirban Mitra and Analava Mitra. Evaluation of wound healing and antimicrobial physiognomiesof alcoholic extract of Ocimum sanctum (basil) in diabetic rabbit model, International Journalof Herbal Medicine 2017; 5(2): 91-97.

Nugroho, A., Hesty, H., Choi, J. S., & Park, H. J. Identification and quantification of flavonoids in Carica papaya leaf and peroxynitrite-scavenging activity. *Asian Pacific Journal of Tropical Biomedicine*, 2017; 7(3), 208–213.

Oluwafemi O. Oguntibeju. Medicinal plants and their effects on diabetic wound healing, 2019;12: 653-63.

Pattanayak, P., Behera, P., Das, D., & Panda, S. K. (2010). Ocimum sanctum Linn. A reservoir plant for therapeutic applications: An overview. *Pharmacognosy Reviews*, 2010; 4(7), 95.

Radhakrishna K, Karri NR, Baskaran M, Kuppusamy G. Potential Use of Herbal Medicines in the Treatment of Diabetic Foot Ulcers. *Medical Science*, 2014; 14(56): 34-42

Risks, R. Promoting Healthy Life. The world health report 2002.

Reidy, B.; Haase, A.; Luch, A.; Dawson, K.; Lynch, I. Mechanisms of Silver Nanoparticle Release, Transformation and Toxicity: A Critical Review of Current Knowledge and Recommendations for Future Studies and Applications. *Materials*. 2013; 6, 2295-2350.

Raja, J., Maturana, M., Kayali, S., Khouzam, A. R., & Efeovbokhan, N. Diabetic foot ulcer: A comprehensive review of pathophysiology and management modalities. *World Journal of Clinical Cases*. 2023; 11(8), 1684–1693.

Rajaram, B., Venkanna, M., Kumaraswamy, B., Kumar, D. R., Maripeddi, K., Puligilla, S., & Reddy, S. THE ROLE OF PAPAYA DRESSINGS IN THE MANAGEMENT OF DIABETIC FOOT ULCERS: a PROSPECTIVE STUDY. *Journal of Evidence Based Medicine and Healthcare*. 2015; 12: 653-63.

Rabari Yash BA, Singh Rakesh BB, Prasad D.V.C, Abraham Arun Alex B. ROLE OF PAPAYA (CARICA PAPAYA) DRESSINGS IN THE MANAGEMENT OF CHRONIC ULCER, *National Journal of Medical and Dental Research*, July-Sep. 2016; Volume-4, Issue-4, Page 329-332.

Rizwan, M., Rasheed, Z., Mirza, N. K., Naz, G., Sayyed, S., Jameel, M. K., & Hameed, F. Comparison of hydrogel and papaya dressings in the treatment of diabetic foot ulcers. *Pakistan Journal of Medical and Health Sciences*. 2023; 17(1), 740–742.

Ramdas, V. M., Botha, I., Krishna, S., & Mbatha, J. N. Enhancing antidiabetic and antimicrobial performance of *Ocimum basilicum*, and *Ocimum sanctum* (L.) using silver nanoparticles. *Saudi Journal of Biological Sciences*. 2017; 24(6), 1294–1305.

Srivastava, A. K., Subhashini, Pandey, V., Yadav, V., Singh, S., & Srivastava, R. Potential of hydroethanolic leaf extract of *Ocimum sanctum* in ameliorating redox status and lung injury in COPD: an in vivo and in silico study. *Scientific Reports*.2023;13(1).

Shetty, S., Udupa, S. L., & Udupa, L. A. Evaluation of Antioxidant and Wound Healing Effects of Alcoholic and Aqueous Extract of *Ocimum sanctum* Linnin Rats. *Evidence-based Complementar and Alternative Medicine*.2008; 5(1),95–101.

Singhal, G., Bhavesh, R., Kasariya, K., Sharma, A. R., & Singh, V. Biosynthesis of silver nanoparticles using *Ocimum sanctum* (Tulsi) leaf extract and screening its antimicrobial activity. *Journal of Nanoparticle Research*.2011;13(7), 2981–2988.

Singh, S. P., Kumar, S., Mathan, S. V., Tomar, M. S., Singh, R. K., Verma, P. K., Ankit, K., Kumar, S., Singh, R. P., & Acharya, A. Therapeutic application of Carica papaya leaf extract in the management of human diseases. *DARU Journal of Pharmaceutical Sciences*.2020; 28(2), 735–744.

Singh, M., Thakur, V., Kumar, V., Raj, M., Gupta, S., Devi, N., Upadhyay, S. K., Macho, M., Banerjee, A., Ewe, D., & Saurav, K. Silver Nanoparticles and its Mechanistic Insight for Chronic Wound Healing: Review on recent progress. *Molecules*.2022; 27(17), 5587.

Tuttolomondo A, Maida C, Pinto A. Diabetic Foot Syndrome as a Possible Cardiovascular Marker in Diabetic Patients. *J Diabetes Res.*, 2015; 2015: 01-12

Vasuki, V., Thanmaran, N. B., Vimala Karan, B., & Madan, K. Comparative study of papaya dressing versus normal saline dressing in healing of ulcers. *International Surgery Journal*. 2017;5883.

Whiting, D., Guariguata, L., Weil, C., & Shaw, J. E. IDF Diabetes Atlas: Global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes Research and Clinical Practice*.2011; 94(3), 311–321.

Yamani, H. A., Pang, E. C. K., Mantri, N., & Deighton, M. Antimicrobial Activity of Tulsi (*Ocimum tenuiflorum*) Essential Oil and Their Major Constituents against Three Species of Bacteria. *Frontiers in Microbiology*, 2016;7.

Yimam, A., Hailu, A., Murugan, R., & Gebretensaye, T. Prevalence of diabetic foot ulcer and associated factors among diabetic patient in Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia. *International Journal of Africa Nursing Sciences*.2021;14, 100285.

ANNEXURE-I

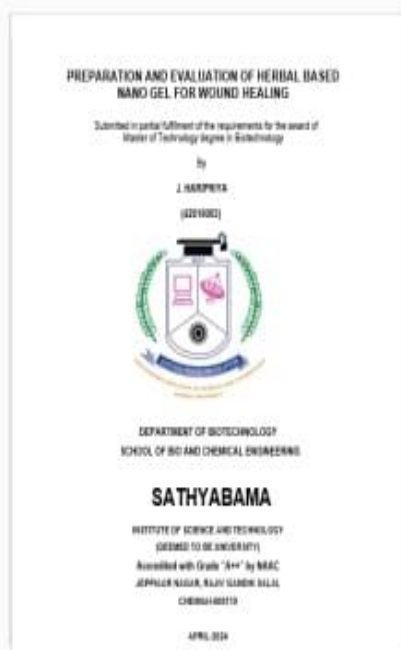


Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

Submission Author: Hari Priya
Submission Title: Hari
File name: PROJECT_THESIS.docx
File size: 4 MB
Page count: 70
Word count: 17,413
Character count: 120,165
Submission date: 15-April-2024 06:18PM (UTC-0700)
Submission ID: 2230032262



Hari

ORIGINALITY REPORT

10%

SIMILARITY INDEX

8%

INTERNET SOURCES

8%

PUBLICATIONS

2%

STUDENT PAPERS

PRIMARY SOURCES

1

worldwidescience.org

Internet Source

1%

2

sci-hub.se

Internet Source

1%

3

www.researchgate.net

Internet Source

2%

4

www.mdpi.com

Internet Source

2%

5

www.science.gov

Internet Source

2%

6

www.frontiersin.org

Internet Source

2%

ANNEXURE-II

Your GATE 2024 Result [BT]

Name

HARIPRIYA J

Registration Number

BT24S57402418

Gender

Female

Parent's/Guardian's name

JAIKUMAR K

Date of Birth (YYYY-MM-DD)

2000-12-14

Examination Paper

Biotechnology (BT)



Photograph



Signature

Marks out of 100[#]

18.33

Qualifying Marks^{##}

38.9

General

35

OBC-
NCL/EWS

25.9

SC/ST/PwD

[#]Normalized marks in case of multisession papers (CE and CS).

^{##}A candidate is considered qualified if the marks secured are greater than or equal to the qualifying marks mentioned for the category, for which a valid category certificate, if applicable, must be produced along with the Score Card.

[FAQ for GATE Score](#)

CLOSE

ANNEXURE-III

Effectiveness of Herbal Extraction for Wound Healing on Diabetic Foot Ulcer

J. Haripriya¹

¹PG student, Department of Biotechnology,
Sathyabama Institute of Science and Technology,
Jeppiaar Nagar, SH 49A,
Chennai, Tamil Nadu 600119, India

T. Rajasekar²

²Assistant professor- Research,
Department of Centre for Drug Discovery and Development,
Sathyabama Institute of Science and Technology,
Jeppiaar Nagar, SH 49A, Chennai,
Tamil Nadu 600119, India.

V. Ramesh Kumar³

³ Professor, Head of the Department of school of Bio and
Chemical Engineering, Sathyabama Institute of Science and
Technology, Jeppiaar Nagar, SH 49A, Chennai,
Tamil Nadu 600119, India.

Abstract:- A serious consequence of diabetes mellitus, diabetic foot ulcers (DFUs) may result in chronic, non-healing sores and increased morbidity. As a result of their resistance to traditional wound healing therapy, DFUs provide a substantial management problem in the healthcare industry. Diabetic foot ulcers (DFUs) pose a significant clinical challenge due to their slow healing and potential for severe complications. Untreated diabetic foot ulcers can result in serious side effects such as osteomyelitis, and, in extreme situations, gangrene, which might need amputation. Cleaning the wound, debridement (the removal of dead tissue), infection control, relieving pressure from the affected area, and other measures are frequently used to treat diabetic foot ulcers. Recent years have seen a rise in interest in herbal-based formulations as potential replacements for DFU wound healing. As the wound heals, several herbal ingredients may help to decrease scarring. The purpose of this review is to determine if a unique herbal extraction can effectively promote wound healing in individuals with diabetic foot ulcers. It's essential for wound healing to maintain the proper amount of moisture around the area. Herbal extracts can assist in controlling moisture and maintaining a healing-friendly environment around the area. Many herbal extracts with anti-inflammatory, antibacterial, and tissue-regenerating effects have been exposed as components in wound healing gels and cream form.

Keywords:- Diabetic foot ulcers (DFUs), DFU wound healing, debridement, anti-inflammatory, antibacterial.

I. INTRODUCTION

A typical chronic condition is diabetes mellitus. One of the most harmful effects of diabetes is diabetic foot ulceration (DFU). The three main factors that contribute to foot and lower leg ulcers are poorly controlled diabetes, distal circulation issues, and neurological impairment. Amputations of the toes, a portion of the foot, or the lower leg may be necessary in severe situations [1]. There are 422 million people with diabetes mellitus globally, and it is thought to be the cause of 2 million fatalities per year [2]. Amputation of the ulcerated foot is necessary in 14%–24%

of diabetics who develop one of these ulcers over time due to complications such as bone infection or other ulcer-related issues. Around 15% of people with diabetes will eventually develop one of these ulcers [3]. Neuropathy, which includes distal symmetrical polyneuropathy (DSPN), mono-neuropathies, and a number of autonomic neuropathies that include erectile dysfunction, urine incontinence, gastroparesis, and nocturnal diarrhoea, affects more than 60% of diabetes patients.

Diabetes mellitus accounts for about 50% of all non-traumatic amputations worldwide due to accelerated lower extremity vascular disease and neuropathy. In people with diabetes, the risk of cardiovascular problems is elevated by a factor of two to six. Because of the higher mortality from diabetic complications, the life expectancy is often 7 to 10 years shorter than for persons without diabetes mellitus. In both type 1 diabetes and type 2 diabetes, many prospective clinical investigations demonstrate a high correlation between glycemia and diabetic microvascular problems [4] [5]. When localized foot issues such as swelling, skin discoloration, discomfort, drainage, or ulceration initially emerge, DFUs should be considered [6] [7]. Cellulitis, myositis, abscesses, necrotizing fasciitis, septic arthritis, tendinitis, and osteomyelitis are among the several infection forms. All diabetic patients should expect thorough foot assessment and examination as a standard aspect of their care. More than half of patients with insensitive feet have no prior history of neuropathic symptoms, thus the clinician should never only depend on the emergence of symptoms to identify high-risk individuals [8].

Most diabetic foot infection (DFI) have a neuropathic DFU as their primary etiology. Therefore, the development of a DFU is frequently one of the first stages in the pathogenesis of DFI. The most important risk factor for DFU production is neuropathy (autonomic, sensory, and/or motor), which is brought on by long-term, poorly managed hyperglycemia [9].