

**SYNTHESIS OF NANOCURCUMIN FROM *CURCUMA
LONGA* AND EVALUATING THE EFFECT OF
TEMPERATURE, pH AND LIGHT OVER NANOCURCUMIN
PROJECT WORK**

Submitted in partial fulfillment of the requirements for the award of Bachelor of
Science degree in Microbiology

By

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**SATHYABAMA
INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)
CATEGORY-1 UNIVERSITY BY UGC**

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This is to certify that this project report is the bonafide work of **ANANTHIKA R (41300004)** and **PREETHI KUMARI A (41300030)** who carried out the project entitled "**SYNTHESIS OF NANOCURCUMIN FROM CURCUMA LONGA AND EVALUATING THE EFFECT OF TEMPERATURE, pH AND LIGHT OVER NANOCURCUMIN**" under our supervision from **January 2024 to April 2024**.

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ABSTRACT

Nanocurcumin, a nanoscale formulation derived from the natural polyphenol curcumin found in *Curcuma longa*, offers promising therapeutic potential due to its enhanced bioavailability and targeted delivery. This project focuses on the synthesis of Nanocurcumin from *Curcuma longa* and evaluates its response to environmental factors including temperature, pH and light exposure. Various synthesis techniques such as precipitation, emulsion and micellar methods are explored to prepare Nanocurcumin formulations with improved solubility and stability. The effects of temperature variations like high, low and room temperature on the physicochemical properties and stability of Nanocurcumin are investigated comprehensively. pH-dependent behavior under acidic, neutral and alkaline conditions is analyzed to understand Nanocurcumin formulations' release kinetics and therapeutic implications. Additionally, the influence of different light spectra including UV, visible and IR light on the photostability and degradation kinetics of Nanocurcumin is assessed. Recent advancements in Nanocurcumin research and potential applications in various biomedical fields are discussed along with future research directions. This project provides valuable insights into Nanocurcumin's synthesis, characterization and environmental effects, paving the way for its development as a promising therapeutic agent in drug delivery and disease management.

Keywords: Curcumin, Nanocurcumin, Extraction, UV, FTIR, Raman spectroscopy, Effects of parameters, Antimicrobial susceptibility test.

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LIST OF ABBREVIATIONS

FESEM	Field Emission Scanning Electron Microscopy
NMR	Nuclear Magnetic Resonance
FTIR	Fourier-transform infrared spectroscopy
AST	Antimicrobial Susceptibility Test
UV	Ultraviolet light
IR	Infrared light
b/w	Between
MIC	Minimum Inhibitory Concentration
NaOH	Sodium hydroxide
HCL	Hydrochloric acid
IMVIC	Indole, Methyl red, Voges Proskauer and Citrate test
COPD	Chronic Obstructive Pulmonary Disease
pH	Potential of Hydrogen
HMA	Mueller Hinton Agar
TSST	Toxic Shock Syndrome Toxin

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CHAPTER 1

INTRODUCTION

1.1 CURCUMIN

Turmeric which is popularly known as the golden spice of India has not only been used as a spice but has also emerged in the pharmacological field with various advantages throughout the world. Curcumin (diferuloylmethane) is a bright yellow colored naturally occurring hydrophobic and polyphenolic compound in turmeric that has a long history as a nutritional spice, food preservative and coloring agent (Maiti, 2007; Karthikeyan, 2020). This introduction presents an overview of curcumin its chemical makeup, uses, drawbacks extraction process as well and Nanoformulations with a focus on Nanocurcumin. Curcumin is a biologically active component that is significantly present in *Curcuma longa*. Curcumin has been known for its multiple activities which include antimicrobial, anti-inflammatory, antioxidant and anti-carcinogenic properties (Aggarwal and Hari Kumar, 2009).

1.2 CURCUMIN CHEMICAL COMPOSITION

Curcumin with its vibrant yellow-orange hue owes its color to a fascinating molecular structure. Comprising two aromatic rings linked by a seven-carbon chain, it forms a conjugated system of double bonds. This structural arrangement not only imparts its distinctive color but also influences its stability and reactivity (Aggarwal *et al.*, 2007). Within this structure, curcumin harbors two phenolic hydroxyl groups and two methoxy groups attached to the aromatic rings. These functional groups play a pivotal role in curcumin's antioxidant properties, enabling it to scavenge free radicals and mitigate oxidative stress in biological systems (Gupta *et al.*, 2011). The molecular formula of curcumin, $C_{21}H_{20}O_6$ encapsulates its chemical composition, providing crucial insights into its stoichiometry and molecular structure. Furthermore, the molecular weight of 368.38 g/mol underscores its compact yet complex nature. It is through this chemical makeup that curcumin exerts its therapeutic effects, ranging from anti-inflammatory and antimicrobial properties to neuroprotective and anticancer activities (Jurenka, 2009; Hewlings & Kalman, 2017).

In recent years, Nanocurcumin has emerged as a promising avenue for improving curcumin's delivery and efficacy. Nanoformulation techniques such as nanoparticle encapsulation and lipid-based carriers have shown promise in enhancing curcumin's solubility, stability and targeted delivery. These advances in formulation technology hold the potential to overcome the limitations associated with conventional curcumin formulation and unlock new opportunities for therapeutic applications. Moreover, improvements in analytical methods like nuclear magnetic resonance (NMR) spectroscopy, UV-spectroscopy, FTIR and X-ray crystallography have enabled researchers to elucidate the finer details of curcumin's chemical structure. These insights into curcumin's molecular architecture facilitate rational drug design and optimization, paving the way for the development of novel curcumin derivatives with enhanced bioactivity and pharmacokinetic properties.

The chemical composition of curcumin serves as the foundation for its diverse pharmacological activities and therapeutic potential. Understanding the intricacies of its molecular structure and functional groups is essential for harnessing its full therapeutic benefits. Further research into curcumin's chemical composition and its implications for drug delivery and formulation holds promise for the development of innovative treatments for various diseases and disorders.

1.3 CURCUMIN USES

Curcumin, with its diverse pharmacological properties, has been utilized for centuries in traditional medicine systems and modern research continues to unveil its potential therapeutic applications. This section explores the wide-ranging uses of curcumin, encompassing its traditional roles as well as its emerging applications in modern medicine.

In traditional Ayurveda and Chinese medicine, turmeric, the source of curcumin has been revered for its medicinal properties. It has been used to treat many conditions like ailments including digestive disorders, respiratory conditions and inflammatory diseases. Curcumin-rich turmeric preparations have been used topically for wound healing and skin conditions as well as internally to support overall health and well-being (Gupta *et al.*, 2011; Hewlings & Kalman, 2017).

In recent years, scientific research has uncovered a myriad of potential therapeutic applications for curcumin. Its anti-inflammatory properties make it a

promising candidate for the management of chronic inflammatory conditions such as arthritis, inflammatory bowel disease and neurodegenerative disorders. Curcumin's antioxidant activity also suggests potential benefits in combating oxidative stress-related diseases including cardiovascular diseases and age-related cognitive decline (Aggarwal *et al.*, 2007; Jurenka, 2009). Furthermore, curcumin has garnered attention for its anticancer properties, with studies demonstrating its ability to inhibit tumor growth, induce apoptosis in cancer cells and modulate various signaling pathways implicated in cancer progression. Additionally, curcumin's antimicrobial activity makes it a valuable agent for combating infectious illnesses brought on by fungi, viruses and bacteria. In addition to its established therapeutic uses, ongoing research is exploring novel applications of curcumin in areas such as wound healing, skin disorders and metabolic diseases. Nano-curcumin formulations are being investigated for their potential to enhance curcumin's bioavailability and targeted delivery, opening up new possibilities for personalized medicine and precision therapeutics (Lestari *et al.*, 2014).

1.4 DRAWBACKS OF CURCUMIN

While curcumin exhibits remarkable pharmacological properties and therapeutic potential, it has limitations. This section examines the drawbacks associated with curcumin including its poor bioavailability, stability issues, potential side effects and challenges in clinical translation.

1.4.1 Poor bioavailability

One of the major challenges in harnessing the therapeutic benefits of curcumin is its poor bioavailability. Curcumin has low water solubility and undergoes rapid metabolism and elimination in the body, leading to limited absorption and systemic exposure (Anand *et al.*, 2007). As a result, achieving therapeutic plasma concentrations of curcumin through oral administration is challenging. This poor bioavailability necessitates the use of high doses or alternative delivery strategies to overcome absorption barriers and enhance curcumin's efficacy.

Various approaches have been explored to improve curcumin bioavailability including the development of novel formulations such as nanoparticles, liposomes, micelles and cyclodextrin complexes (Yadav *et al.*, 2012). These delivery systems

aim to enhance curcumin solubility, stability and absorption, thereby maximizing its therapeutic potential. Additionally, co-administration with bio-enhancers such as piperine, found in black pepper has been shown to enhance curcumin bioavailability by inhibiting its rapid metabolism and increasing gastrointestinal absorption (Shoba *et al.*, 1998).

1.4.2 Stability issues

Curcumin is susceptible to degradation under various environmental conditions including exposure to light, heat, oxygen and pH fluctuations. Degradation products such as vanillin and ferulic acid may form during storage or processing, compromising curcumin's potency and efficacy (Gupta *et al.*, 2013). Additionally, curcumin may undergo chemical modifications such as oxidation, conjugation and hydrolysis, which can affect its bioactivity and therapeutic properties.

Strategies to enhance curcumin stability include encapsulation in protective matrices such as liposomes, polymers and proteins, which shield curcumin from degradation and improve its stability during storage and transportation (Anand *et al.*, 2017). Furthermore, the addition of natural antioxidants such as tocopherols and ascorbic acid can help mitigate oxidative degradation and preserve curcumin's integrity.

1.4.3 Potential side effects

While curcumin is generally considered safe at recommended doses, high doses or prolonged use may lead to adverse effects in some individuals. Gastrointestinal symptoms like nausea, diarrhea and abdominal pain have been recorded with highdose curcumin supplementation. Moreover, curcumin may interact with certain medications, particularly anticoagulants and antiplatelet drugs, increasing the risk of bleeding (Hewlings & Kalman, 2017).

1.5 NANOFORMULATION OF CURCUMIN

Nanoformulation represents a promising approach to overcome the limitations of conventional curcumin delivery, including poor bioavailability and

stability. This section explores the principles and potential applications of Nanoformulation in enhancing the therapeutic efficacy of curcumin.

1.6 PRINCIPLES OF NANOFORMULATION

Nanoformulation involves the encapsulation of curcumin molecules within nanosized carriers such as nanoparticles, liposomes, micelles and Nano gels, to improve their solubility, stability and bioavailability. These carriers provide a protective environment for curcumin, shielding it from degradation and facilitating controlled release at the target site (Kumar *et al.*, 2019). Nanoformulation exploits various physicochemical properties including particle size, surface charge and surface chemistry, to optimize drug delivery and enhance therapeutic outcomes.

The selection of appropriate nano-carriers is crucial in nanoformulation to ensure compatibility with curcumin and desired drug release kinetics. Nanoparticles composed of polymers, lipids, or inorganic materials, offer versatility in encapsulating hydrophobic curcumin molecules and controlling their release profile. Liposomes, consisting of phospholipid bilayers, provide a biocompatible and biodegradable platform for curcumin delivery, enabling both hydrophilic and lipophilic drug encapsulation. Micelles, formed by amphiphilic surfactants, enhance curcumin solubility and stability in aqueous environments, promoting its systemic circulation and tissue penetration. Nano gels, cross-linked hydrogel networks, offer sustained release and site-specific targeting capabilities, making them suitable for localized delivery of curcumin to diseased tissues or organs.

1.7 APPLICATION OF NANOFORMULATION

Nanoformulation of curcumin offers a versatile platform for targeted drug delivery and site-specific release, enabling enhanced therapeutic efficacy and reduced systemic toxicity. Nano-sized carriers can encapsulate curcumin and facilitate its transport across biological barriers such as the blood-brain barrier, to target specific tissues or organs affected by disease (Liu *et al.*, 2020). Additionally, Nanoformulated curcumin can exhibit prolonged circulation time in the bloodstream, leading to sustained release and prolonged therapeutic effects.

Moreover, Nanoformulation allows for the co-delivery of curcumin with other therapeutic agents such as anticancer drugs, antioxidants and anti-inflammatory

agents, to synergistically enhance treatment outcomes and overcome drug resistance (Rai *et al.*, 2020). Co-encapsulation of curcumin with complementary compounds can potentiate its pharmacological effects and broaden its therapeutic applications in various disease conditions including cancer, inflammation, neurodegenerative disorders and metabolic diseases.

1.8 CHALLENGES AND FUTURE PERSPECTIVES

Despite the promising potential of nanoformulated curcumin, several obstacles remain to be resolved for clinical translation and commercialization. These include scalability of production, reproducibility of formulations, regulatory considerations and long-term safety assessment (Yallapu *et al.*, 2019). Additionally, the selection of appropriate Nano-carriers, formulation parameters and manufacturing techniques must be optimized to ensure optimal drug loading, stability and release kinetics.

1.9 NANOCURCUMIN STRUCTURE

Nanocurcumin is a nanoformulation of curcumin that exhibits distinct structural characteristics compared to its conventional counterpart. This section explores the structural features of Nanocurcumin and their implications for its pharmacokinetics, bioavailability and therapeutic efficacy.

1.10 NANO-SIZED PARTICLES

Nanocurcumin is composed of curcumin molecules encapsulated within nanosized carriers such as nanoparticles, liposomes or micelles. These carriers serve as protective matrices that entrap curcumin molecules, preventing their degradation and enhancing their stability in biological environments (Shen *et al.*, 2019). The nano-sized particles provide a large surface area-to-volume ratio, facilitating efficient drug loading and controlled release of curcumin.

1.11 CORE-SHELL STRUCTURES

Many nanocurcumin formulations exhibit a core-shell structure where curcumin molecules are encapsulated within a core material and surrounded by a shell or coating layer. The core material is often composed of polymers or lipids and

serves as a reservoir for curcumin while the shell provides stability and facilitates controlled release of the drug (Sahu *et al.*, 2020). This core-shell structure enables sustained drug release and prolonged circulation time in the bloodstream thereby enhancing the bioavailability and therapeutic efficacy of Nanocurcumin.

1.12 SURFACE MODIFICATIONS

Surface modification of Nanocurcumin particles allows for the introduction of functional groups or ligands that confer specific properties such as targeting ligands for site-specific delivery or stealth coatings for prolonged circulation in the bloodstream. Surface-modified Nanocurcumin particles can be engineered to target specific tissues, organs or cellular receptors, thereby enhancing drug accumulation at the site of action and minimizing off-target effects (Khan *et al.*, 2021). Additionally, surface modification can improve colloidal stability, biocompatibility and cellular uptake of nano curcumin, further enhancing its therapeutic potential.

1.13 ADVANTAGES OF NANOCURCUMIN

Nanocurcumin offers a plethora of advantages over conventional curcumin formulations, making it a promising candidate for various biomedical applications. This section further explores and elaborates on the distinct advantages of Nanocurcumin, highlighting their significance in improving therapeutic outcomes and addressing unmet medical needs.

1.14 ENHANCED CELLULAR UPTAKE

Nanocurcumin formulations exhibit superior cellular uptake compared to free curcumin, owing to their Nano-sized dimensions and enhanced solubility. The small particle size of Nanocurcumin enables efficient penetration of biological barriers such as cell membranes, leading to increased intracellular concentrations and enhanced pharmacological effects (Khan *et al.*, 2020). This enhanced cellular uptake enhances curcumin's bioavailability and efficacy, thereby enabling potent modulation of intracellular signaling pathways and biological processes involved in disease pathogenesis.

1.15 SYNERGISTIC EFFECTS WITH OTHER THERAPEUTICS

Nanocurcumin can be co-formulated with other therapeutic agents such as chemotherapeutic drugs, antioxidants and anti-inflammatory agents to synergistically enhance the treatment outcomes. Co-delivery of curcumin with complementary compounds allows the simultaneous targeting of multiple disease pathways or molecular targets, leading to enhanced therapeutic efficacy and reduced drug resistance (Chen *et al.*, 2021). Synergistic combinations of Nanocurcumin with other therapeutics hold promise for overcoming treatment resistance and improving patient outcomes in various diseases including cancer, inflammatory disorders and neurodegenerative diseases.

1.16 BIOCOMPATIBILITY AND SAFETY

Nanocurcumin formulations exhibit excellent biocompatibility and safety profiles, minimizing adverse effects and toxicity associated with conventional curcumin formulations. The biodegradable and biocompatible nature of Nano-carriers used in Nanocurcumin formulations ensures minimal immunogenicity and cytotoxicity thereby making them suitable for clinical applications (Jain *et al.*, 2020). Furthermore, the controlled release kinetics of Nanocurcumin minimize fluctuations in plasma drug levels, reducing the risk of systemic toxicity and enhancing patient tolerance and compliance.

1.17 VERSATILE FORMULATION OPTIONS

Nanocurcumin offers versatile formulation options to meet diverse therapeutic needs and application requirements. Nano-carriers can be tailored to optimize drug loading capacity, release kinetics and target specificity, enabling customized formulations for specific disease conditions or patient populations (Pandey *et al.*, 2021). Moreover, Nanocurcumin formulations can be adapted for various routes of administration including oral, intravenous, transdermal and intranasal delivery, providing flexibility and convenience in treatment regimens.

1.18 ENVIRONMENTAL SUSTAINABILITY

Nanocurcumin formulations present environmentally sustainable alternatives to conventional drug delivery systems with reduced resource

consumption, waste generation and environmental impact. The use of biodegradable and biocompatible materials in Nano-carriers minimizes environmental pollution and the ecological footprint associated with drug manufacturing and disposal (Manju & Sreenivasan, 2011). Additionally, the efficient drug delivery and targeted release afforded by Nanocurcumin formulations contribute to reduced drug dosages and improved treatment outcomes, further enhancing their environmental sustainability.

1.19 DIFFERENCE BETWEEN CURCUMIN AND NANOCURCUMIN

Curcumin and Nanocurcumin represent distinct formulations of the same bioactive compound, each offering unique characteristics and advantages. This section compares and contrasts the key differences between curcumin and nanocurcumin in terms of their structure, properties, pharmacokinetics and therapeutic applications.

1.20 STRUCTURAL DIFFERENCE

Curcumin is a naturally occurring polyphenolic compound derived from the turmeric plant (*Curcuma longa*). It is characterized by its lipophilic nature and limited aqueous solubility, which poses challenges for its formulation and delivery. Curcumin exists in various chemical forms including curcumin I, curcumin II and curcumin III, each with distinct structural configurations and bioactivities (Aggarwal *et al.*, 2007). Nanocurcumin, on the other hand, refers to curcumin formulated into nano-sized carriers such as nanoparticles, liposomes, micelles or nano-gels. These nanocarriers encapsulate the curcumin molecules providing protection, stability and enhanced delivery to target tissues or organs. Nanocurcumin formulations may exhibit core-shell structures, surface modifications and controlled release properties, which differentiate them from free curcumin (Yallapu *et al.*, 2019).

1.21 PROPERTIES AND PHARMACOKINETICS

Curcumin exhibits poor aqueous solubility, low bioavailability and rapid metabolism in vivo, limiting its therapeutic efficacy and clinical translation. Free curcumin is prone to degradation under various environmental conditions including

light, heat and pH fluctuations, leading to reduced stability and potency (Anand *et al.*, 2007). In contrast, Nanocurcumin formulations overcome the limitations of free curcumin by improving its solubility, stability and bioavailability. Nano-sized carriers protect curcumin from degradation, facilitate its transport across biological barriers and enable targeted delivery to specific tissues or cells. Nano curcumin exhibits enhanced cellular uptake, prolonged circulation time, and controlled release kinetics compared to free curcumin, leading to improved pharmacokinetics and therapeutic outcomes (Sharma *et al.*, 2020).

Nanocurcumin formulations offer advantages over free curcumin in terms of targeted delivery, sustained release and enhanced efficacy, making them attractive candidates for drug development and biomedical applications. Nanocurcumin has been investigated for the treatment of cancer, inflammatory diseases, neurodegenerative disorders, metabolic syndrome and cardiovascular diseases, demonstrating promising results in preclinical studies and clinical trials (Ganeshpurkar *et al.*, 2021).

1.22 THERAPEUTICS APPLICATIONS

Both curcumin and nanocurcumin possess pharmacological properties with diverse therapeutic applications including anti-inflammatory, antioxidant, anticancer, neuroprotective, and cardio-protective effects. Free curcumin has been extensively studied for its therapeutic potential in various disease conditions but its clinical utility is limited by poor bioavailability and stability (Hewlings & Kalman, 2017).

Nanocurcumin formulations offer advantages over free curcumin in terms of targeted delivery, sustained release and enhanced efficacy, making them attractive candidates for drug development and biomedical applications. Nanocurcumin has been looked into for the treatment of carcinogenic activity, inflammatory illnesses, neurodegenerative disorders, metabolic syndrome and cardiovascular diseases demonstrating promising results in preclinical studies and clinical trials (Ganeshpurkar *et al.*, 2021).

1.23 EFFECTS OF LIGHT, pH AND TEMPERATURE OVER CURCUMIN AND NANOCURCUMIN

Exposure to light can have a significant impact on the stability and degradation of curcumin which is a primary bioactive compound found in turmeric. Curcumin is known to be photosensitive which means it can degrade upon exposure to light, particularly ultraviolet (UV) light (Gupta *et al.*, 2012). When curcumin is formulated into nanoparticles known as nano curcumin, its stability against light degradation is improved. The encapsulation of curcumin in nanoparticle structures can protect against photodegradation thereby enhancing its stability and extending its shelf-life (Anand *et al.*, 2010).

1.23.1 Effect of pH on curcumin

Curcumin is relatively stable under neutral to slightly acidic conditions. However, it can undergo degradation under extreme pH conditions especially alkaline conditions. At high pH levels, curcumin can undergo hydrolysis which leads to the formation of degradation products that may have reduced bioactivity.

1.23.2 Effect of pH on nanocurcumin

Nanocurcumin formulations can be designed to be stable over a broader pH range compared to free curcumin. The encapsulation of curcumin in nanoparticles can protect it from the external environment including pH-induced degradation. The nanoparticle structure can maintain the stability of curcumin even under varying pH conditions ensuring better bioavailability and therapeutic efficacy (Yallapu *et al.*, 2012).

1.23.3 Effect of temperature on curcumin

Temperature plays a significant role in the stability of curcumin. High temperatures can accelerate the degradation of curcumin leading to reduced bioactivity. Curcumin is relatively stable at room temperature; however, exposure to elevated temperatures can result in degradation and the formation of degradation products, affecting its therapeutic efficacy (Aggarwal & Harikumar, 2009).

1.23.4 Effect of temperature on nanocurcumin

Nanocurcumin formulations offer enhanced stability against temperature-induced degradation compared to free curcumin. The encapsulation of curcumin in nanoparticles can protect against thermal degradation, preserving its bioactivity and improving its stability under varying temperature conditions (Bisht *et al.*, 2007).

1.23.5 Effect of light on curcumin and nanocurcumin

Curcumin is photosensitive and can degrade upon exposure to light, particularly ultraviolet (UV) light. This photodegradation can lead to a reduction in curcumin's therapeutic properties. Nanocurcumin however, exhibits improved stability against light-induced degradation due to its nanoparticle formulation. The encapsulation of curcumin in nanoparticles protects against photodegradation thereby maintaining its bioactivity and extending its shelf-life (Anand *et al.*, 2010).

In summary, Curcumin and Nanocurcumin represent distinct formulations of the same bioactive compound, each with unique characteristics and advantages. While curcumin exhibits inherent limitations in terms of solubility, stability and bioavailability, Nanocurcumin formulations overcome these challenges and offer enhanced pharmacokinetics and therapeutic efficacy. Nanocurcumin holds great promise for the development of novel drug delivery systems and personalized medicine approaches, offering tailored solutions for various biomedical applications.

CHAPTER 2

LITERATURE SURVEY

Recent years have seen a major increase in interest in curcumin, a bioactive polyphenolic molecule derived from the rhizomes of *Curcuma longa*, due to its varied pharmacological properties which include anti-inflammatory, antioxidant, antibacterial and anticancer effects (Manju and Sreenivasan, 2011). Curcumin's medicinal efficacy is limited in clinical applications due to its low bioavailability and poor water solubility. By improving solubility, stability and targeted administration, nanocurcumin produced using a variety of synthetic techniques offers potentially effective ways to address these issues.

2.1 CONVENTIONAL EXTRACTION TECHNIQUES

To extract curcumin from turmeric rhizomes, conventional techniques like soxhlet extraction, maceration and steam distillation have been applied extensively. (Gupta and Misra, 2019). These techniques provide extracts rich in curcumin that can be processed further by extracting the material using a solvent and then evaporating the solvent.

2.2 CHEMICAL SYNTHESIS

Curcumin structure and purity can be precisely controlled by chemical synthesis methods. Recent developments in chemical synthesis methods such as condensation reactions and alterations of natural substrates (Singh and Pai, 2019). These techniques make it possible to create curcumin analogs with improved pharmacokinetic and bioactive characteristics.

2.3 BIOTECHNOLOGICAL APPROACHES

Growing curcumin sustainably is possible with the use of biotechnological techniques like plant cell culture and microbial fermentation. Biotechnological approaches that offer benefits including better yields, less environmental effect and predictable production conditions are emphasized as viable methods for producing curcumin on a large scale (Mishra and Singh, 2016).

2.4 SYNTHESIS TECHNIQUES FOR NANOCURCUMIN

2.4.1 Precipitation techniques

Solvent shifting and anti-solvent precipitation are two techniques that can be used to produce nanocurcumin. The precipitation of curcumin nanoparticles from emulsions shows better stability and control over particle size. These techniques allow for the synthesis of Nanocurcumin with improved therapeutic efficacy and bioavailability (Ahmadi and Nourbakhsh, 2019).

2.4.2 Emulsion techniques

For the synthesis of nano curcumin, emulsion-based techniques such as sonication and high-pressure homogenization are frequently used. Nanoemulsions which have improved solubility, stability and controlled release qualities can be used as efficient delivery systems for curcumin. (Tan and Li, 2017). Scalable methods for creating Nanocurcumin formulations appropriate for a range of pharmacological and biological uses are provided by emulsion procedures.

To improve the curcumin molecule's water solubility and stability, curcumin molecules are encapsulated within surfactant micelles by the use of micellar solubilization techniques showing enhanced dispersibility and bioavailability. Micellar techniques provide easy and affordable ways to prepare Nano curcumin that can be used in both industrial and research settings (Kurniati and Utami, 2020).

2.5 EFFECTS OF TEMPERATURE

The stability and efficacy of curcumin and nano curcumin are significantly influenced by temperature. Many studies were conducted on the impacts of varying temperature ranges including high, low and room temperature.

2.5.1 High temperature

Curcumin can degrade more quickly at temperatures over 40°C, which can result in lower stability and bioactivity (Aggarwal *et al.*, 2006). For Nanocurcumin, higher synthesis temperatures (> 60°C) can improve reaction kinetics and produce smaller particle sizes. On the other hand, too much heat can also cause

nanoparticles to aggregate or degrade, which would lower the quality of the final product (Singh *et al.*, 2017)

2.5.2 Low temperature

Products containing curcumin may have a longer shelf life and slower breakdown processes at lower temperatures (< 10°C) (Li *et al.*, 2018). Although colder temperatures can cause slower reaction rates and larger particle sizes during the nanoparticle production process for nano curcumin, they can also increase stability and prevent agglomeration in nano curcumin dispersions (Chen *et al.*, 2020).

2.5.3 Room temperature

Products based on curcumin are often handled and stored at room temperature (20–25°C) (Sharma *et al.*, 2019). Similarly, Nanocurcumin is typically achieved at moderate temperatures (~25°C) under regulated synthesis circumstances (Gupta *et al.*, 2018).

2.6 EFFECTS OF LIGHT

Curcumin formulation's photochemical behaviour and stability may be affected by exposure to light. The effects of various light types such as UV, visible and infrared light on the stability of curcumin and nano curcumin were investigated.

2.6.1 UV light

Curcumin can undergo photodegradation upon exposure to UV radiation resulting in the production of degradation products and a reduction in bioactivity (Jovanovic *et al.*, 1999). On the other hand, because of their smaller particle size and greater dispersion, formulations of nanocurcumin may be more photostable than bulk curcumin.

2.6.2 Visible light

Curcumin formulations may experience color changes as a result of exposure to visible light which may indicate structural changes and degradation (Singh *et al.*, 2019). In a similar vein, exposure to visible light may cause color fading

in micro curcumin dispersions indicating possible photochemical reactions or degradation.

2.6.3 IR light

Little impact of infrared (IR) radiation on the stability of curcumin when we expose curcumin directly to it. However, heating caused by infrared radiation may alter the integrity of nanoparticles and modify the colloidal characteristics of nanocurcumin (Gupta *et al.*, 2018).

2.7 EFFECTS OF pH

The solubility, stability and release kinetics of formulations containing curcumin and nanocurcumin are influenced by pH levels. The impacts of various pH ranges acidic, neutral and alkaline on the performance and stability of the product were examined.

2.7.1 Acidic pH

Curcumin solubility can be increased in acidic pH circumstances ($< \text{pH } 3$) but these conditions may also hasten the degradation process, especially in the presence of oxidative stress (Gupta *et al.*, 2019). Similar to this, formulations of acid-responsive nanocurcumin, emphasizing pH-triggered release mechanisms for applications involving targeted drug delivery.

2.7.2 Neutral pH

Curcumin stability is comparatively better at pH 7 which permits sustained release and systemic absorption. Formulations intended for systemic administration of Nanocurcumin generally strive for neutral pH levels of stability and regulated release (Singh *et al.*, 2022).

2.7.3 Alkaline pH

Because of their higher rates of chemical reactivity and degradation, alkaline environments ($\text{pH} > 9$) may be problematic for the stability of curcumin (Sharma *et al.*, 2021). However, methods for maintaining nanocurcumin under alkaline circumstances, stress the significance of encapsulation methods and pH-responsive formulations.

2.8 FUTURE DIRECTIONS

To improve tumor targeting and treatment efficacy, future research should clarify the underlying mechanisms of nanocurcumin's anticancer activities and investigate potential synergistic combinations with traditional medicines. Furthermore, the goals of translational research should be to regulatory clearances for formulations based on nanocurcumin, validate preclinical findings in clinical settings and carry out extensive clinical trials to assess safety and efficacy profiles. All things considered, nanocurcumin has great potential as a medicine.

CHAPTER 3

AIM AND OBJECTIVES

3.1 AIM

To synthesize curcumin and nanocurcumin from *Curcuma longa* and evaluate the effects of temperature, pH and light over curcumin and nanocurcumin.

3.2 OBJECTIVES

- To synthesize nano-scaled curcumin from raw *Curcuma longa* using conventional laboratory techniques.
- Characterization of the as-prepared nanocurcumin using optical spectroscopic techniques like UV, FTIR and RAMAN spectroscopy.
- Characterization of the prepared Nanocurcumin using morphological techniques like FESEM.
- Evaluating the effect of different temperatures (high to low), light (UV, IR and visible light) and pH (acidic to basic) over curcumin and nanocurcumin.
- Evaluating the effect of curcumin and nanocurcumin on gram-positive and gram-negative bacteria.

CHAPTER 4

MATERIALS AND METHODS

4.1 MATERIALS REQUIRED

The materials required for the various methodology employed in the experiment are Turmeric rhizome, Distilled water, Hot air oven, Grinder, Soxhlet apparatus, Weighing machine, Ethanol, Filter paper, Rotary evaporator, Petri plates, Ultra sonicator, Aluminium foil, UV blocker flim, Hydrochloric acid, Sodium hydroxide, Mueller hinton agar, Bacterial cultures, Nutrient broth, Deionized water, Grinder, Conical flask.

Table 4.1 : Nutrient broth

Medium	Concentration (g/ml)
Beef extract	3g
Peptone	5g
Sodium chloride	5g
Distilled water	1000ml

Table 4.2 : Mueller Hinton agar

Medium	Concentration g/ml
Beef infusion	300ml
Casein hydrolysate	1.6g
Starch	1.5g
Agar	1.5g
Distilled water	1000ml
pH	7.2 ± 0.2

4.2 METHODOLOGY

4.2.1 Sample collection and preparation

Freshly cultivated turmeric rhizomes are collected either from farms or marketplaces. The collected turmeric rhizomes are then washed with salt water which is believed to remove dirt, debris and pesticides and also helps to prevent oxidation, followed by this, the turmeric is peeled thoroughly and partially sun-dried and kept under a hot air oven at 60°C for 6 hours to remove moisture content present in it. The dried sample is finely ground and the powder is stored in an airtight container.

4.2.2 Curcumin extraction

4.2.2.1 Manual extraction

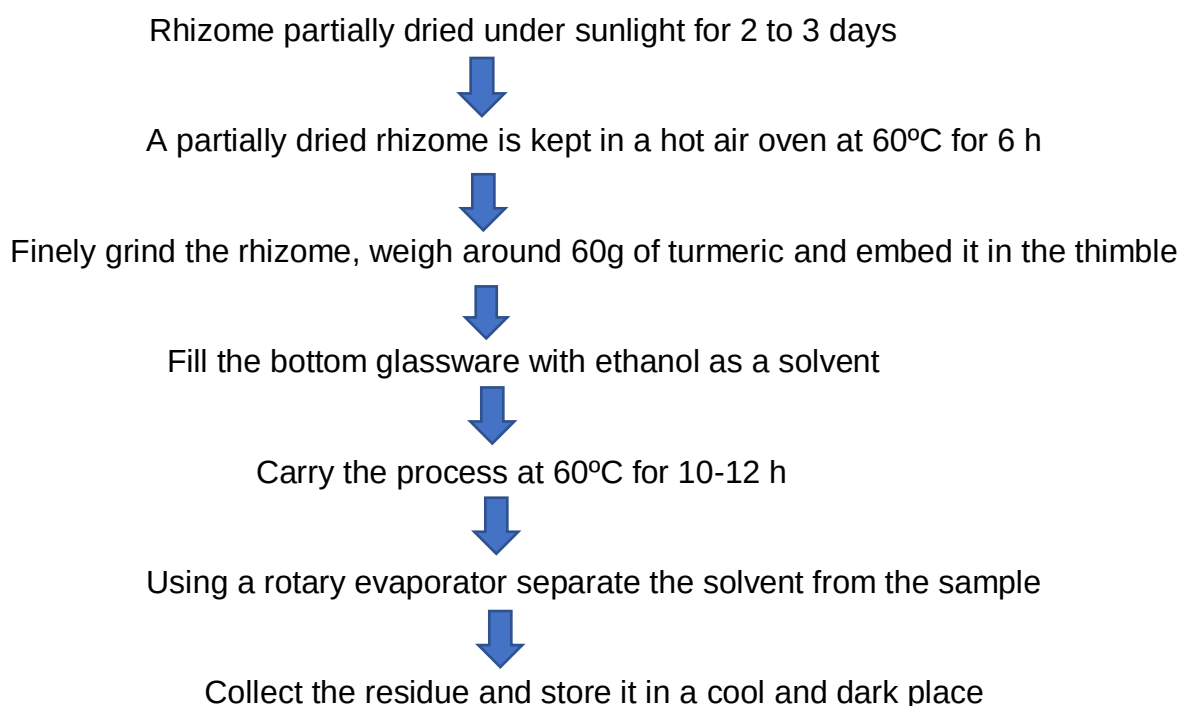
One of the easily available process to synthesize curcumin is the manual extraction method. Commonly used solvents are ethanol or acetone. Weigh around 50g of powdered turmeric and add it to a sterile screwcap glass container containing 100 ml of ethanol to make a solution. Now vigorously shake or stir it for 3 to 4 hrs completely until the curcumin thoroughly dissolves into the solvent after that leave this for about 1-2 h. Then use a filter paper to filter the liquid part containing the curcumin compound from the unwanted solid residue. Now transfer the liquid extract into sterile petri plates for solvent evaporation in a dark room. This evaporation process may take around 4 to 5 days after that scrape the semi-dried curcumin powder from the plates gently by using a sterile spatula or any clean tool and store it in a sterile clean airtight container to preserve and keep up with its stability and potency.

4.2.2.2 Soxhlet extraction

Soxhlet extraction is one of the most conventional methods used for any plant extraction. First of all, properly assemble the soxhlet apparatus and fill the chosen solvent which is ethanol of 700-800ml in the round-bottom flask. Now using a weighing machine weigh around 60g of powder turmeric, pack it in a filter paper embed it in a thimble and place the thimble in the soxhlet apparatus. Now heat the solvent, as the solvent reaches its boiling point, it will vaporize and get into the

condenser and again drip down into the thimble which contains the curcumin and this process is a continuous cycle, generally curcumin extraction process will be carried out at 60°C for 8 to 12 h. Once the extraction is completed by using a rotary evaporator, the ethanol will be evaporated from the extract under a vacuum of 35°C. An alternative method can also be used for solvent evaporation which is leaving the sample in a sterile room for evaporation of solvent and once the solvent is evaporated, the residue is collected and stored in an airtight container in the refrigerator. The better option is to lyophilize the extract for preserving the components and it also ensures the stability of the components.

FLOW CHART OF CURCUMIN SYNTHESIS



4.2.3 Nanocurcumin extraction

4.2.3.1 Ultrasonication

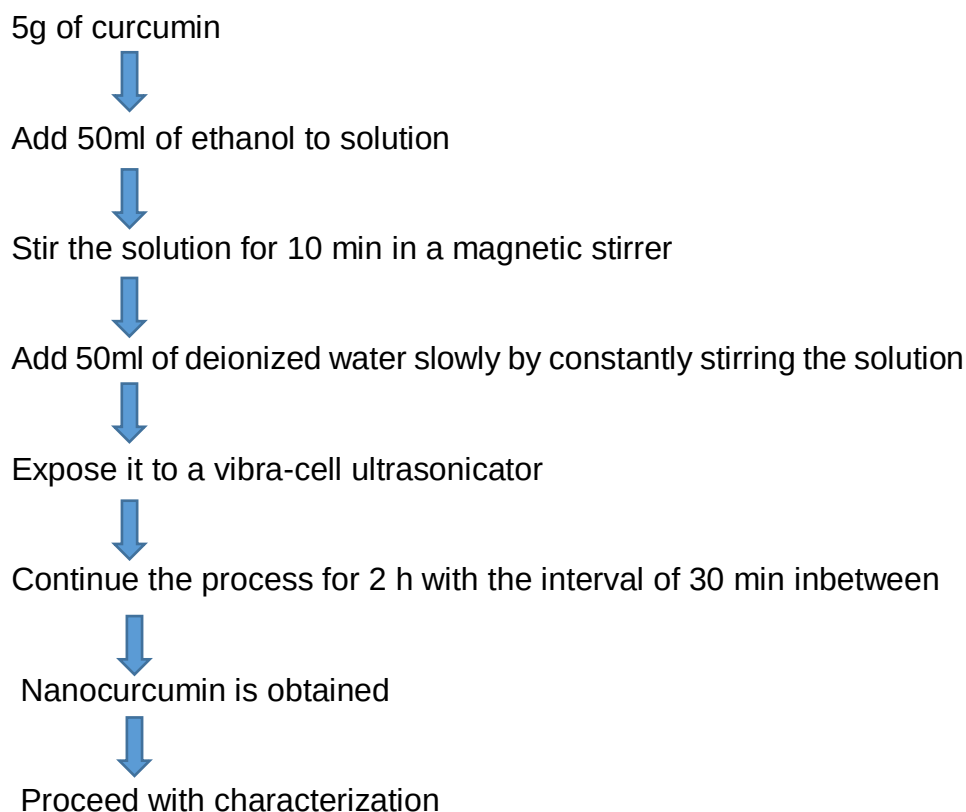
Various methods can be followed for the extraction of nanocurcumin. Among the various ways available, one method that can be used is the ultrasonication method. For this process, curcumin is dissolved in a homogeneous solution. Following this, the solution is subjected to ultrasonication which uses a water bath-type sonicator equipped with an ultrasonic frequency of 50kHz at a temperature of

50 ± 5°C with a time interval of 2 h to produce curcumin nanoparticles. After the sonication process, the resultant nanocurcumin containing mixture is subjected to stirring for 30 min to 1 h at 800 rpm and the extract-containing tubes are covered with aluminium foil and stored in a dark and cool environment to prevent photodegradation.

4.2.3.2 Anti-solvent precipitation

Another comfortable and conducive method for nano curcumin synthesis is antisolvent precipitation, a widely used method for poorly water-soluble compounds like curcumin. In this procedure, the curcumin is first dissolved in an organic solvent(ethanol) and stirred for 10 min. Subsequently, add this solution into deionized water in a ratio of 1:10 (10ml curcumin-ethanol solution in 100ml deionized water) followed by introducing this solution into ultracentrifugation with a rpm of 20,000 for about 10 min. After that cover the tubes with aluminium foil and store it for further use. These two methods easily scale up the production of high quality curcumin nanoparticles.

FLOW CHART OF NANOCURCUMIN SYNTHESIS



4.2.4 CHARACTERIZATION OF CURCUMIN AND NANOCURCUMIN

Ultraviolet-vis spectroscopy calculates the amount of visible and UV light absorbed by the sample. Electrons reach higher energy when molecules absorb light. The rate of absorption completely depends on the concentration of the sample and its molecular structure. UV spectroscopy works based on beer-lambert's law. The law states that "absorbance of the solution is directly proportional to the concentration of the absorbing material present in the solution and path length". The average absorption band for curcumin ranges from 360 to 460nm and the strongest band wavelength is at 424nm. For nanocurcumin, the absorption band ranges from 300 to 500nm and the maximum wavelength range is around 420 to 430nm. FTIR spectroscopy calculates the absorption of IR by sample. FTIR technique is used to collect data about the presence of functional groups present in the sample and its molecular structure. Due to its high accuracy rate and sensitivity bits are a quite reliable technique (Jaggi and Vij, 2006). For performing FTIR, the sample can be in solid, liquid or powder form. FTIR results are analyzed as "absorption versus wavenumber" or "transmission versus wavenumber". The presence of more than five bonds indicates complex molecules. The presence of single bonds ranges from 2500-4000 cm^{-1} , double bonds 1500-2000 cm^{-1} and triple bonds range from 2000-2500 cm^{-1} (Nandiyanto *et al.* 2019).

The inelastic scattering of monochromatic light by a material is measured using Raman spectroscopy. A shifted Raman spectrum is the consequence of energy being exchanged between photons and molecular vibrations when molecules scatter light. The material's structural characteristics and molecular vibrations are revealed by the Raman spectra. An effective method for evaluating nanocurcumin formulations is Raman spectroscopy. It provides information about the interactions, crystallinity and molecular makeup of the nanoparticles. To learn more about the structural properties of nanocurcumin particles, one can investigate the Raman bands that correspond to particular vibrational modes of the chemical bonds within the particles. Raman spectroscopy gives precise information about the molecular vibrations and structural characteristics of formulations of nanocurcumin; it typically operates in the 500–2000 cm^{-1} range (Maiti and Mukherjee, 2007).

Researchers can study the surface morphology of nanocurcumin particles with nanometre-scale resolution thanks to FESEM. Surface characteristics including

texture, porosity and roughness are visible in high-resolution pictures captured by FESEM. These characteristics affect the physicochemical characteristics and interactions of nanocurcumin with biological systems. Quantitative information on the size and form of nanocurcumin particles can be obtained from FESEM pictures. Image analysis tools can be used to get diameter and aspect ratio measurements of the particles from FESEM micrographs. Evaluation of the size distribution and homogeneity of nanocurcumin formulations, which affect their stability and biological activity, requires this information.

4.2.5 Evaluating the effect of temperature, pH and light over curcumin and nanocurcumin

It is believed that curcumin and nanocurcumin are very sensitive to light, they are photodegradable in nature. To understand the stability and improve the storage, it's important to understand the reactivity of curcumin and nanocurcumin when they are exposed to certain parameters for a certain period of time. To evaluate the effect of light, the curcumin and nanocurcumin samples are exposed to visible light, UV light and IR light for a period of three days with intervals of 30 minutes for every 3 hours. As for UV light, the curcumin and nanocurcumin samples were poured into cuvettes and placed under direct sunlight only UV rays could penetrate through the cuvette and other samples were also placed under UV rays for better results. As for IR light, commercially available lights were purchased and a setup was made, then the samples were exposed under the IR lights. To evaluate the effect of temperature, the samples were exposed in a hot-air oven at a temperature of 85-90°C for three days at intervals of 30 minutes for every 3 hours. As for low temperatures, the samples were kept in a normal refrigerator for continuous three days without any intervals. For evaluating the effect of pH, the samples were exposed to both acidic and alkaline conditions for creating an acidic environment, HCL was used and for an alkaline environment, NaOH was used and the samples were kept under these conditions for the decided amount of time and then for evaluating UV spectroscopy method was used to check the degradation of the sample.

4.2.6 Antimicrobial susceptibility test

AST is a type of test used in the medical field to analyze the effectiveness of antibiotics and the amount of dosage required to inhibit the growth of desired bacteria. For the growth of these organisms, a proper growth media that is a “culture media” is required. AST test can be performed in two ways one is by MIC and the other is by disk diffusion method (Marlon Bayot and Bradley Bragg, 2020)

4.2.7 Preparation of broth culture

For preparing around 100ml of nutrient broth measure 0.3g of peptone, 0.5g of beef extract and 0.5g of sodium chloride, then add around 100ml of water to the measured content. Peptone is rich in source of organic nitrogen which acts as a food supplement to the bacteria. Bacteria grow well in the presence of nitrogen. Beef extract is rich in source of carbon and they are extracted from bovine tissue. Sodium chloride maintains the osmotic balance and prevents the cell lysis of bacteria. To prevent any sort of cross-contamination, sterilizing the broth is necessary. To sterilize this, auto-clave it for 15 minutes at 121°C and once the broth is autoclaved, allow it to reach normal room temperature and then inoculate the desired microorganism. Here we have used gram-positive bacteria, *Staphylococcus aureus* and gram-negative bacteria, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

4.2.8 Overview of selected strains of bacteria

4.2.8.1 *Staphylococcus aureus*

It is a type of gram-positive bacteria that usually appears as clusters of grapes under the microscope. These bacteria are very commonly found on the mucosal membranes and skin of humans. They are usually responsible for causing various skin-related diseases in humans (Tracey Tylor and Chandrashekhar Unkal, 2023). They cause localized pyogenic lesions. A person who is affected by *staphylococcus* exhibits pyrogenic, mutagenic and cytotoxic effects. Tampon users are detected to suffer from TSST. *Staphylococcus* is usually grown under temperatures ranging between 35-40°C. *Staphylococcus* is resistant to beta-lactamase, vancomycin and methicillin (Ananthanarayan; Paniker, 2020) due to this

curcumin and nanocurcumin are good alternatives for exploring the sensitivity against such bacteria.

4.2.8.2 *Klebsiella pneumoniae*

Klebsiella pneumoniae is a gram-negative bacteria that is non-motile in nature. They are capsulated bacteria which is straight and plump in appearance. They form mucoidal colonies in normal agar and lactose fermenting in MacConkey agar. As for the IMVIC biochemical test, the results are like - - + +. *Klebsiella* causes bloodstream infections (Ananthanarayan; Paniker, 2020).

4.2.8.3 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a gram-negative bacteria that is found majorly in hospital environments. They appear with flagella and they are motile in nature. They are highly resistant to high temperatures. *Pseudomonas* are related to localized infections, eye infections, COPD and secondary infections in burn patients. In macconkey agar, they form lactose non-fermenting colonies. It's believed that *Pseudomonas aeruginosa* and various other strains of *pseudomonas* are pan-drug-resistant. (Ananthanarayan; Paniker, 2020). For such drug-resistant bacteria, traditional and ayurvedic methods could be proven as better alternatives since turmeric has been used for a very long period without proper knowledge about its benefits and the way it should be used.

4.2.9 Streak plate for AST

For testing antimicrobial sensitivity, MHC agar is used. For preparing around 1000ml of agar: weigh around 1.5g of agar, 16g of casein hydrolysate, 1.5g of starch, and 300 ml of beef infusion. Beef extract, acid casein hydrolysate starch and agar provide carbon, vitamins, nitrogen and amino acids whereas starch acts as a colloid and absorbs any toxin metabolites. Autoclave this solution and the petri plates at 121°C for 15 minutes and after that allow them to settle at room temperature. Once they become normal, pour that into the petri plates and allow it to solidify and mark the petri plates with relevant data about the bacteria, once the solution is solidified using a sterile or cotton swab, obtain a small amount of culture from the broth and begin to swab in back and forth motion from one edge to another with soft hand

without applying too much pressure. Discard the previously used swab and by using a new swab obtain some sample, rotate the plate at a 90° angle and again start to streak back and forth, repeat the process 2 to 3 times. After completing the swabbing process make some wells in the agar and pour the curcumin and nanocurcumin samples in the well with the help of a micropipette and an alternative to this is using a sterile disk, firstly either dip or pour some curcumin and nanocurcumin sample over a sterile disk and allow them to absorb the content for few minutes. Once they absorbed the samples, place the disk on the agar plate and then place them in an incubator for 24-48 hr at a temperature of 37°C and observe the growth and zone of inhibition, measure the zone using a ruler. The zone indicates the sensitivity of the bacteria toward the curcumin or nanocurcumin sample.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 SYNTHESIS OF CURCUMIN



Fig 5.1 : Soxhlet extraction of curcumin



Fig 5.2 : Sample left for solvent evaporation (ethanol)



Fig 5.3 : Curcumin sample after solvent evaporation and residue collection

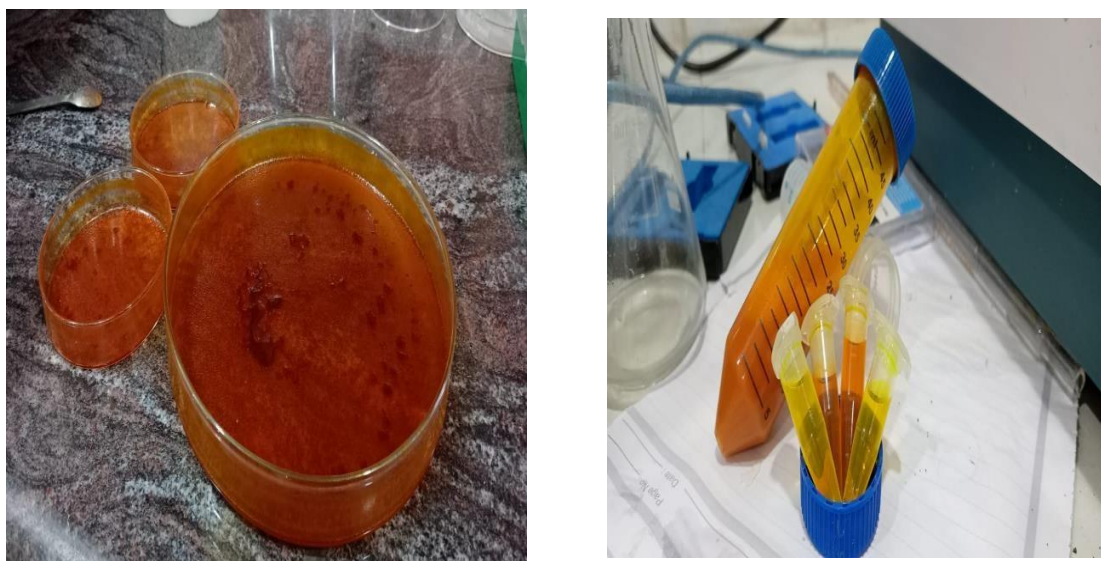


Fig 5.4 : Residue is mixed with ethanol



Fig 5.5 : Lyophilized curcumin



Fig 5.6 : Manually synthesized curcumin

5.2 SYNTHESIS OF NANOCURCUMIN



Fig 5.7 : Nanocurcumin synthesis



Fig 5.8 : Lyophilized nanocurcumin

5.3 CHARACTERIZATION OF CURCUMIN AND NANOCURCUMIN



Fig 5.9 : Curcumin and nanocurcumin samples for characterization

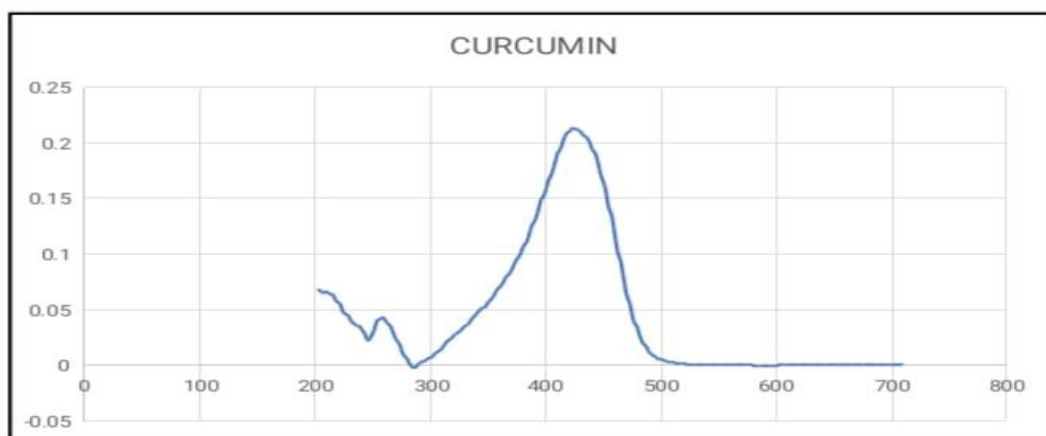


Fig 5.10 : UV spectroscopy result for curcumin, peak range is 425nm

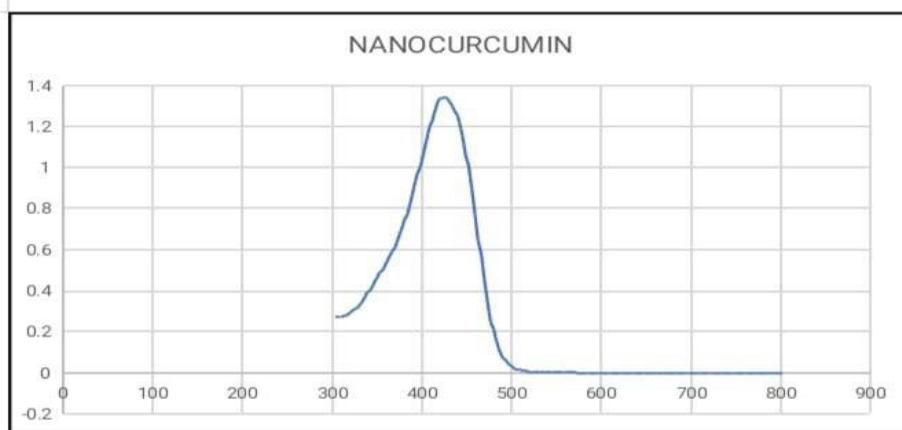


Fig:5.11: UV spectroscopy result for nanocurcumin, peak range is 424nm

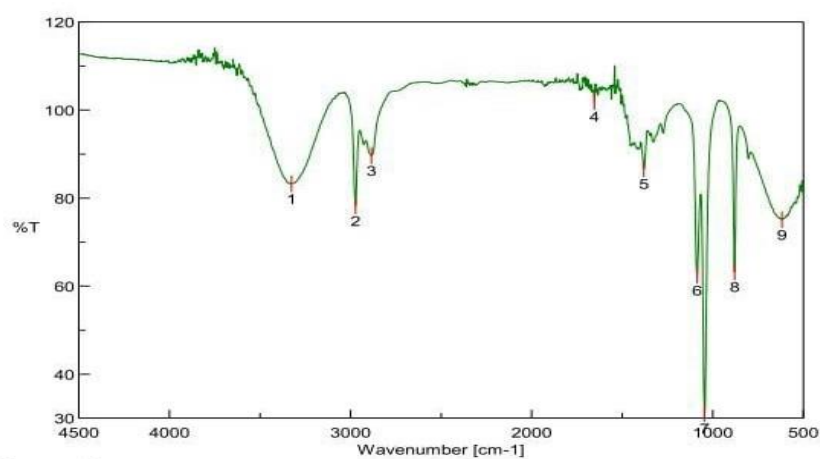


Fig 5.12 : FTIR result for curcumin

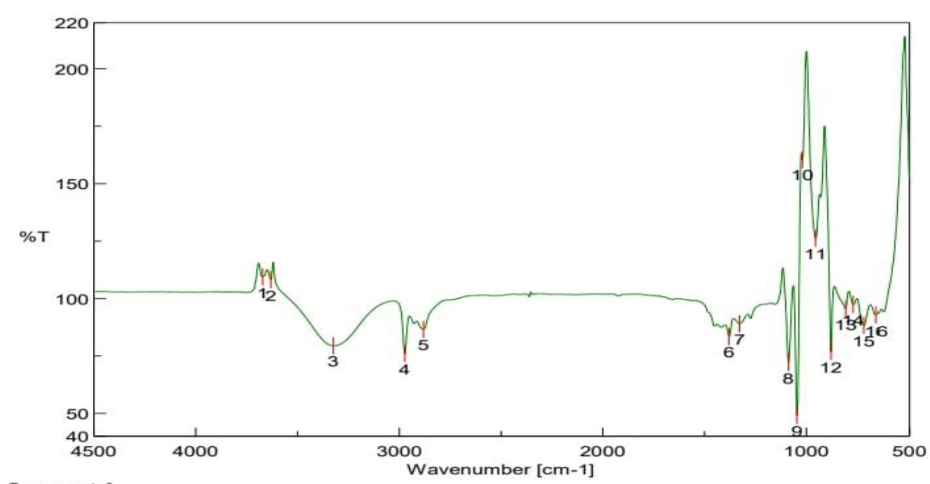


Fig 5.13 : FTIR result for nanocurcumin

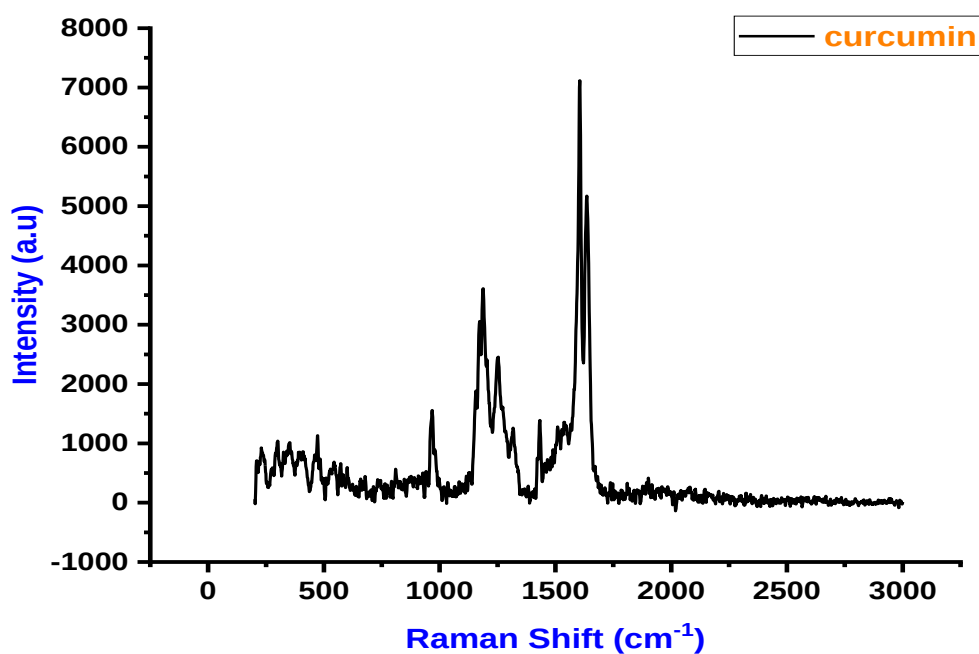


Fig 5.14 : Raman spectroscopy result for curcumin

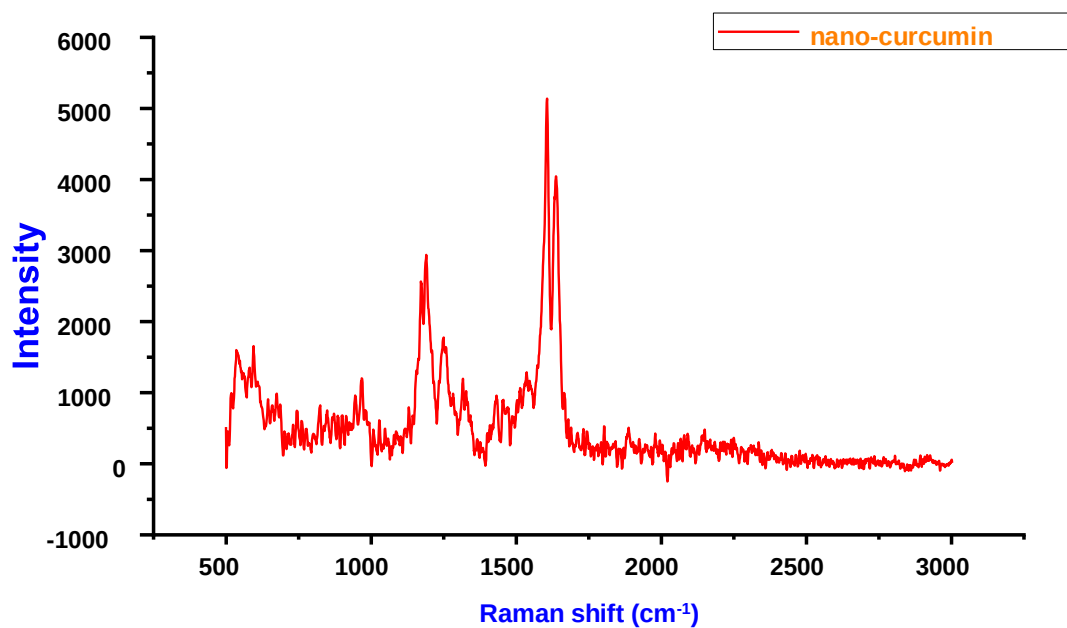


Fig 5.15 : Raman spectroscopy result for nanocurcumin

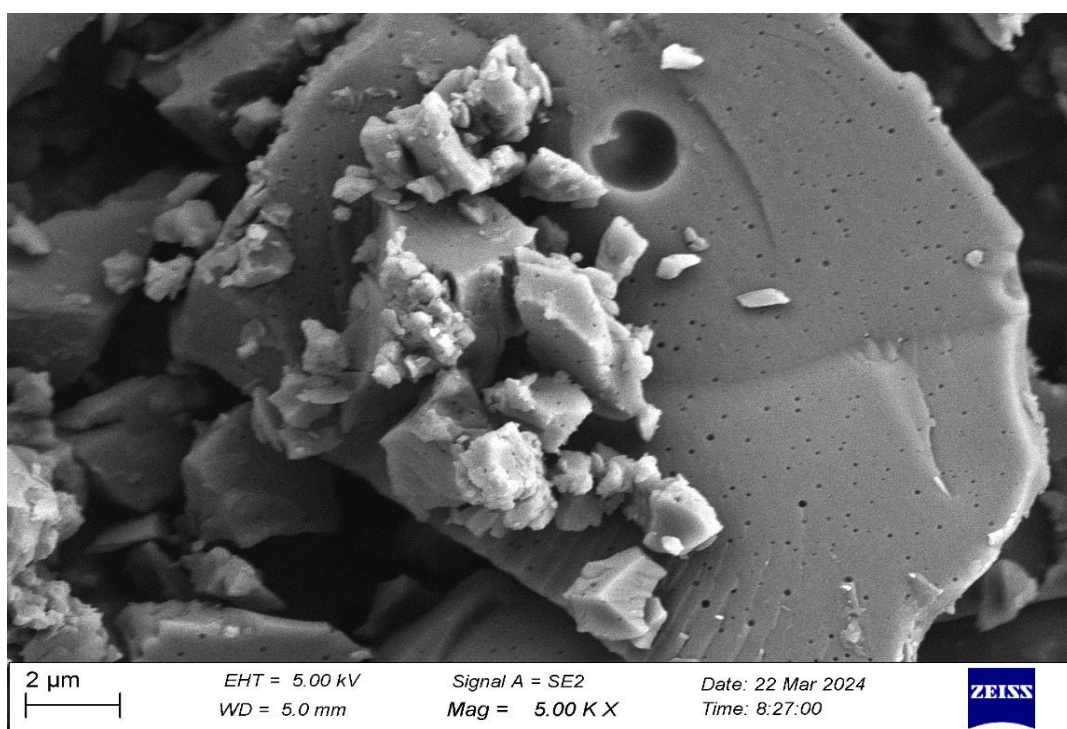


FIG 5.16 : FESEM result of nanocurcumin at 2μm

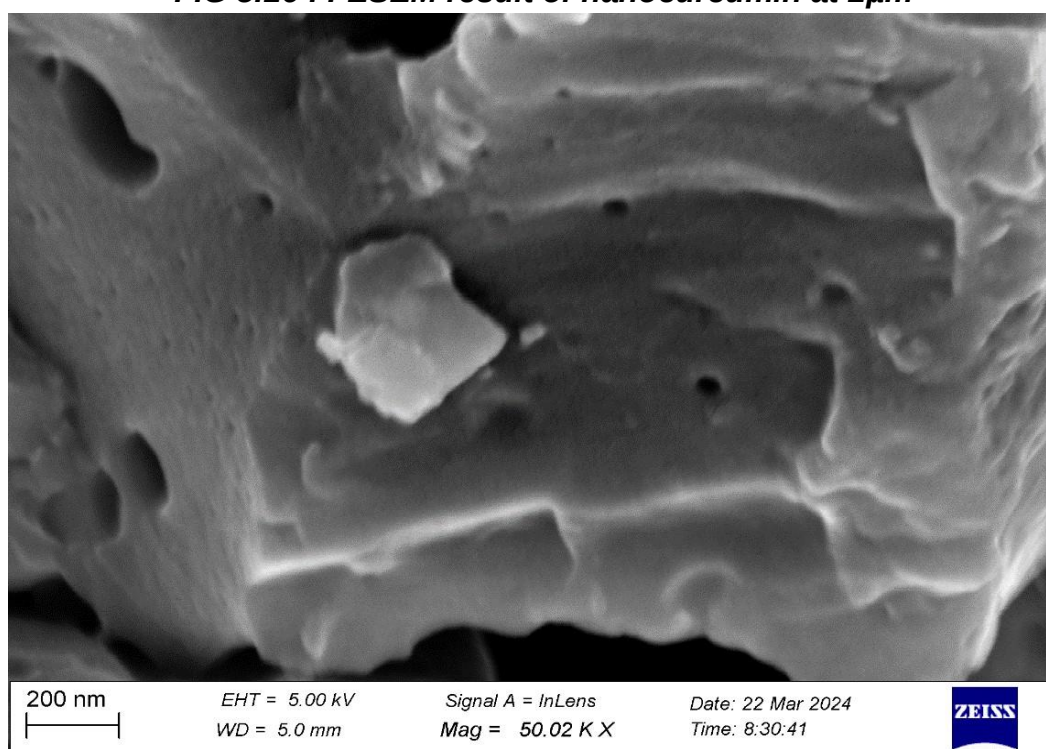


FIG 5.17 : FESEM result of nanocurcumin at 200nm

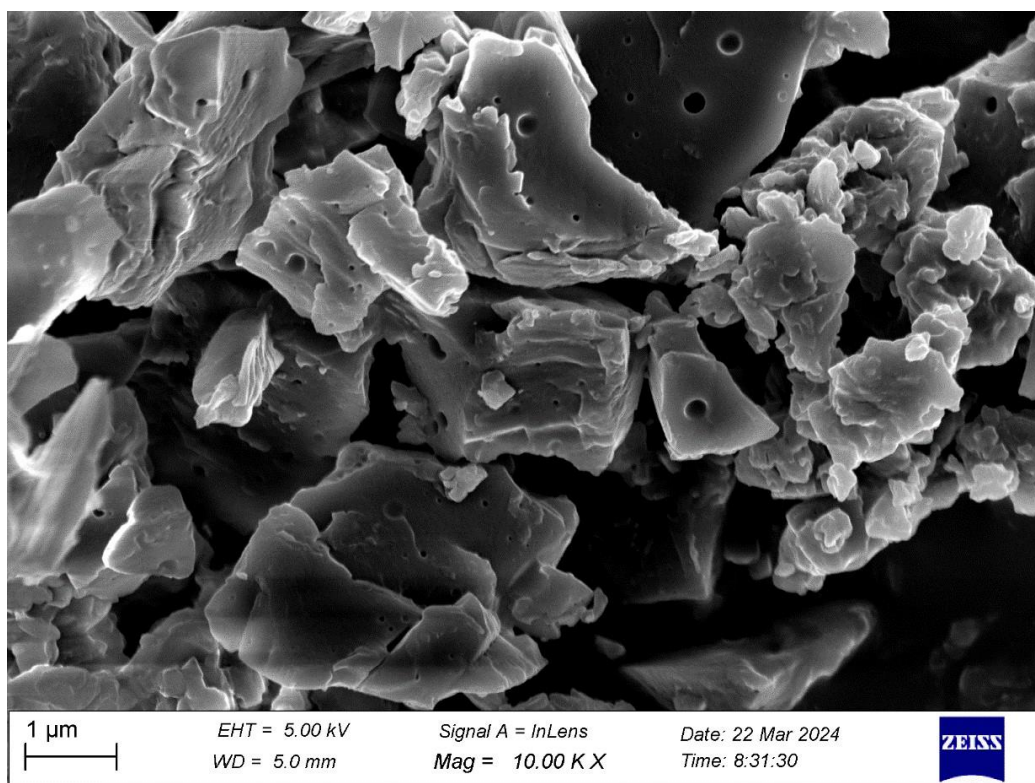


Fig 5.18 : FESEM result of nanocurcumin at 1 μm

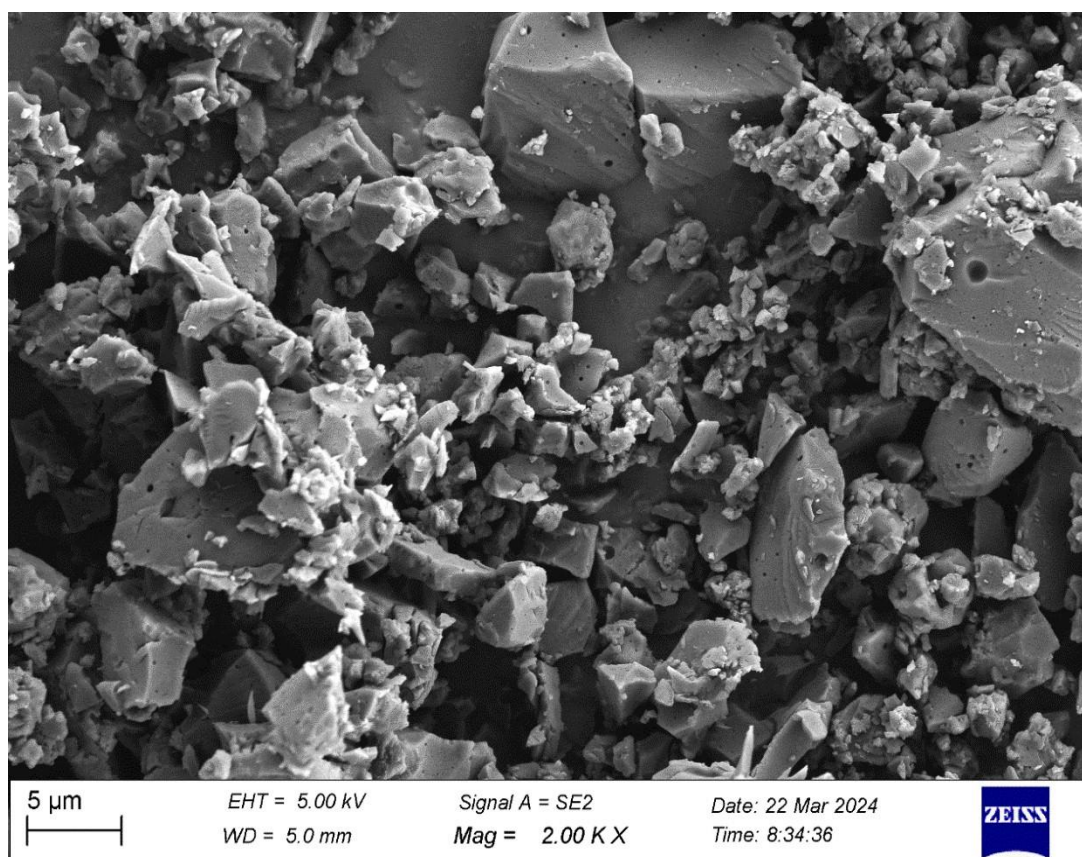


Fig 5.19 : FESEM result of nanocurcumin at 5 μm

5.4 EVALUATING THE EFFECT OF TEMPERATURE, pH AND LIGHT OVER CURCUMIN AND NANOCURCUMIN

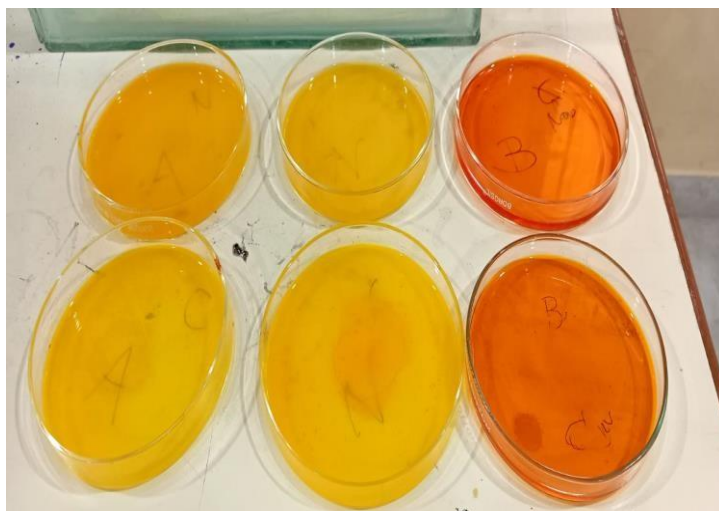


Fig 5.20 : Effect of Ph on Curcumin and nanocurcumin samples

In alkaline conditions, color change has been noticed whereas in acidic conditions crystalline structure was noticed.

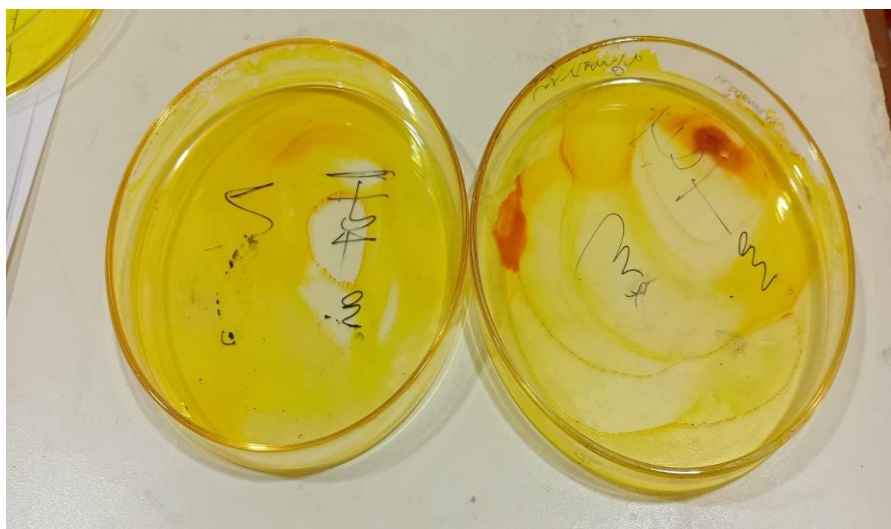


Fig 5.21 : Effect of high temperature on curcumin and nanocurcumin sample



Fig 5.22 : Effect of room temperature on curcumin and nanocurcumin sample

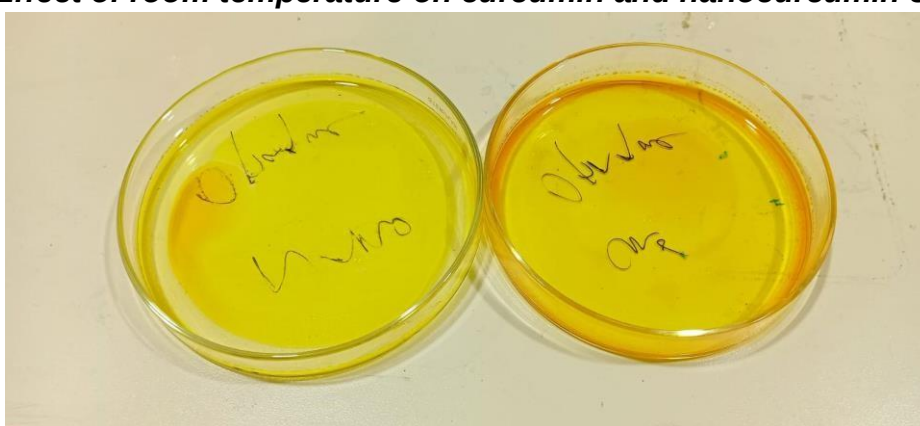


Fig 5.23 : Effect of low temperature on curcumin and nanocurcumin sample

For high temperature, samples were kept in a hot air oven at 80°C. For low temperatures, it was kept in the fridge and samples were degraded. The results were confirmed by performing UV spectroscopy.



Fig 5.24 : Effect of UV light on curcumin and nanocurcumin sample



Fig 5.25 : Effect of Visible light on curcumin and nanocurcumin sample



Fig 5.26 : Effect of IR light on curcumin and nanocurcumin sample



Fig 5.27 : Total samples after evaluated the parameters

5.5 ANTIMICROBIAL SUSCEPTIBILITY TEST

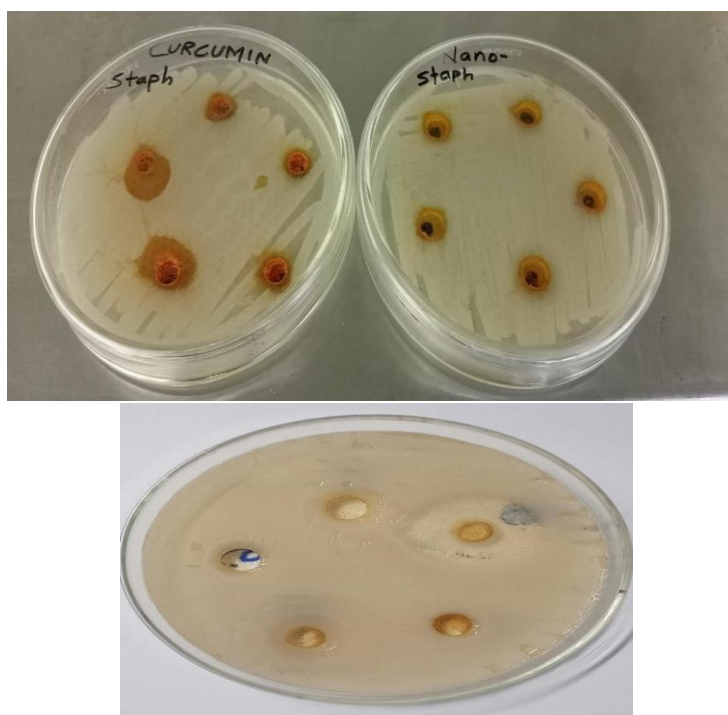


Fig 28: *S. aureus* swabbed plates with curcumin and nanocurcumin loaded wells

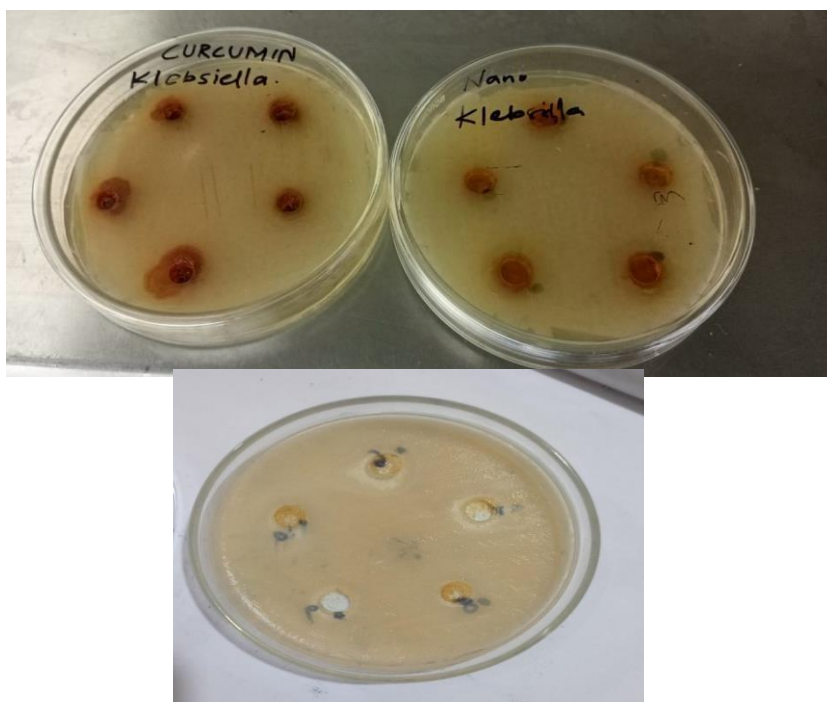


Fig 5.29 : *K. pneumoniae* swabbed plates with sample-loaded wells

CHAPTER 6

SUMMARY AND CONCLUSION

The objective of this work was to create Nanocurcumin from *Curcuma longa* and examine how it reacts to temperature, pH and light variations. There were multiple steps in the synthesis process such as extraction, purification and nanoparticle production. Systematic experiments were conducted to assess the impact of temperature changes, pH levels and light exposure on the stability and characteristics of the produced Nanocurcumin. The degradation rate of Nanocurcumin was found to be affected by light exposure with higher intensities resulting in a faster rate of deterioration. It was discovered that the stability of Nanocurcumin was influenced by pH levels, with acidic settings showing higher stability than alkaline ones. The stability and characteristics of Nanocurcumin were similarly impacted by temperature, with higher temperatures hastening deterioration. All things considered, this work offers insightful information about how Nanocurcumin behaves in various environmental settings.

In conclusion, it was shown that it was possible to synthesize Nanocurcumin from *Curcuma longa* and the resulting nanoparticles showed sensitivity to temperature, pH and light. The results of this investigation highlight how crucial it is to take these variables into account when creating and utilizing formulations based on Nanocurcumin. The stability and effectiveness of Nanocurcumin are specifically dependent upon limiting light exposure, retaining ideal pH levels and averting extreme temperatures. These discoveries add to the expanding corpus of information about Nanocurcumin and its possible uses in fields like materials engineering, food science and medicine. Going forward, more investigation is necessary to determine other elements impacting Nanocurcumin's behavior and to improve its formulations for particular uses.

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ANNEXURE

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