Development of Flaxseed Hydrogel based, Dodonaea Angustifolia Induced and Ag-Nanoparticles Encapsulated Biofilm for Antibacterial Application

Komies Tekle Baraki

Thesis Paper Submitted to the Department of Chemical Engineering

School of Mechanical, Chemical, and Material Engineering

Presented in Partial fulfillment of the Requirement for the Degree of Master's in Chemical Engineering (Specialization in Process Engineering)

Office of Graduate Studies

Adama Science and Technology University

Adama, Ethiopia

July, 2022

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Declaration

I hereby declare that this Master Thesis entitled "**Development of Flaxseed Hydrogel based, Dodonaea Angustifolia Induced and Ag-Nanoparticles Encapsulated Biofilm for Antibacterial Application"** is my original work. That is, it has not been submitted for the award of any academic degree, diploma or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citation.

Komies Tekle _____ _____

Name of the student Signature Date

Recommendation

We, the advisor(s) of this thesis, hereby certify that we have read the revised version of the thesis entitled **"Development of Flaxseed Hydrogel based, Dodonaea Angustifolia Induced and Ag-Nanoparticles Encapsulated Biofilm for Antibacterial Application"** prepared under our guidance by Komies Tekle is submitted in partial fulfillment of the requirements for the degree of Mater's of Science in Chemical Engineering (Specialization Process Engineering). Therefore, we recommend the submission of revised version of the thesis to the department following the applicable procedures.

Major Advisor	Signature	Date
Co-advisor	Signature	Date

Approval Page

We, the advisors of the thesis entitled **"Development of Flaxseed Hydrogel based, Dodonaea Angustifolia Induced and Ag-Nanoparticles Encapsulated Biofilm for Antibacterial Application**" and developed by Komies Tekle certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

_____	_____	_____
Major Advisor	Signature	Date
_____	_____	_____
Co-advisor	Signature	Date

We, the undersigned, members of the Board of Examiners of the thesis by _____have read and evaluated the thesis **entitled "Development of Flaxseed Hydrogel based, Dodonaea Angustifolia Induced and Ag-Nanoparticles Encapsulated Biofilm for Antibacterial Application"** and examined the candidate during open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Chemical Engineering (Specialization in process engineering).

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LIST OF ABBREVIATIONS

AgNPs	Silver nanoparticles
DA	*Dodonaea Angustifolia*
DW	Distilled water
GLY	Glycerol
E. coli	*Escherichia coli*
S. aureus	*Staphylococcus aureus*
P. aeruginosa	*Pseudomonas aeruginosa*
S. pyogenes	*Streptococcus pyogenes*
FSH	Flaxseed hydrogel
RSM	Response Surface Methodology
FTIR	Fourier-transform infrared spectroscopy
XRD	X-ray diffraction spectroscopy

LIST OF NOTATIONS

mM	millimolar
mg	milligram
mL	milliliters
μg	microgram
%	Percentage
hr	hour
CFU/mL	colony-forming unit per milliliter

ABSTRACT

Pathogen infections are causing major challenge in the current world. The need to management of pathogen infection using rapid, environmentally friendly and cost-effective method leads to a need for a continuous research. In this research Flaxseed Hydrogel based, Dodonaea Angustifolia Induced and Ag-Nanoparticles Encapsulated Biofilm was developed for Antibacterial Application. Leaf extract of Dodonaea Angustifolia was used as filler on flaxseed hydrogel (due to its antimicrobial activity), reducing and capping agents for the synthesis of silver nanoparticles (AgNPs). Distilled Water DW was used solvent for the extraction process and the effect of different operational parameters (temperature and time) was tested. Operational parameter was also optimized for extraction of Flaxseed hydrogel (FSH) by using RSM (BBD).

For the Dodonaea Angustifolia mediated green synthesis of AgNPs, the effect of temperature, time, volume of plant extract, and precursor concentration as operation parameters was investigated for the synthesis of AgNPs. Among the tested parameters, the effect of precursor concentration was confirmed as the dominant parameter that affects the AgNPs solution. Then the synthesized AgNPs were homogeneously dispersed into FS-GLY-DA biofilm and used as filler to prepare AgNPs dispersed FS-based biofilms.

Greenly synthesized AgNPs were confirmed using UV-Vis spectroscopy and characterized by XRD, PSA, FT-IR. And its antimicrobial activities were also evaluated. Phytochemical screening test and FT-IR analysis of Dodonaea Angustifolia leaves extract revealed the existence of active components which capped the nanoparticles during the synthesis. The antibacterial activities of synthesized AgNPs were evaluated against Gram-positive bacteria (S. pyogenes and S. aureus) and Gram-negative bacteria (E. coli and P. aeruginosa) and observed that a higher zone of inhibition. The study results revealed that the antimicrobial activity of FS biofilms was enhanced by dispersing Dodonaea Angustifolia leaves extract and green synthesized AgNPs.

CHAPTER ONE

INTRODUCTION

1.1. Background

Hydrogels are especially described as three-dimensional cross-linked networks of polymers with properties like solid and liquids (Gong, 2006) and are capable of taking huge amount of water because of the presence of hydrophilic groups which include _OH, _CONH, _CONH2, _SO3H. Hydrogels are new and advanced polymeric substances and researchers have explored their application in distinct areas because of their water-rich nature. They can be prepared by chemical and physical crosslinking methods. Chemical methods involve the formation of new covalent bonds among the polymer chains of hydrogel, whereas physical interactions were existing between the polymer chains of hydrogel (G. Sharma et al., 2018). Crosslinking of polymer can be done using cross linker, chemical modification, grafting or using high energy radiation (Gamma or UV rays) conjugation (Hennink & van Nostrum, 2012). In the recent era hydrogel technology used in the different application such as metal ion adsorption, drug delivery, antibacterial drug release for wound dressings, degradation of dyes (Singh, Sharma, & Dhiman, 2017), Agriculture, Nano fibers, Hygienic Products (Ahmad, Ahmad, Manzoor, Purwar, & Ikram, 2019), cell encapsulation, tissue engineering, soft electronics, and actuators (Zhang & Khademhosseini, 2017) .Those widely applicaple hydrogels can be prepared from different raw materials or origins.

Based on their origin there are two major sources of hydrogels such as synthetic polymers, and natural polymers. Synthetic polymers are interesting because it is easy to control their chemistry and structure and thus alter their properties. Synthetic materials which can be used to form hydrogels are poly(ethylene oxide) (PEO), poly(vinyl alcohol) (PVA) and poly(propylene fumarate) (PPF) , poly(hydroxyalkyl methacrylates), Poloxamers, poly(acrylate), poly(acrylic acid),poly(acrylamide) and poly(methacrylamide) (Varaprasad, Raghavendra, Jayaramudu, Yallapu, & Sadiku, 2017).But large disposals of synthetic polymers have been causing serious environmental impact. Natural polymers are produced from natural origins such as plants, animals, algae, and microorganisms. Those nature derived polymers used as source of hydrogels are polysaccharide-based natural hydrogels, protein-based natural hydrogels, lignin-based

hydrogels, algae-based hydrogels, and bacteria-based hydrogels (Mohammadinejad et al., 2019), (Varghese, Rangappa, Siengchin, & Parameswaranpillai, 2020). As compared both classes of source of hydrogels, natural polymers are economical, readily available, easy to modify, high degree of flexibility, potentially biodegradable, and biocompatible due to their origin(Varghese et al., 2020).among those natural sources of hydrogels polysaccharides are commonly used sources for hydrogel production.

Polysaccharides are most abundant class of biopolymer, they are obtained from animal, plant, microbes, and Algaa (Kosaraju, 2005). Polysaccharides are highly stable, renewable, nontoxic, intrinsically biodegradable, relatively cheap, and hydrophilic in nature due to the presence of functional groups such as hydroxyl amino and carboxylic acid and these functional groups in polysaccharides that offer the floor for the modification of polysaccharide (Y. Wen & Oh, 2014). Diversity in structures and in properties of polysaccharides are attributed to the wide range of molecular weights and different chemical compositions (Z. Liu, Jiao, Wang, Zhou, & Zhang, 2008).Despite that wide range of molecular weight distributions and different chemical compositions of polysaccharides still they suffer from several drawbacks. Polysaccharides exhibit limited solubility in organic solvent, short shelf life, and poor mechanical stability. In the direction of targeted application and to overcome the drawbacks various techniques have been used (Arakawa et al., 2017). Nowadays, the polysaccharide is being modified in order to obtain novel biomaterial for Controlled advanced applications by dispersing advanced functionalized materials which can meet a multiplicity of requirements and to decrease the draw backs of pure polysaccharide hydrogels. The advanced biomedical region, the degradation of natural polymers into physiological metabolites creates them tremendous candidates for a wide range of advanced applications. Developed from natural, renewable, nontoxic and biodegradable sources, polysaccharide-based hydrogels are justifiably viewed as 'ecologically-friendly' products (Varaprasad et al., 2017).one of the most used polysaccharide are Natural mucilage's composed of polysaccharides with numerous sugar units, which are linked together to form huge polymers with large molecules.

Flaxseed (*Linum usitatissimum* L.) hydrogel is one of the natural mucilage's which is by-product of the flax oil industry and occurs mainly at the outermost layer of flaxseed hulls (J. Liu, Shim, Timothy, Wang, & Reaney, 2018) .Flaxseed (*Linum usitatissimum* L.) contains approximately 3.0–9.0% mucilage, whose polysaccharide is heterogeneous and composed of

galacturonic acid (21.0–36.0%), xylose (19.0–38.0%), rhamnose (11.0–16.0%), galactose (12.0–16.0%), arabinose (8.0–13.0%) and glucose (4.0–6.0).flax seed can be considered as a hydrocolloid, thickener (Wu, Li, Wang, Zhou, & Mao, 2010) and gelling agent (Chen, Xu, & Wang, 2006). Furthermore, the intake of flaxseed mucilage (FSM) has been recommended for the blood glucose and cholesterol reduction in diabetics (Thakur, Mitra, Pal, & Rousseau, 2009) and increase fecal fat excretion (Basiri, Haidary, Shekarforoush, & Niakousari, 2018).But FSH have some draw backs such as low mechanical strength, low plasticity, low antimicrobial activity, and dropping of its viscosity during storage. Those drawbacks of FSH was improved using methods of adding of fillers, crosslinking, esterification, and blending. To improve its antimicrobial activity it is possible to use plant extract as fillers from medicinal plants which possessed antimicrobial activity. However, plants are very complex in their compositions and their therapeutic activity depends on their major active chemical constituents(Pandey et al., 2014). Essential oil of different plants has been reported to possess antibacterial and antifungal activities(Oladimeji, Akinkunmi, Raheem, Abiodun, & Bankole, 2015).One of the most important plants used as medicinal herpes in the traditional and modern medicine is *Dodonaea Angustifolia* (DA). Recent studies on the DA prove that the extracted leaves of DA plant have antimicrobial activity against Gram-negative (Escherichia coli), Gram-positive (*Staphylococcus aureus and Bacillus pumilus*) bacteria and the fungus *Sacchromyces cerevisiae*(Omosa et al., 2014).The most recent study shows that extracts from DA leave by using 80% methanol as solvent showed a significant wound healing activity against control as supported by an increase in % of wound contraction and decreasing healing period(Tessema, Yibeltal, & Molla, 2021).In the way of using bio films specially in extending shelf life of fruits, Nano particles as fillers are widely used materials to enhance the property of the film. The green synthesis of nanoparticles is a newly advanced technique that is broadly utilized in both the scientific and industrial fields. Green synthesis nanoparticles have sizes among 1 to 100 nm and their synthesis procedure is environmentally friendly and non-poisonous as compared to chemical or physical techniques of nanoparticle synthesis. They are usually utilized in extension of food shelf life, medical, biomedical and cosmetic industries and also are important to exhibit antibacterial properties. Various inorganic metallic materials including silver, gold, zinc, platinum and nickel, can be used in the green synthesis of nanoparticles. In this study, silver will be used because of its low

cost and accessibility. Plant extracts can be used as capping material for the stabilization and reduction of silver ion to form silver nanoparticles (Joseph et al., 2021).

Due to the nature of flaxseed hydrogel, the activities of DA extract as well as Ag NPs against microorganisms, the aim of this research is developing of flaxseed hydrogel based DA extract induced and green AgNPs encapsulated biofilm for antibacterial application.

1.2. Statement of the problem

The need for management of pathogen control using a rapid and cost-effective method presents a major quest for the research in the area. Wide ranges of bacteria, molds, and yeast contamination as well as infections are known to inflict mess on various items. Different infectious diseases and the deterioration of different products due to microbial contamination in the food packaging industries and agricultural areas are undeniably major issues, resulting in considerable economic losses and health issues. Most of the time in developing countries, severe burn injuries, surgical operations, skin infections are causes of morbidity and mortality. As a result, the rising incidence of bacterial resistance leads to severe systematic complications. These have an impact on an individual's and a country's economy.

Antibacterial agents are crucial in several areas of science, such as medicine, industrial materials, food packaging, and water treatment. These antimicrobial agents are active agents that inhibit bacterial growth, prevent the formation of microbial colonies, and may destroy microorganisms. Plants extracts mediated Nanoparticle (NPs) synthesis enhances antibacterial activity without creating toxicity to surrounding tissues is preferable. Because different synthetic methods, such as the micro emulsion technique, sol–gel method, and aerosol techniques are time-consuming, have high cost of production, and possibility of contamination with toxic chemicals. To overcome these limitations, low-cost green synthetic techniques were developed in which substances with low toxicity are utilized. Researchers have been studying the antibacterial properties of nanoparticles for a long time. Among these, AgNPs are of high value because they have low toxicity.

Biofilm preparation which contains NPs and plant extract as fillers become ultra-efficient for various applications such as bio-medical, active packaging, agricultural, and heat transmission application. The majority of packaging materials used across the world are petroleum-based polymers. Theses plastic polymers trash has significant drawbacks in terms of posing a threat to the environment due to higher levels of toxic emissions, changes in the carbon dioxide cycle, and composting issues. Therefore the application of nono-based biofilms could replace these limitations.

1.3. Research gap

According to different literatures, various flaxseed hydrogel based biofilms been developed for various applications. To improve the characteristics of the film such flexibility and plasticity the flaxseed hydrogel was blended with materials like glycerol and polyvinylchloride as cross linker and plasticizer. But this is not enough to improve the microbial inhibition performance of the biofilm. Based on the literature survey, no work was observed by the authors of this thesis on:

- Developing a biofilm with good anti-microbial property which contains DA extract and Ag nanoparticles using flaxseed hydrogel as matrix.

1.4. Research question, hypothesis and choice of system

Research questions: the present work aims to answer the following major questions

- Is it possible to develop DA extracting induced flaxseed hydrogel biofilm?
- Is it possible to encapsulate Ag-nanoparticles in flaxseed hydrogel matrix?
- What will be the role of Ag-nanoparticles and DA extract on the improvement of flaxseed hydrogel based biofilm?
- What role can AgNPs and DA extract plays on the inhibition of bacteria's that mostly facilitate infection around wounded skin?

Based on the above research questions the choice of the systems is formed as follows:

- Flax seed hydrogel is chosen as matrix because it is a polysaccharide based polymer and have the ability to form gel.
- DA is selected due to its high antimicrobial activates and it is highly applicable in as traditional medicine for wound dressing.
- Ag-nanoparticles are commonly known for their high antimicrobial activities.

Hypothesis: due to the collective properties of those raw materials it may be possible to develop sustainable and green AgNps encapsulated DA induced flaxseed hydrogel biofilm.

1.5. Objective of the study

1.5.1. General objective

The major objective of this research work is to develop flaxseed hydrogel based, Dodonaea Angustifolia induced and Ag-nanoparticles encapsulated biofilm for antimicrobial application.

1.5.1. Specific objective

- Extraction and Characterization of active components from Dodonaea Angustifolia.
- Green synthesis and characterization of AgNPs
- Preparation and characterization of flax seed hydrogel
- Preparation and characterization of Dodonaea Angustifolia induced flax seed hydrogel.
- Encapsulation of Ag nanoparticles in to Dodonaea Angustifolia induced flax seed hydrogel and characterization.
- Investigation of the antimicrobial performance of the final biofilm on the skin infection causing bacteria

1.6. Significance of the study

In developing countries wound infection is serious problem because of poor hygienic condition. DA plant is commonly used traditionally for wound healing purpose. Thus, the production of this biofilm also enables to modernize the traditional way of using of DA plant for its antimicrobial application.

Plant-meditated synthesis of NPs leads the development of environmentally friendly nanoparticle synthesis processes. It helps to prevent the creation of hazardous by-products due to its low cost, zero contamination, and high reduction potential and stable nanoparticles. AgNPs synthesized by this method play effective antimicrobial actions however, unique characteristics like size, shape, and phases influence bacterial inactivation. But Suspensions of nanoparticles in the fluid make smaller particles, high surface area, and the interaction among the particles became dominant. In this research, AgNPs were dispersed in DA encapsulated FSH, which can be used for further applications. Nanoparticles dispersed flaxseed-based biofilms are eco-friendly materials and could replace petroleum-derived films.

This research will contribute to the knowledge of Ethiopia's production of a biofilm in the near future and reduce the environmental treat that waste plastic accumulation pose. It will initiate the consumption of flaxseed hydrogel as best raw material for the production of biofilm. the production of this natural biofilm not only be reduced or cease the imported synthetic polymer films but also mitigate the waste plastic problems and generate a foreign currency too. For many kinds of fruits such tomato, coating with edible film continues to be one of the most cost effective ways to maintain their quality and shelf life.

1.7. Scope of the Study

The scope of this research was focused on the following bulleted points:

- Studying the effect of operational parameters on (extraction temperature and time) of *Dodonaea Angustifolia*, locally known as Kitkita by indirect method.
- Optimization of extraction parameters of flaxseed hydrogel using design expert software.
- Green synthesis of AgNPs using leaves extract of *Dodonaea Angustifolia*
- Effect of different operational parameters (precursor concentration, volume ratios of AgNO3 solution to plant extract, temperature, and time) on greenly synthesized AgNPs was clearly investigated.
- Characterization of *Dodonaea Angustifolia* extract, flaxseed hydrogel, greenly synthesized AgNPs and their antimicrobial activity were investigated.
- The antimicrobial activity of prepared flaxseed based biofilm was also investigated.

CHAPTER TWO

LITRATURE REVIEW

2.1. Back ground of hydrogel

Hydrogels were designed biomaterials in 1960 for the first time by Wichterle and Lim as a hydrophilic network of poly (2-hydroxyethyl methacrylate) (PHEMA) in contact lenses and developed for human use. Hydrogels are cross-linked, three-dimensional, hydrophilic polymer structures, which can absorb, swell and retain huge amounts of water or aqueous fluids (Thakur, Thakur, & Arotiba, 2018). Their affinity to absorb water is due the presences of polar functional groups/ hydrophilic groups on the skeleton of macromolecule such as –OH, –CONH–, –$CONH_2$–, and –SO_3H in polymers forming hydrogel structures. Due to the contribution of these groups and domains in the network, the polymer is thus hydrated to different degrees (sometimes, more than 90%wt.), depending on the nature of the aqueous environment and polymer composition. Hydrogels show a swelling behavior instead of being dissolved in the aqueous surrounding environment as a consequence of the critical crosslink's present in the hydrogel structure. Hydrogels can be prepared by chemical and physical crosslinking methods. Chemical methods involve the formation of new covalent bonds among the polymer chains of hydrogel, whereas physical interactions were existing between the polymer chains of hydrogel (G. Sharma et al., 2018). Crosslinking of polymer can be done using cross linker, chemical modification, grafting or using high energy radiation (Gamma or UV rays) conjugation (Hennink & van Nostrum, 2012).The biophysical similarity of these to soft biological tissues makes them useful biomaterials for the research community. These are used as slabs, microparticles, nanoparticles, coatings, and films (Auriemma et al., 2020). In the recent era hydrogel technology used in the different application such as metal ion adsorption, drug delivery, antibacterial drug release for wound dressings, degradation of dyes (Singh et al., 2017), Agriculture, Nano fibers, Hygienic Products, (Ahmad et al., 2019), cell encapsulation, tissue engineering, soft electronics, and actuators (Zhang & Khademhosseini, 2017) .

2.1.2. Classification of hydrogel

Hydrogels can be classified based on different ways. Some of the classifications are based on source(Ismail, Irani, & Ahmad, 2013), crosslinking, (Auriemma et al., 2020),degradability ,ionic charge, and, crosslinking (Peppas & Mikos, 2019) .

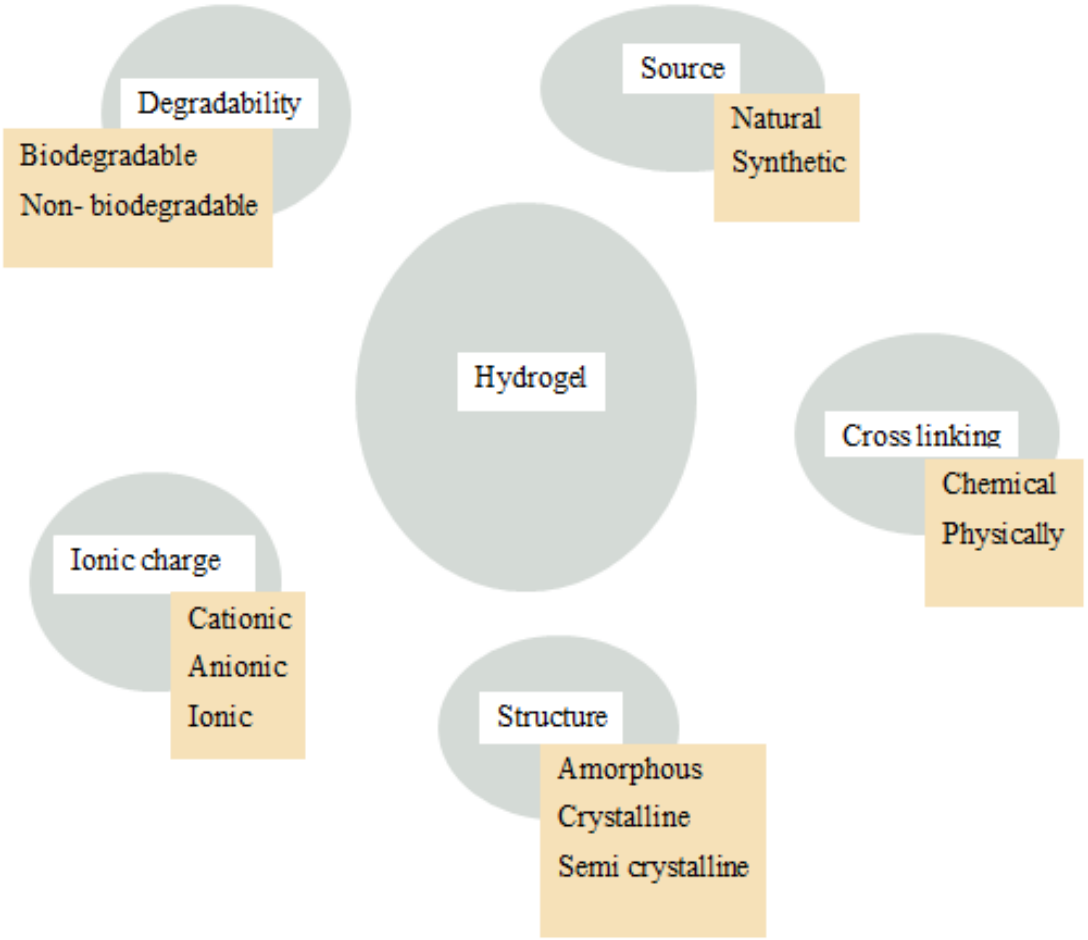


FIGURE2.1. Classification of hydrogels

2.1.2.1. Classification of hydrogel depending on source

Hydrogels can be arising from Natural and Synthetic sources. Those nature derived polymers used as source of hydrogels are polysaccharide-based natural hydrogels (Starch, Pectin, Cellulose, Alginate, chitosan, chitin,) ,protein-based natural hydrogels(elastin, keratin, Collagen and gelatin), Lignin-based hydrogels, algae-based hydrogels (agar, alginate, and carrageenan) , Bacteria-based hydrogels such levan, gellan, and cellulose (Mohammadinejad et al., 2019), (Varghese et al., 2020).

2.1.2.1.1. Polysaccharide based hydrogel

Polysaccharides are most abundant class of biopolymer, they are obtained from animal, plant, microbes, and Algaa (Kosaraju, 2005). Polysaccharides are highly stable, renewable, nontoxic, intrinsically biodegradable, relatively cheap, and hydrophilic in nature due to the presence of functional groups such as hydroxyl amino and carboxylic acid and these functional groups in polysaccharides that offer the floor for the modification of polysaccharide (Y. Wen & Oh, 2014). Diversity in structures and in properties of polysaccharides are attributed to the wide range of molecular weights and different chemical compositions (Z. Liu et al., 2008).Despite that wide range of molecular weight distributions and different chemical compositions of polysaccharides still they suffer from several drawbacks.one of the most used polysaccharide are Natural mucilage's composed of polysaccharides with numerous sugar units, which are linked together to form huge polymers with large molecules. Polysaccharides are the most broadly used components found in hydrogels as they're found in most commercially available formulations. In the case of producing hydrogel biofilms from Polysaccharides, it shows powerful gas barrier properties even though they're quite hydrophilic and show excessive water vapor permeability in contrast with commercial plastic films. The important polysaccharides that may be included in edible coating formulations are starch and starch derivate, cellulose derivate, alginate, carrageenan, chitosan, pectin, and several gums and mucilage's.(Vargas, Pastor, Chiralt, McClements, & Gonzalez-Martinez, 2008).one of the most common source of natural polysaccharide based mucilage (hydrogel) is flaxseed.

2.2. Preparation of hydrogel from flaxseed

Different scholars undergo the study of flux seed hydrogel on its preparation, compostion, characterstics, and its application. Cui et al reported that the extracted Flaxseed gum under optimized aqueous extraction was contained over 80.3% carbohydrates. The crude gum was dialyzed and separated by ion exchange chromatography into an acidic (AFG) and a neutral fraction (NFG). The neutral fraction composed mainly of arabinoxylans with large molecular sizes, exhibited shear thinning behavior at higher concentrations. The acidic fraction contained pectic-like material with much smaller molecular sizes and consequently exhibited Newtonian flow behavior even at concentrations up to 2.0%. Arabinoxylans were the major component responsible for the shear thinning and weak gel properties of flaxseed gum, although possible polymer-polymer interactions may exist (Cui, Mazza, & Biliaderis, 1994) .Qian et al. proves that because of Soluble flaxseed gum (SFG) have low viscosity, it has potential in applications as a fiber fortifier because Soluble dietary fiber with low viscosity has the potential to deliver acceptable mouth feel and texture when included in the diet in a significant amount to show health benefits. According to Qian et al. the gum (SFG) extracted from flaxseed hulls was further separated into neutral (NFG) and acidic (AFG) fraction gums using ion exchange chromatography (IEC). The chemical (neutral monosaccharide composition, uronic acid and protein content), physical (molecular weight distribution and heat stability), rheological (viscosity, viscoelasticity, intrinsic viscosity and critical concentration,) and functional (surface tension and emulsification) characteristics of SFG and its two fractions was reported (Qian, Cui, Wu, & Goff, 2012).The composition and yield of flaxseed gum has been reported to vary with extraction conditions such as time, temperature, PH and water to seed ratio. the optimum gum extraction condition to achieve higher yield and quality was found that water to seed ratio of 13 at 85-95 ^{0}C and pH from 6.5 to 7.0 for 3 h using response surface methodology .The neutral monosaccharide composition of SFG, NFG and AFG was comparable with previous results (Cui et al., 1994);(Fedeniuk & Biliaderis, 1994).neutral flaxseed gum (NFG) was mainly composed of xylose (68.2%) and arabinose (20.2%) therefore being identified as arabinoxylans (Warrand et al., 2003). Its minor components included galactose (7.9%) and glucose (3.7%), but no rhamnose or fucose. In contrast, acidic flaxseed gum(AFG) mainly consisted of rhamnose (38.3%), galactose (35.2%) and fucose (14.7%). With most galactose and all fucose existing as terminals in side chains (Naran et al., 2008), AFG was referred to as rhamonogalacturonans for its high

content of rhamnose and galacturonic acid (38.7 %). The presence of small amount of xylose (8.9%) and arabinose (2.9%) in AFG might be due to the residual fraction of NFG (Cui et al., 1994)or might be derived from side chain components covalently linked to its backbone (Naran, Chen, & Carpita, 2008).Oomah and its coworkers also study on quantitative and qualitative information on the distribution and variation of component phytochemicals in existing aqueous extract flax accessions or cultivars with the aims of providing new information on the carbohydrate composition of a large set of flaxseed accessions (Oomah, Kenaschuk, Cui, & Mazza, 1995).Karami et al produces flaxseed mucilage by hot aqueous extraction and the results obtained in the reveals that polysaccharide mucilage extracted from different flaxseed cultivars exhibited significant variations in their chemical composition and functional properties. All mucilage samples had presence of high proteinaceous components in them which contributed to their foaming and rheological properties. The rheological properties of mucilage solutions exhibited their shear thinning behavior and suggested their use to provide texture and structure to various food products. Frequency sweep tests on mucilage solution indicated their viscoelastic fluid behavior. Power Law Model could be used for analyzing and modeling of mucilage flow properties. All mucilage's showed high decomposition onset temperature thereby indicating their thermal stability. Accordingly suitable cultivar can be selected as this variation in properties offers opportunities to produce flaxseed mucilage for specific applications in food and non-food industries(Karami, Kamkar, Shahbazi, & Misaghi, 2019).

2.3. Application of hydrogel from flaxseed

Flax is one of the crops targeted for genetic manipulation for seed oil (Rowland et al., 1995); (Rowland & Bhatty, 1990) and nutritional therapeutics (Oomah et al., 1995). (O'Mullane & Hayter, 1993);(Oomah et al., 1995) have been issued for the use of flaxseed gum for cosmetic field (Warrand, 2003), therapeutic, and medical preparations(Oomah et al., 1995).Soluble flaxseed gum (SFG) have low viscosity, it has potential in applications as a fiber fortifier because Soluble dietary fiber with low viscosity has the potential to deliver acceptable mouth feel and texture when included in the diet in a significant amount to show health benefits (Qian et al., 2012).

2.4. Limitation of hydrogel from flaxseed regarding its application

FG has some limitations such as low dissolution rate in cold water, poor mechanical stability, and low storage stability and such properties might limit FG utilization(J. Liu et al., 2018).Yang et al found that Flaxseed gum had the less effect on the formation and maintenance of a rigid structure than KGM, in that the samples with a higher FSG ratio in a fixed AG ratio showed the formation of a more complex structure of the compound gel(Yang, Kim, Choi, & Hahn, 2021).

According to Tee et al studies one possible drawback of pure flaxseed film is its low elasticity. Recent studies on basil seed and chia seed mucilage-based film showed that the addition of plasticizer aided the formation of film with a notable reduction in the films' brittleness (Khazaei, Jafari, Ghorbani, & Kakhki, 2014).

2.5. Modification of flaxseed hydrogel

Polysaccharide gums show significant advantages over synthetic materials due to their sustainability, biodegradability, and biosafety. Many natural gums can form three-dimensional interconnected molecular networks known as 'gels', which enables their application in different areas based on specific functional properties. However, natural gums do have inherent problems associated with their use including uncontrolled rates of hydration, pH dependent solubility, thickening, drop in viscosity on storage, and the possibility of microbial(Rana et al., 2011) .Chemical modification of gums not only minimizes those drawbacks but also enables their application for specific purposes. Due to the reason of flax seed hydrogel is one of the most remarkable polysaccharide, and many scientists use different techniques to modify the properties of flaxseed hydrogel such as cross linking, esterification, filler dispersion, and blending.

2.5.1. Crosslinking and esterification

Polysaccharides may be cross-linked to alter their swelling properties and to prepare novel hydrogels. The properties of cross-linked gum derivatives depend mainly on cross-linking density (the ratio of moles of cross-linking agent to moles of polymer repeating units). Cross-linking of gums requires availability of active functional groups, including free alcohols, carboxylic acids, polyacrylamide, and phosphate. Ionic bonds are the main interactions of network forming reactions but other interactions, such as hydrogen bonds and hydrophobic interactions also influence properties (Rana *et al.*, 2011).Many cross linkers such as multivalent metal ions (Ca2+ and Ba2+), disodium trimetapolyphosphate, acrylamide, glutaraldehyde, and

epichlorohydrin have been used for crosslinking polysaccharides of a sodium alginate-carboxymethyl guar gum mixture (Bajpai, Saxena, & Sharma, 2006), konjac glucomanan(Gao, Guo, Wu, & Wang, 2008); (L. Wen et al., 2009) a hyaluronan-guar gum mixture (Gliko-Kabir, Yagen, Penhasi, & Rubinstein, 2000),hyaluronic (Dulong et al., 2004),alginate-guar gum mixture (George & Abraham, 2007),guar gum(Soppirnath & Aminabhavi, 2002) ,and cashew gum(Silva, Feitosa, Maciel, Paula, & de Paula, 2006).Chen et al studies on Flaxseed gum solutions and observed that the dissolution temperature, pH value, addition of differential valence mineral salts as cross linking agents affected the gel strength of flaxseed gum. According Chen et al Effect of salts was observed by the responses in gel strength of flaxseed gum with addition of different valence mineral salts. Different salts had different effects on the gel strength. Initial addition of NaCl resulted in reduction of the gel strength, which attributed to compression of the double electric layer of molecules. While addition of CaCl2 up to 0.3% resulted in increase of gel strength and the converse was obtained above 0.3%. As the Zeta potential was lowered, the intramolecular charge repulsions decreased progressively. The polysaccharide molecules were contracted and the junction zone numbers were decreased due to the addition of monovalent and divalent cations (Mazza & Biliaderis, 1989). Flaxseed gum possesses 14% galacturonic acid which attaches to the backbone of molecules(Y.-Y. Chen & Chang, 2004).Ca2+-induced crosslinking between flaxseed gum molecules promoted to form three-dimensional networks when adding small amounts of CaCl2 to the gels, as commonly observed for pectin (BeMiller, 1996). But the shielding effect of electrostatic repulsion was predominant when adding large amounts of CaCl2. Thus, the formation of three-dimensional networks was inhibited and gel strength decreased correspondingly(H.-H. Chen, Xu, & Wang, 2006).

Esterification has been employed as a common method to modify polysaccharide gum physic Chemical properties and improve utilization of the starting material. Polysaccharide gums may be handled as thermoplastic materials after esterification to facilitate processing. Esterification has additional effects on polysaccharide gum properties as esterified products may have better thermal stability, reduced water absorbency, increased solubility, and reduced crystallinity(J. Liu et al., 2018).Polysaccharide gum structures can be modified using esterification protocols such as acetylation (Teramoto, Arai, & Shibata, 2006), succinylation (Fang, Sun, Tomkinson, & Fowler, 2000) ,sulfation (Abatangelo, Barbucci, Brun, & Lamponi, 1997), and tosylation (Gericke et al., 2012).

Devi et al studies on mucoadhesive potential of flaxseed mucilage after thiol(an organic compound containing –SH) functionalization. Thiol-functionalization of flaxseed mucilage (FSM) polysaccharide was carried out by the esterification reaction employing thioglycolic acid (TGA) in the presence of catalytic amount of hydrochloric acid. Thiolation of FSM was confirmed by –SH stretch in FTIR spectra at 2549.01 cm-1. The formation of disulfide bonds between thiomers and cysteine rich subdomaine of mucus leads to mucoadhesion (Bernkop-Schnurch, 2005); (Devi & Bhatia, 2019). Thiomers possess many advantages apart from mucoadhesion that include prolonged residence time of delivery system, site specific targeting, avoidance of first pass metabolism, reduced side effects due to localization of drug at disease site and significant reduction in cost (Andrews, Laverty, & Jones, 2009);(R. Sharma & Ahuja, 2011). the result also shows greater ex-vivo bioadhesion time of TFSM as compared to FSM. This improvement in mucoadhesion property of TFSM over FSM can be attributed to the formation of disulphide bond between mucus and thiolated mucilage. Further, the in-vitro dissolution study conducted in phosphate buffer (pH 6.8) provided release of diclofenac sodium for a prolonged period of 12h for TFSM pellets by anomalous transport mechanism of drug release following zero order model of release kinetics. The thiol functionalization of Flaxseed mucilage has been proved to impart the mucoadhesive properties to it and also sustained the drug release over a period of 12 h. Thus it was concluded that TFSM conjugate owned potential as a bioadhesive carrier (Devi & Bhatia, 2019).

2.5.2. Blending

Polymer blends are physical mixtures of two or more polymers with/without any chemical bonding between them. The objective of polymer blending is achieving commercially viable product through either unique properties or lower cost than some other means might provide. At least two polymers are blended together to create a new material with different physical properties.

de Paiva et al. observed that Mixing flaxseed mucilage with polyvinyl alcohol (PVA)significantly influenced the mechanical properties, providing less rigid films by reducing youngs modules (YM) and more flexibility with increasing elongation at break with better thermal stability but did not change water vapor barrier properties compared with pure mucilage films and pure PVA films. These films obtained with mixtures of flaxseed and PVA mucilage extracts can be an interesting and advantageous alternative for producing bio-based packaging

for different application (de Paiva et al., 2020).The mechanical properties of food packaging are relevant and of great interest to the packaging industry, as they reflect the ability to protect the material from food integrity during storage and marketing. For this reason, the strength and flexibility of packaging are necessary to maintain this integrity(Beigomi, Mohsenzadeh, & Salari, 2018); (Gheribi et al., 2018).

Tee et al also studies effect of adding various concentrations of glycerol as a plasticizer, with characterizations in terms of physical, mechanical, and morphological properties of the developed film. A flaxseed mucilage-based edible film was developed with the addition of glycerol as a plasticizer. Various concentrations of glycerol were blended into the extracted mucilage, and the developed films were studied in terms of physical, mechanical, and morphological properties. As the glycerol concentration was increased from 1 to 5 wt%, the elongation at break of the films prominently increased, whereas the tensile strength and Young's modulus decreased. The film failed to form at 6 wt% glycerol inclusion. The developed film was slightly reddish and yellowish in color, with enhanced transparency as the glycerol concentration in the film increased. Overall, this work demonstrated that with the addition of glycerol as an plasticizer up to 5 wt%, a flaxseed mucilage-based edible film could be developed as a sustainable alternative for food and bioproduct coating or packaging (Tee, Wong, Tan, & Talib, 2016).

Yang et al prepared hydrocolloid gels in which flaxseed gum (FSG), konjac glucomannan (KGM), and agar (AG) were blended in different ratios for use as a viscoelastic food becauset FSG had the less effect on the formation and maintenance of a rigid structure than KGM, in that the samples with a higher FSG ratio in a fixed AG ratio showed the formation of a more complex structure of the compound gel. In this study, as a potential strategy for the use of FSG as a food replacement, a simple FSG extraction process was proposed, and hydrogels were prepared by mixing FSG with KGM and AG. Then, the hydrogels that were mixed at different FSG, KGM, and AG ratios were tested to determine their water solubility index and swelling power values, viscoelastic properties, and microstructures. The result reveals that the relatively high swelling power (SP) value appeared according to the specific KGM/FSG/AG mixing ratio (FKA820.5, FKA821, FKA641, FKA821.5, and KFA641.5). the microstructure of FKA821.5 had a smaller pore size and a denser structure than the other samples. Water solubility index (WSI) value indicates how soluble the gel sample is in water. All of the gel samples with respective FSG and

KGM ratios of 10:0 and 8:2 (FKA1000.5, FKA1001, FKA1001.5, FKA820.5, FKA821, and FKA821.5) exhibited significantly lower water solubility than the other samples. In addition, the test gels tended to have lower solubility in water as the proportion of FSG was increased. FKA821.5 had high elasticity and water holding capacity. Finally FKA821.5 at the proposed ratios was found to have a synergistic effect that yields solid properties, such as rigid and elastic structures.The results of this study indicates that possibility of the applicability of FSG as a gelling agent in the food industry (Yang et al., 2021).

2.5.3. Fillers

Sheikh et al developed novel gastro retentive drug delivery system as floating matrix tablets based on a polysaccharide material from linseeds (Linum usitatissimum L.) for fluoroquinolone antibiotics. A number of formulations were designed with a combination of linseed hydrogel (LSH) and different excipients to obtain a desired sustained release profile of moxifloxacin (Sheikh, Hussain, Ashraf, Haseeb, & Farid-ul-Haq, 2020). LSH based gastroretentive drug delivery system was successfully developed which sustained the release of moxifloxacin in the stomach and tablets remained floating for 6 h .These findings indicated that LSH can be used to develop novel gastroretentive sustained release drug delivery systems with the double advantage of sustained drug release at all pH of GIT (Sheikh et al., 2020).to improve the desired properties like flouting, swelling and drug release ability of flaxseed hydrogel different materials was introduced. As duration of time increase, the rate of drug release from a polymeric system is reduced due to the greasy nature of the polymers. To overcome this difficulty, a channeling agent is introduced in the formulation design which eliminates the chances of a potential decrease in drug release. b-cyclodextrin, a pH-independent highly soluble channeling agent, was used which not only increases the drug release at the later stage of the study but also improves the compressibility and physical texture of the tablets (Chavanpatil, Jain, Chaudhari, Shear, & Vavia, 2006). Drug release was increased by adding b-cyclodextrin, mainly due to the formation of channels that facilitates the approach of dissolution media in the deep area of the tablet. Sodium bicarbonate was also used as a gas-generating agent that is liberated in contact with the acidic media of the stomach. drug release is mainly due to the increase in the swelling of the tablet which ultimately increases the diffusion path length.

To improve the antimicrobial activity of flaxseed hydrogel different type of essential oils was dispersed. *Ziziphora clinopodioides* is one of the most common used spice in dairy and meat

products in western provinces of Iran. Several studies have reported the antibacterial, antifungal and antioxidant effects of *Z. clinopodioides* essential oil (ZEO)(Naeeji, Shahbazi, & Shavisi, 2020) and Sesame oil (SO) has abundant, pleasant and significant aroma and it has been considered as an effective antimicrobial agent against some foodborne pathogens (Malhan, Najeeb, & Buniya, 2019). Chitosan is one of the most abundant renewable polymers used in the medical, food, agricultural and chemical industries mainly due to its unique properties including intrinsic wound-healing, antioxidant and antimicrobial properties, hemostatic activity, film-forming ability, biodegradability, biocompatibility, availability and non-toxicity (Shahbazi, 2017).by Combining those three components and incorporating into flaxseed hydrogel Karami et al. produces good film with antimicrobial and antioxidant property.

Table 3.1.Summary of flax seed based hydrogels

Bio-polymer source	Unique Features	application	Reference
Pure flaxseed	- 3D network structure - optical transparency - viscoelastic fluid behavior - good thermal stability - soluble at high temperature	- food and non-food industries.	(Kaur, Kaur, & Punia, 2018)
chitosan-flaxseed mucilage film with dispersed *Ziziphora clinopodioides* essential oil (ZEO)and sesame oil (SO	- good antioxidant - Antimicrobial property - bio-compatable for foods - can be processed as eadible film or coatings	- antioxidant and Antimicrobial against *microorganisms*	(Karami et al., 2019)
Flaxseed gum solutions and different mineral salts	- strong gel property	- packaging	(H.-H. Chen et al., 2006)
Flaxseed hydrogel and thioglycolic acid (TGA)	- excellent mucoadhesive potential - prolonged residence time of delivery system	- drug release	(Devi & Bhatia, 2019).

	- the ability to form gels		
Flaxseed hydrogel and glycerol	- excellent oxygen barrier properties - can be processed as eadible film or coatings - enhanced transparency and plasticity - excellent thermal stability	- food and bio product coating or packaging	(Tee et al., 2016).
Flaxseed hydrogel and polyvinyl alcohol	- less rigid films - reducing young's modules (YM) and - excellent flexibility with good elongation at break - with thermal stability	- food packaging	(de Paiva et al., 2020).
flaxseed gum (FSG), konjac glucomannan (KGM), and agar	- high swelling power - high elasticity - high water holding capacity - rigid structure	gelling agent in the food industry	(Yang et al., 2021)
Flaxseed hydrogel and (alginate, pectin ,chitosan and alginate)	- potential to act as wall material for the encapsulation of probiotic - high encapsulation	- microencapsulation of lactobacillus rhamnosus GG - encapsulating probiotic *Lactobacillus casei*	(Lai, How, & Pui, 2021);(Lai et al., 2021)

	efficiency - reduces the pore size of the microbeads - positively affect for growth of *L. casei*		
Flaxseed hydrogel and cyclodextrin	- porous nature - elongated channels - good swelling property tablet - facilitated discharge of moxifloxacin from the core of the tablet	- gastroretentive drug delivery(fluoroquinolone antibiotics)	(Sheikh et al., 2020)

2.6. Application of flaxseed based hydrogel

Flax is one of the oldest crops and its seeds (flaxseeds/linseeds) are the rich source of carbohydrates (Barbary, Al-Sohaimy, El-Saadani, & Zeitoun, 2009);(Kaewmanee et al., 2014).Hot water extracted linseed hydrogel/mucilage (LSH) is considered a safe excipient (Haseeb et al., 2018) as well as exhibiting diverse biomedical applications such as reducing and capping agent for the synthesis of silver nanoparticles and incorporated in wound healing dressing ,stimuli responsive oral sustained release drug delivery system (Haseeb, Hussain, Bashir, Ashraf, & Ahmad, 2017), development of nanoparticle carrier system for anticancer drug (Haseeb, Hussain, Abbas, et al., 2017),as a component for the reduction of cholesterol and blood glucose in diabetics and for the increase of fecal fat excretion in humans Bustamante, Villarroel, Rubilar, & Shene, 2015. Flaxseed hydrogel has also been introduced as a suitable agent for enhancing the starter bacterial count of stirred yogurt (Basiri et al., 2018) and also as an effective wall material for encapsulating flaxseed oil (Mohseni & Goli, 2019).

2.6.1. Medicinal

2.6.1.1. Drug delivery

Hydrogellable biomaterials are being widely used in sustained release formulations nowadays (Lungan et al., 2015; Samantha & Ray,) like other natural mucilages, flaxseed mucilage has the potential to be used as excipient of modified release systems, as reported by Nerkar and Gattani ,(Haseeb, Hussain, Bashir, et al., 2017), (Sheikh et al., 2020), and (Nayak & Hasnain, 2019). Rocha et al develops feature matrix tablets by using gross linseed mucilage (not precipitated by alcohol) as a retardant polymer. gross linseed mucilage using as excipient of modified release systems was developed (Rocha et al., 2021).Sheikh et al also developed novel gastro retentive drug delivery system as floating matrix tablets based on a polysaccharide material from linseeds (Linum usitatissimum L.) for fluoroquinolone antibiotics (Sheikh et al., 2020). The thiol functionalization of Flaxseed mucilage has been proved to impart the mucoadhesive properties and also sustained the drug release over a period of 12 h. (Devi & Bhatia, 2019).

2.6.1.2. Antimicrobial and antioxidant effect

Flaxseed based biofilm was used as Antimicrobial and antioxidant properties by incorporating of chitosan, *Ziziphora clinopodioides* essential oil and sesame oil. chitosan-flaxseed mucilage (CH

FM) film against *Listeria monocytogenes*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Escherichia coli* O157:H7 *in vitro* condition and raw minced trout fillets gives positive result.The *in vitro* antibacterial and antioxidant properties of CH-FM films were evaluated using agar disk diffusion method and free radical scavenging activity assay, respectively. The antioxidant property of CH-FM based films were found to be ranged 5.45% ± 0.04-37% ± 0.45.The designated films had good antibacterial effect against some food borne pathogenic bacteria including *L. monocytogenes*, *S. aureus*, *S. typhimurium* and *E. coli* O157:H7 in raw rainbow trout fillets(Karami et al., 2019) .

2.6.1.3. Encapsulation of prebiotics

Improving health through modulation of the micro biome is a part of a comprehensive method for human wellness *Lactobacilluscasei* (*L. casei*) is one of the most important probiotic species which have wide applications in food products. However, a high percentage of these probiotic bacteria lose their viability during passage through the gastrointestinal tract. This has encouraged researchers to find effective approaches to protect the viability of probiotic cultures (Granato, Branco, Nazzaro, Cruz, & Faria, 2010). Microencapsulation is one of the latest techniques used for this purpose. Alginate (ALG) has remained the most commonly used hydrocolloid for microencapsulation. The non-toxicity, low cost and the ability of ALG to simply form gentle matrices around living microbial cells are some of the advantages of using this biopolymer for encapsulation purposes(Bhagwat, Bhushette, & Annapure, 2020). However, it has low stability in the acidic conditions which has limited its applications (Granato et al., 2010). To overcome this drawback, a mixture of ALG and a soluble fiber can be used for creating microcapsules. In recent years, flaxseed, owing to its excellent health benefits, has become an attractive ingredient in the diets specially designed for specific health benefits. The mucilage extracted from flaxseed contains arabinose, xylose and galactose and trace amounts of acidic rhamnose (Hall Iii, Tulbek, & Xu, 2006) .Shafizadeh et al was studies the effect of FM as a nutritive source, on the growth of *L. casei* bacteria.In addition, the effect of incorporating FM into ALG microcapsules on the morphology, dimension, and viability of the free and microencapsulated *L. casei* before and after exposure to simulated gastrointestinal conditions was investigated.the result shown that FM can be used as a nutritive source for improving the growth of *L. casei* probiotic bacteria. It has also been observed that microencapsulation of *L. casei* with a combination of ALG (2%) and FM

(0.9%) provides a relatively good protection of probiotic bacteria against gastrointestinal simulated conditions and enhances their viability. However, investigation of the stability of this microencapsulated probiotic in the food model during storage is an area that needs further research (Shafizadeh, Golestan, Ahmadi, Darjani, & Ghorbani-HasanSaraei, 2020).Lai et al also studies on microencapsulation of lactobacillus rhamnosus GG with flaxseed mucilage using co-extrusion technique.FM mainly contains non-nitrogen compounds, proteins,fat, fiber, ash and different monosaccharides including xylose, galactose, arabinose, fucose, glucose and maltose(Shafizadeh et al., 2020). Therefore, it can act as a carbon source promoting bacterial growth and increasing the OD. Hadi Nezhad et al reported that the soluble fiber of flaxseed improved the viability and growth of probiotic bacteria.flax Seed mucilage is a soluble fibre that was hydrated and produced by mucilage secreting cells (Kreitschitz & Gorb, 2018). The research claimed that seed mucilage showed good prebiotic potential as probiotics could utilize it for growth (Mueller, Čavarkapa, Unger, Viernstein, & Praznik, 2017). Its prebiotic properties are associated with the presence of arabinoxylan and were capable of stimulating the growth of *L. acidophilus* La-05 and has the potential to act as wall material for the encapsulation of probiotic (Bustamante, Villarroel, Rubilar, & Shene, 2015).

2.6.2. Encapsulation of flaxseed oil

Flaxseed oil have high amount of unsaturation and it is susceptible to oxidation which could reduce oil nutritional value and cause off-flavor. Due to this problem Mohseni et al studies on flaxseed oil encapsulation in new wall materials of tertiary conjugate of gelatin-flaxseed mucilage (FM)-oxidized tannic acid (OTA) which are available and food grade with nutritional value, controlled-release ability. The findings revealed that the oil is physically entrapped into the complex and no chemical interaction with the functional groups of wall materials was created. The complex was successful to protect and improve stability index as well as controlled release of flaxseed oil. Since the tertiary complex of gelatin flaxseed mucilage-tannic acid could be fabricated from available, food grade and functional materials depending on this result Mohseni et al conclude that flaxseed mucilage might have great potential for application in food and drug industries to encapsulate bioactive components(Mohseni & Goli, 2019).

2.6.3. Green synthesis of nanoparticles

NPs have been synthesized from a large variety of metals; silver nanoparticles (Ag NPs) have gained special importance over the last few years. Silver is also known to show antimicrobial properties, and several silver salts and their derivatives have been used as commercial antimicrobial agents. Polysaccharides are being extensively employed for the synthesis of silver nanoparticles (Ag NPs) having diverse morphology and applications. Haseeb et al develops a novel and green synthesis of Ag NPs without using any physical reaction conditions using Linseed hydrogel (LSH) used as a template to reduce Ag+ to Ag^0. The reason to use linseed is LSH is composed of two major polysaccharidal fractions: rhamnogalacturonan and arabinoxylan hydrogel (LSH) ,thus it can be utilized a green reducing and self-capping agent for the formation of Ag NPs. Ag NPs were stored in LSH thin films, and UV/Vis spectra recorded after 6 months indicated that Ag NPs retained their texture over the storage period. Significant antimicrobial activity was observed when microbial cultures (bacteria and fungi) were exposed to the synthesized Ag NPs. Wound-healing studies revealed that Ag NP–impregnated LSH thin films could have potential applications as an antimicrobial dressing in wound management procedures(Haseeb, Hussain, Abbas, et al., 2017).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Materials, chemicals and reagent

Dodonaea Angustifolia leaves were collected from Adama Science and Technology University premises, Ethiopia and Flaxseed was purchased from local market in Adama city. The major material that was used for performing various experimental works in this study are pH meter, Analytical balance, magnetic stirrer, beaker, heating mantle, Beakers , Measuring Cylinders, Conical Flasks, Funnels, Petri Dishes, Test Tubes, Centrifuge Tubes, Samples Holder, Centrifuge, Sieves, Ultra-Sonicator, and oven. For analysis purposes, UV-Vis Spectroscopy, X-RAY Diffraction (XRD), particle size analyzer (PSA) and Fourier-Transform Infrared Spectroscopy (FT-IR) have been used.

Chemicals used in this research are silver nitrate ($AgNO_3$), ferric Chloride, Mercury (II) Chloride, potassium Iodide, ethanol (C_2H_6O, purity ≥ 97%), sulfuric acid (H_2SO_4, 99%), chloroform, sodium hydroxide (NaOH, purity ≥ 97%), hydrochloric acid (HCl, 37%), Glycerol (99%), and distilled water were used for different stages of this study. All the chemicals, reagents, and equipment used to carry out various procedures and characterize the samples were analytical grade and standard Laboratory tools.

3.2. Methodology

3.2.1 Sample collection and preparation of *Dodonaea Angustifolia* (DA) leaves powder

The *Dodonaea Angustifolia* fresh leaves (DAFL) were collected from Adama Science and Technology University premises. The required mass of leaves was thoroughly washed once with tap water and then washed twice with distilled water to remove debris and dust particles. Subsequently, the cleaned leaves were shade dried at room temperature for dried for at least 10 days to reach constant weight. The dried leaves were crushed into powder using POLYMIX® PX-MFC 90D laboratory miller, equipped with hammer grinding attachment and sieve with 0.2 mm mesh. The prepared powder was packed with an airtight container and stored under dry conditions for further use. The overall procedure of powder preparation from *DA leaves* is illustrated in Fig. 3.1.

FIGURE 3.1 Stages of *DA* leaves powder preparation

3.2.2. Extraction of DA leaves powder

DW based DA leafs powder extraction parameters selection was conducted by indirect method (by synthesizing AgNPs) and observing the intensity peaks using UV-Vis spectrophotometer. Fixing one parameter at a time was takes place during selection factors. Factors affect the extraction of DA leafs was extraction time (0.5-2 hr), temperature (40-70 ^{0}c), and mass of DA leafs powder to volume of DW was fixed at 1/20 wt% and mixing speed was also fixed at 1200 rpm. DA Leafs extract has sticky nature which is difficult for filtration due to this reason 1/20 wt% was selected because this combination is suitable to solve the problem. The overall extraction of DA leafs is illustrated in Fig. 3.2 (Anode, Abraha, & Araya, 2018).

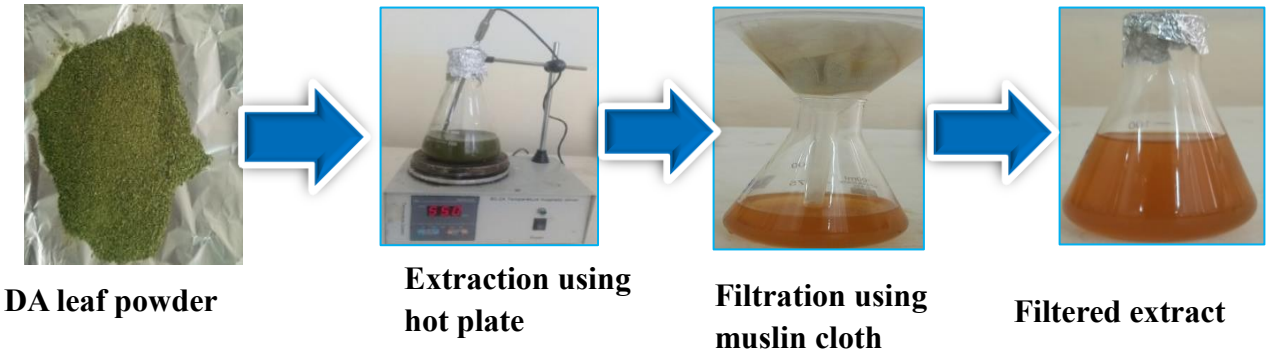


FIGURE 3.2 Stages of *DA* leafs powder extract preparation

3.2.3. Study the effect of operational parameters on the extraction of DA leafs powder

In the extraction of DA, various factors were needed to be maintained at optimum condition to attain the desired quality of product. In this study, the effect of operational parameters such as DA extraction temperature and time was analyzed for fixed ratio of mass of DA powder to volume of DW.

3.2.3.1. Effect of DA extraction temperature

Temperature has potential to degrade phytochemical constituents on the plant extract if increase beyond capacity of the components. Effect of DA extraction temperature was studied by synthesizing AgNPs using oil extracted on 40°C, 55°C and 70 °C. the reason to use temperature from 40-70 ^{0}c is, most of the time active components in plant extract are start to degrade at higher temperature (Harshiny, Iswarya, & Matheswaran, 2015) .The experiment was conducted separately by adding 5 mL of extract in to a 250 mL Erlenmeyer flask containing 50 mL of precursor solution and kept it for 30 minute by stirring speed of 1200 rpm. The AgNPs synthesized by oil extracted at various temperatures were confirmed using UV-Vis spectrophotometer. Based on the results observed by the UV-Vis spectrophotometer, the optimum DA extraction temperature was selected and used for further experiments.

3.2.3.2. Effect of DA extraction Time

The DA extraction time has great role on formation of good contact between solvent and DA powder. Contact time was varied as (30 min, 60 min, and 90 min) (Harshiny et al., 2015) . The experiment was conducted separately at different times by adding 5 mL of extract in to 250 mL Erlenmeyer flask containing 50 mL of precursor solution and kept at 40°C under stirring speed of 1200 rpm for 30 minute. The AgNPs synthesized by oil extracted at various times were confirmed using UV-Vis spectrophotometer. Based on the results observed by the UV-Vis spectrophotometer, the optimum DA extraction time was selected and used for further experiments.

3.2.4. Green synthesis of silver nanoparticles from the powdered leaves of DA

Green-based nanoparticle synthesis is a biologically mediated process in which biologically active compounds acting as a capping and stabilizing agent. In this study, the green synthesis of AgNPs was performed using leaves extract of DA and metal salt ($AgNO_3$). the mixture was stirred at speed of 1200 rpm using hot plate. The overall procedure of synthesis of AgNPs is illustrated in Fig. 3.3 (Revathi & Dhanaraj, 2019).

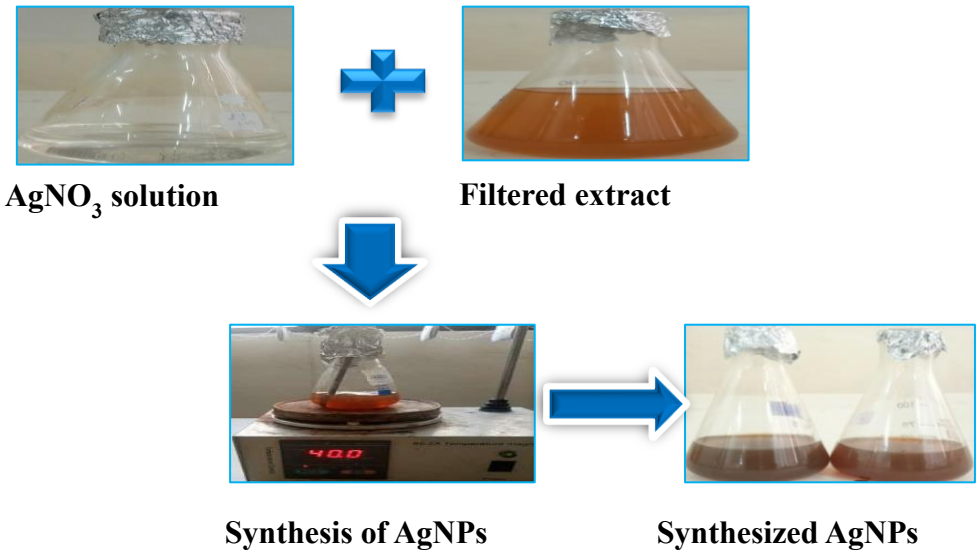


FIGURE 3.3 Stages in the preparation of AgNPs using DA leaves extract

3.2.5. Study the effect of operational parameters on the synthesis of AgNPs

In the synthesis of metal nanoparticles, various factors need to be maintained at an optimum condition to attain the desired quality of products. In this study, the effect of various operational parameters such as temperature, time, volume of plant extract, and precursor concentration was analyzed for different ranges on the AgNPs synthesis.

3.2.5.1. Effect of temperature on the synthesis of AgNPs

Temperature is a potential controlled parameter that affects the growth of AgNPs by controlling the reaction kinetics of the synthesis process (Bhagat, Anand, Datt, Gupta, & Arya, 2019) .Effect of synthesis temperature was studied at 30°C, 40°C, 50°C and 55 °C (Sun et al., 2014).The experiment was conducted separately by adding 5 mL of extract in a 250 mL Erlenmeyer flask containing 50 mL of precursor solution and kept it for 30 minute under stirring speed of 1200

rpm. The synthesized AgNPs were confirmed using UV-Vis spectrophotometer. Based on the results observed by the UV-Vis spectrophotometer, the suitable synthesis temperature was selected and used for further experiments.

3.2.5.2. Effect of time on the synthesis of AgNPs

Most of the time, synthesis time or reaction stirring time were related to temperature and had great effects on nanoparticle synthesis. Reaction time or contact time was done by the varying time taken for the formation of nanoparticles (Dada et al., 2018).The reaction time of synthesis was varied as (15 min, 30 min, 45 min, 60 min, and 75 min) (Sun et al., 2014). The experiment was conducted separately at different times by adding 5 mL of extract in a 250 mL Erlenmeyer flask containing 50 mL of precursor solution and kept it at 40°C under stirring speed of 1200 rpm. The synthesized AgNPs were confirmed using UV-Vis spectrophotometer. Based on the results observed by the UV-Vis spectrophotometer, the suitable synthesis time was selected and used for further experiments.

3.2.5.3. Effect of extract volume on the synthesis of AgNPs

The volume of plant extract, which serves as reducing and stabilizing agent affects the concentration of Ag^{+} reduced to Ag^{0} (Dada et al., 2018). Due to this 50 ml of 1mM $AgNO_3$ solution was mixed with various extract volume as (5 ml, 10 ml, 15 ml, 20 ml, and 25 ml). Then the experiment was done at 40 ^{0}c for 30 minute under stirring speed of 1200 rpm.The synthesized AgNPs were confirmed using UV-Vis spectrophotometer. Based on the results observed by the UV-Vis spectrophotometer, the suitable precursor concentration was selected and used for further experiments (Dada et al., 2018).

3.2.5.4. Effect of precursor concentration on the synthesis of AgNPs

The precursor concentration affects the NPs characteristics, namely shape, size, stability, and concentration of NPs in the sample (Femi-Adepoju, Dada, Otun, Adepoju, & Fatoba, 2019) ;(Saxena, Sharma, Sharma, & Singh, 2016) . the precursor ($AgNO_3$) concentration was varied from 1 to 11 mM to study their effect on the AgNPs synthesis. The synthesized AgNPs were confirmed using UV-Vis spectrophotometer. Based on the results observed by the UV-Vis spectrophotometer, the suitable precursor concentration was selected and used for further experiments.

3.2.6. Synthesis of powdered AgNPs

The Colloidal AgNPs was purified from unconverted Silver ions and some impurities using Centrifuge. Figure 3.4 showed that the synthesized AgNPs in the solution were transported to 15 ml centrifuge tubes. The nanoparticle-filled centrifuge tubes were put into the centrifugation machine's holes (NF 200 Bench Top Centrifuge). The particles were centrifuged for 15 minutes at 5000 rpm followed washing by ethanol and distilled water for further purification. Then, to eliminate any contamination, Petridis was rinsed with distilled water. AgNPs that had settled were put onto Petridish and dried in the oven (Moodley et al., 2018).

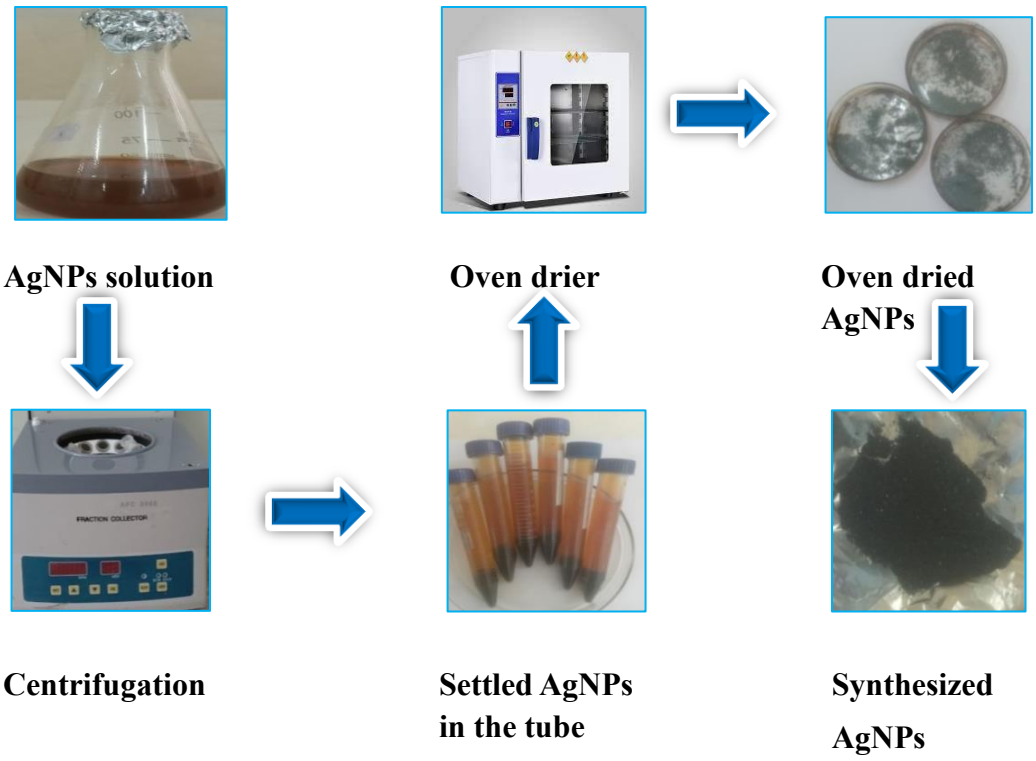


FIGURE 3.4 Synthesizing technique of powdered AgNPs

3.2.7. Flax seed hydrogel extraction

Experimental was conducted using hot plate equipment by solvent extraction process. The solvent used during extraction was water. Temperature, extraction time, and volume of solvent were used as major parameters affecting mass of extracted Flaxseed hydrogel and should be controlled highly. The extracted FSH was oven dried and grinded into powder form as illustrated in figure 3.5.

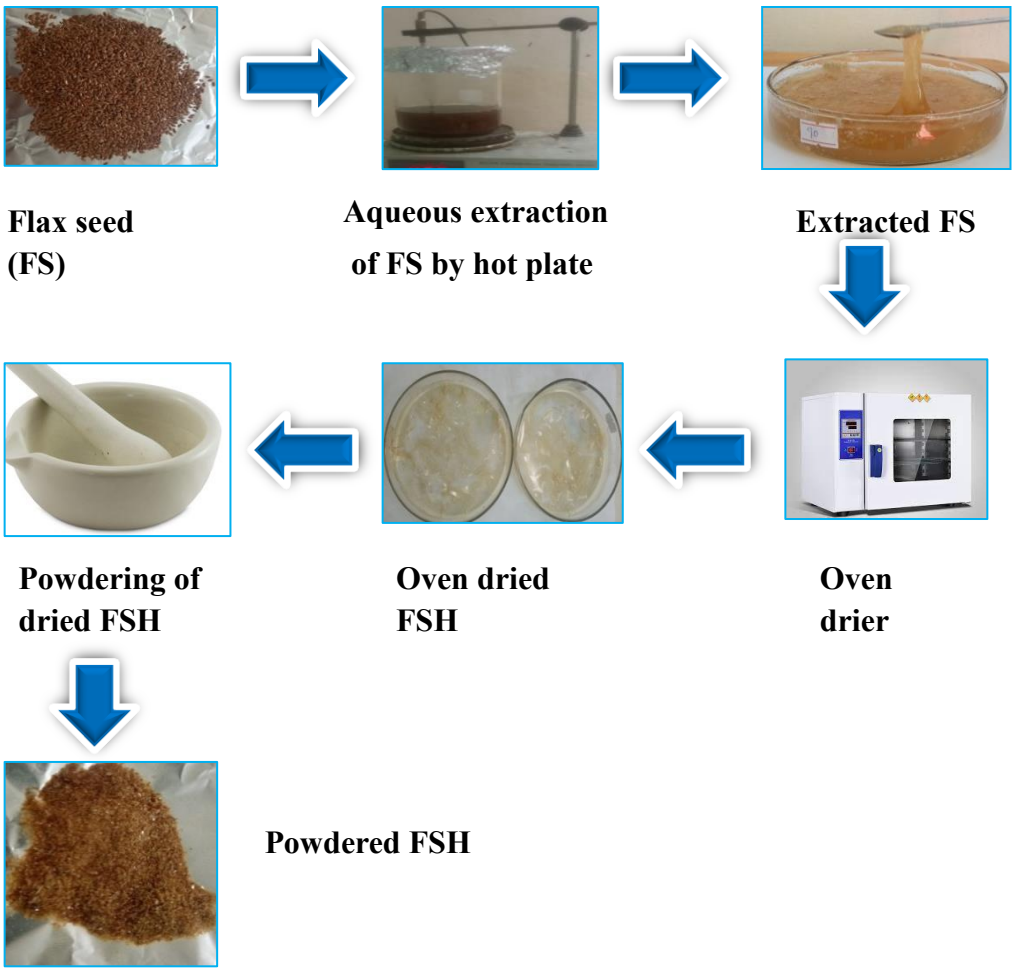


FIGURE 3.5 The diagrammatic representation of powder flaxseed hydrogel extraction

3.2.7.1. Optimization of flaxseed hydrogel extraction

Flaxseed hydrogel extraction was optimized by response surface method (box benkhen method).But before using RSM one parameter at a time was investigated to select the upper and lower bounds.

Table 3.1. Box Behnken experimental design matrix of Flaxseed hydrogel extraction from RSM

Name	Units	Low	High
Temperature	C	90	110
Time	hr	2	4
water volume	ml	150	900

Table 3.2. The summary of Box Behnken design of main and interaction effect and the responses/ yield of powder Flaxseed hydrogel /

Run	Factor 1 A:Temprature c	Factor 2 B:Time hr	Factor 3 C:Water Volume ml	Response 1 Yield of FSH(powder) %
1	100	4	900	
2	100	3	525	
3	90	3	900	
4	100	2	150	
5	90	3	150	
6	110	3	900	
7	90	4	525	
8	110	4	525	
9	90	2	525	
10	110	2	525	
11	110	3	150	
12	100	2	900	
13	100	3	525	
14	100	3	525	
15	100	3	525	
16	100	4	150	
17	100	3	525	

3.2.8. Development plasticized flaxseed hydrogel biofilm

1 gm of FSH powder was added to 150 ml of distilled water and stirred at speed of 1200 rpm for 2 hr until the powder dissolved in the water properly. After the formation of hydrogel 5 % of glycerol was added and continuously stirred for 5 minutes at temperature of 55 ^{0}c. The mixture was stirred for 30 minute without heating to homogenise the mixture. 30 gm of the solution was cast in Petridis and was put in oven at 35 ^{0}c for 16 hr for the purpose of thin film production (Tee et al., 2016).

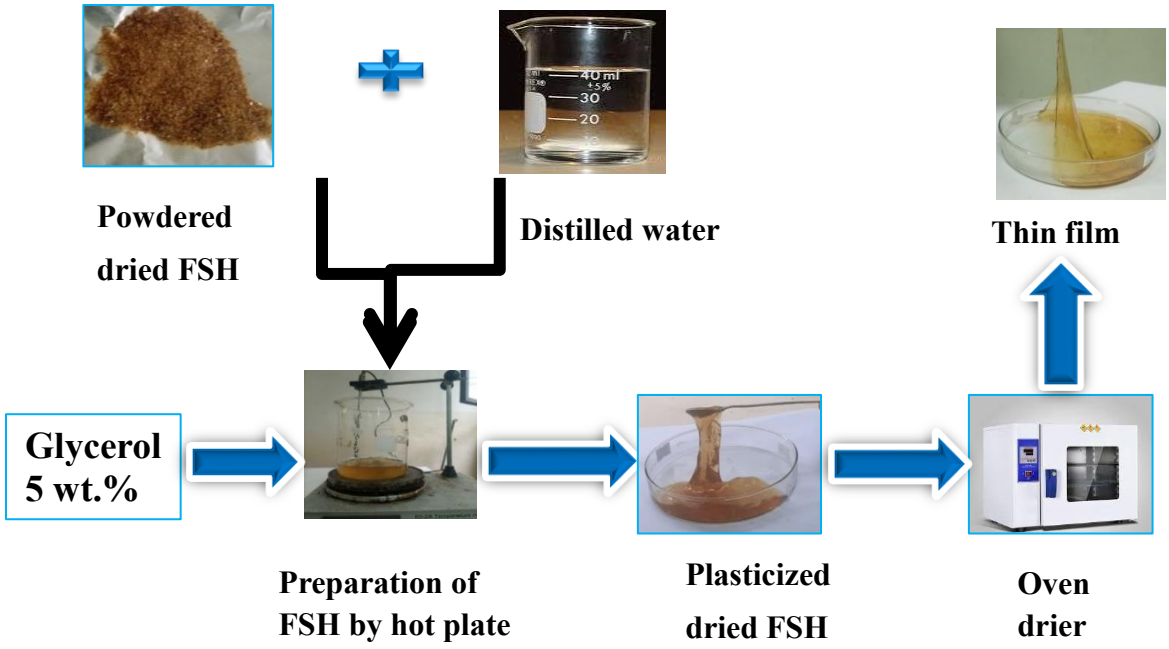


FIGURE 3.6 The diagrammatic representation of plasticized FSH preparation

3.2.9. Development of DA induced flaxseed hydrogel biofilm

1 gm of FSH powder was added to mixture which contains 75 ml of distilled water and 75ml of DA extract followed by continuous stirring for 2 hr. 5 % of glycerol was added and continuously stirred for 5 minutes at temperature of 55 ^{0}c. The mixture was stirred for 30 minute without heating for good homogeneity. 30 gm of the solution was cast in Petridis and was put in oven at 35 ^{0}c for 16 hr for the purpose of thin film production (Tee et al., 2016).

3.2.10. In situ dispersion of AgNps into DA extract

AgNPs synthesized from DA powdered leave was mixed with 75ml of DW followed by sonication for 2 hr to produce stable dispersion. 75 ml of DA extract was added drop wise for purpose of encapsulation of the AgNps with DA extract.

3.2.11. Development of DA induced and AgNPs encapsulated flaxseed hydrogel biofilm

150 ml of The AgNps encapsulated DA extract mixture was kept on hot plate till temperature reached 50 ^{0}c.1 gm of FSH powder was added followed by continuous stirring for 2 hr. 5 % of glycerol was added and continuously stirred for 5 minutes at temperature of 55 ^{0}c to the AgNps encapsulated DA extract induced hydrogel. The mixture was stirred for 30 minute without heating for good homogeneity. 30 gm of the solution was cast in Petridis and was put in oven at 35 ^{0}c for 16 hr for the purpose of thin film production (Tee et al., 2016).

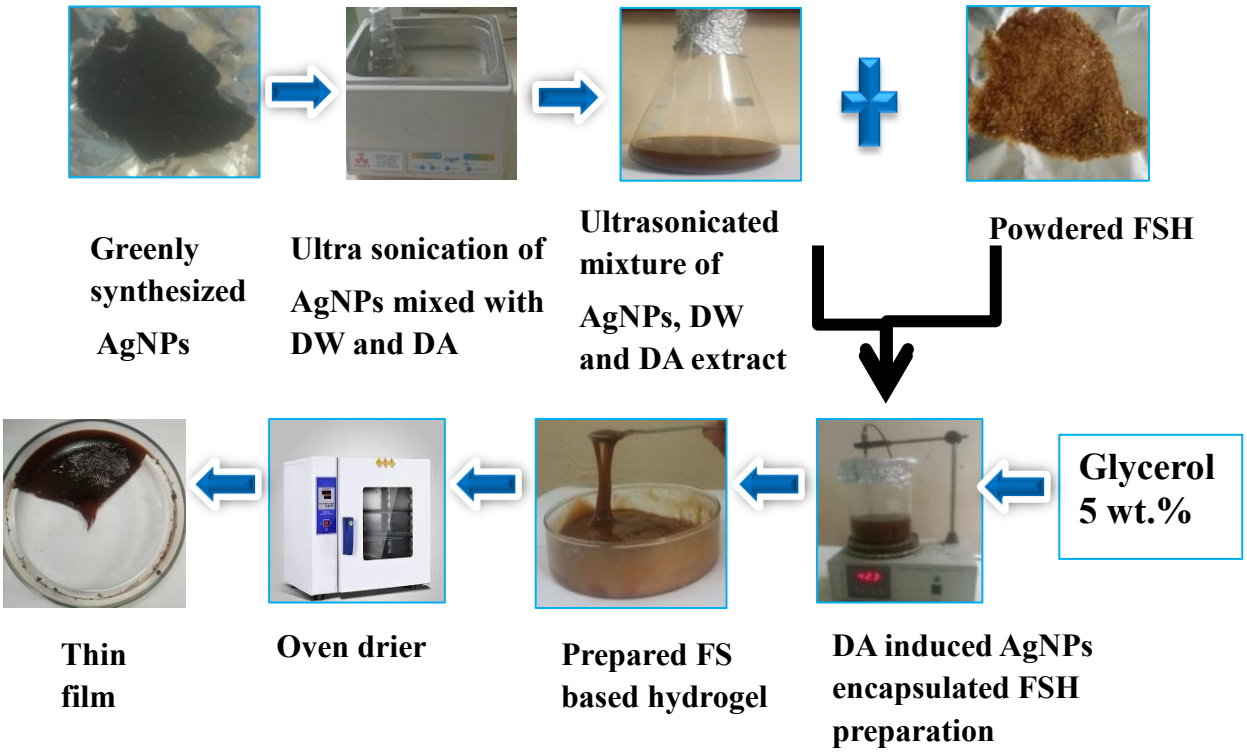


FIGURE 3.7 The diagrammatic representation of DA induced AgNPs encapsulated FSH preparation

3.3. Characterization

3.3.1. Characterization of DA leaf extract

3.3.1.1. UV-Vis analysis

UV-Vis analysis was conducted for selection of of DA leave extraction parameters.

3.3.1.2. Phytochemical Screening of Extract

Phytochemical Screening for the extraction helps for the identification of bioactive compounds like phenolic, Saponins, Flavonoids, Tannins, and Terpenoids, alkaloids (Anode et al., 2018). Phytochemical screening tests of *DA* leaf extract were carried out on the selected plant extract extracted on 1.5 hours and 55 ^{0}c. The phytochemical screening was done using the following standard procedures to identify the constituents

i. Test for Phenolic Compounds

1 ml of plant extract was transferred to the test tube and diluted to 2 ml of distilled water. To this, a few drops of neutral 10% ferric chloride solution were added. The dark green color indicates the presence of phenolic compounds.

ii. Test for Saponins

5 ml of extract was vigorously shaken with 5 ml of distilled water in a test tube and then heated to boil. Production of froths from the test solution was treated as a preliminary evidence for the presence of the saponins.

iii. Test for Flavonoids

The Phytochemical test for flavonoids was done by using Alkaline Reagent Test. 2 ml of 2.0% NaOH mixture was mixed with aqueous plant leaves extract.a concentrated yellow color was produced, which became colorless when we added 2 drops of diluted acid to the mixture. This result showed the presence of flavonoids.

iv. Test for Tannins

1 mL of plant extract was transferred to the test tube by using a dropper. 2 mL of 5% ferric chloride was added. The formation of dark blue or greenish-black colour confirms the presence of tannins.

v. Test for Terpenoids

5ml of plant extract sample was transferred to a test tube and treated with 2ml of chloroform after and evaporated on the water bath then boiled with 3 ml of H_2SO4 concentrated. Red brown color was formation showes the presence of terpenoids.

vi. Test for alkaloids

1ml of the extract was stirred with 1% HCl on a water bath for 5min and filtered. Mayer‘s reagent (the mercury (II) chloride solution was added followed by potassium iodide) 1ml to the filtrate. The formation of orange-colored precipitate gives an indication of the presence of alkaloids.

3.3.1.3. Fourier transform infrared (FTIR) spectroscopy

The functional groups found in the DA extract will be analyzed using FTIR attenuated total reflectance (ATR) mode in the range of 4000 to 500 cm^{-1}.

3.3.1.4. Antimicrobial test

Powdered DA plant extract will dissolve in dimethyl sulfoxide to the concentration of 500µg/mL to determine the zones of inhibition. For the minimum inhibitory concentration measurements, the stock solution (500µg/mL) will be serially diluted to give the desired concentrations of 25 µg/mL, 50 µg/mL, 75 µg/mL, and 100 µg/mL. The test is performed by applying a microbial inoculum of approximately 1-2×108CFU/mL to the surface of Mueller-Hinton agar plate. Commercially-prepared with fixed concentration, paper antibiotic disks will be placed on the inoculated agar surface. Plates will be incubated for 16-24 h at 35°C prior to determination of results. The zones of growth inhibition around each of the antibiotic disks will be measured to the nearest millimeter. The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium. The zone diameters of each product will be interpreted using the criteria published by the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards (Kebede, Gadisa, & Tufa, 2021).

3.3.2. AgNPs

3.3.2.1. UV-Vis spectroscopy

UV-Vis analysis was used for the indication of the presence of silver NPs and their concentration. The reduction of pure Ag+ ions was monitored by measuring the UV-Vis spectrum of the medium for after diluting a small aliquot of the sample into distilled water. The spectrophotometer was equipped with ―UVWin lab‖ software to record and analyze data. Baseline correction of the spectrophotometer was carried out by using a blank reference. The synthesized AgNPS was poured into UV- cuvette and the cuvette was placed in a spectrophotometer for testing. Then, the UV-Vis absorption spectra of the samples characterization were recorded and numerical data plotted in the ―Origin v-18‖.

3.3.2.2. Fourier transform infrared (FTIR) spectroscopy

The chemical structure of the AgNPs was analyzed using FTIR attenuated total reflectance (ATR) mode in the range of 4000 to 500 cm^{-1}.

3.3.2.3. X-Ray Diffraction (XRD)

X-ray diffraction is a powerful technique that helps to identify the structure, phase purity, degree of crystallinity and unit cell parameters of a given sample (Arya et al., 2019). The XRD patterns are recorded by the measurements of the angles at which the X-ray beams are diffracted by the periodicity of the sample. The relation between the distance between two planes (D-spacing) *(d)* and the angle of diffraction (2θ) is given by Bragg's equation (equation 3.4) **.**

3.3.2.4. Particle size analysis

The size of the applied AgNps was examined through Malvern Nano-Zetasizer ZS (Malvern, UK) at 25 °C with a model number of ZEN 3600 (Malvern Instrument Ltd, UK). The scattering angle was maintained at 173°. The AgNps suspension was diluted at a solid content 0.2–0.1% was sonicated for 30 s before measurement. Dynamic light scattering (DLS) measurements provided a Z-average size that was applied to estimate the size of the particle. The zeta sizer nano series performs measurements using a process called dynamic light scattering (DLS). Particle size distribution (PSD) was determined from the distribution of the scattered light energy

using the rigorous Mie light scattering theory. Zetasizer Nano range options for particle size measurements size range with maximum diameter 0.3 nm to 10 µm.

3.3.2.5. Antimicrobial activity

Synthesized powder of AgNPs was dissolved in dimethyl sulfoxide to the concentration of 500µg/mL to determine the zones of inhibition. For the minimum inhibitory concentration measurements, the stock solution (500µg/mL) will be serially diluted to give the desired concentrations of 25 µg/mL, 50 µg/mL, 75 µg/mL, and 100 µg/mL. The test is performed by applying a microbial inoculum of approximately 1-2×108CFU/mL to the surface of Mueller-Hinton agar plate. Commercially-prepared with fixed concentration, paper antibiotic disks will be placed on the inoculated agar surface. Plates will be incubated for 16-24 h at 35°C prior to determination of results. The zones of growth inhibition around each of the antibiotic disks will be measured to the nearest millimeter. The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium. The zone diameters of each product will be interpreted using the criteria published by the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards (Kebede et al., 2021).

3.3.3. Powder Flaxseed hydrogel (FSH)

3.3.3.1. Fourier transform infrared (FTIR) spectroscopy

The chemical structure of the flaxseed hydrogel was analyzed using FTIR attenuated total reflectance (ATR) mode in the range of 4000 to 650 cm^{-1}.

3.3.3.2. X-Ray Diffraction (XRD)

X-ray diffraction is a powerful technique that helps to identify the structure, phase purity, degree of crystallinity and unit cell parameters of a given sample (Arya et al., 2019). The XRD patterns are recorded by the measurements of the angles at which the X-ray beams are diffracted by the periodicity of the sample. The relation between the distance between two planes (D-spacing) *(d)* and the angle of diffraction (2θ) is given by Bragg's equation (equation 3.4)

$n\lambda = 2d\sin\theta$ .....Equation 3.4

Where λ = wavelength of X-rays, n= an integer known as the order of reflection (*h, k,* and *l* represent Miller indices of respective planes). From the diffraction patterns, the uniqueness of the mesoporous structures, phase purity, degree of crystallinity, and unit cell parameters can be determined. The average crystalline or grain size of FSH powder were calculated using scherrer equation (equation 3.4)

$$D = \frac{k\lambda}{\beta \cos \theta} \quad \text{.....Equation 3.5}$$

Where D is average crystalline size: K is the shape factor (0.9), λ is the wavelength of X-ray radiation, is Bragg angle of corresponding diffraction peak, β is full width at half maxima (FWHM) of main diffraction pick. In this work, FSH powder was performed with an X-ray diffraction technique (Philips PAN analytical, The Netherland) using Cu Kα radiation.

3.3.3.3. Particle size analysis

Particle size of the greenly synthesized AgNPs powder was analysed by DLS machine.It is used to characterize the size distribution of nanoparticles. The DLS technique uses light to measure the size of particles in a solution. The diameter that is measured in dynamic light scattering is called the hydrodynamic diameter and refers to how a particle diffuses within fluid (Manosalva et al., 2019).

3.3.4. Plasticized Flaxseed hydrogel (FSH)

3.3.4.1. Fourier transform infrared (FTIR) spectroscopy

The chemical structure of the plasticized flaxseed hydrogel was analyzed using FTIR attenuated total reflectance (ATR) mode in the range of 4000 to 650 cm^{-1}.

3.3.4.2. Antimicrobial test

Antimicrobial analysis was applied to check the antimicrobial effect of FSH with 5% of glycerol.*in vitro* antibacterial susceptibility test for prepared plasticized film was determined by disc diffusion method (Iconaru et al., 2014). The agar medium was prepared within dissolving 38 g of Mueller Hinton agar medium (MHA) in 1000 mL of distilled water autoclaved at 121 °C for 15 min. The autoclaved medium was poured into sterile plates (20-25 mL/plate) and the plates

were allowed to solidify under sterile condition at room temperature. After solidification, the plates were seeded with overnight grown culture approximately 1.5 X 108 CFU/mL by swabbing evenly on to the surface of the medium with a sterile cotton swab.

According to the methods described by CLSI with slight modification the synthesized FS-GLY 5 Wt% Film were cut in the diameter of approximately 6 mm (as discs) using puncher. The 6mm FS biofilm were placed and position on the surface of the medium with sterile forceps. gently pressed down to ensure contact with the MHA. Ampicillin was used as standard antibiotics and then plates were inverted and then incubated at 37 ℃ for 24 hrs. After incubation the inhibition the zones of growth inhibition around each of the antibiotic disks against *E.coli* , *S.pyogen, P. aeruginosa* and *S.aureus* were measured to the nearest millimeter. Zone halo of growth produced by the synthesized biofilms were evaluated by measuring the diameter (mm) of the clear zone around the disc against the test organisms using a ruler. The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium.

3.3.5. DA induced flaxseed hydrogel biofilm

3.3.5.1. Fourier transform infrared (FTIR) spectroscopy

The chemical structure of the of DA induced flaxseed hydrogel was analyzed using FTIR attenuated total reflectance (ATR) mode in the range of 4000 to 650 cm^{-1}.

3.3.5.2. Antimicrobial test

Antimicrobial analysis was applied to check the antimicrobial effect of DA induced flaxseed hydrogel. *in vitro* antibacterial susceptibility test for prepared film was determined by disc diffusion method (Iconaru et al., 2014). The agar medium was prepared within dissolving 38 g of Mueller Hinton agar medium (MHA) in 1000 mL of distilled water autoclaved at 121 ℃ for 15 min. The autoclaved medium was poured into sterile plates (20-25 mL/plate) and the plates were allowed to solidify under sterile condition at room temperature. After solidification, the plates were seeded with overnight grown culture approximately 1.5 X 108 CFU/mL by swabbing evenly on to the surface of the medium with a sterile cotton swab.

According to the methods described by CLSI with slight modification the synthesized FS-GLY-DA 25 ml , FS-GLY-DA 50 ml, and FS-GLY-DA 75 ml Films were cut in the diameter of approximately 6 mm (as discs) using puncher. The 6mm FS biofilm were placed and position on

the surface of the medium with sterile forceps. gently pressed down to ensure contact with the MHA. Ampicillin was used as standard antibiotics and then plates were inverted and then incubated at 37 ℃ for 24 hrs. After incubation the inhibition the zones of growth inhibition around each of the antibiotic disks against *E.coli* , *S.pyogen, P. aeruginosa* and *S.aureus* were measured to the nearest millimeter. Zone halo of growth produced by the synthesized biofilms were evaluated by measuring the diameter (mm) of the clear zone around the disc against the test organisms using a ruler. The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium.

3.3.6. AgNPs encapsulated and DA induced flaxseed hydrogel biofilm

3.3.6.1. Fourier transform infrared (FTIR) spectroscopy

The FTIR spectra of the formulated hydrogel biofilm was recorded by using FTIR spectrophotometer in the range of between 400 and 4000 cm^{-1} .

3.3.6.2. Antimicrobial test

As we have been working before *in vitro* antibacterial susceptibility test for prepared film was determined by disc diffusion method (Iconaru et al., 2014). The agar medium was prepared within dissolving 38 g of Mueller Hinton agar medium (MHA) in 1000 mL of distilled water autoclaved at 121 °C for 15 min. The autoclaved medium was poured into sterile plates (20-25 mL/plate) and the plates were allowed to solidify under sterile condition at room temperature. After solidification, the plates were seeded with overnight grown culture approximately 1.5 X 108 CFU/mL by swabbing evenly on to the surface of the medium with a sterile cotton swab.

According to the methods described by CLSI with slight modification the synthesized FS-GLY, FS-GLY-DA , FS-GLY-DA-Ag 1Wt%, FS-GLY-DA-Ag 1.5Wt%, and FS-GLY-DA-Ag 3 Wt% Films were cut in the diameter of approximately 6 mm (as discs) using puncher. The 6mm FS biofilm were placed and position on the surface of the medium with sterile forceps. gently pressed down to ensure contact with the MHA. Ampicillin was used as standard antibiotics and then plates were inverted and then incubated at 37 ℃ for 24 hrs. After incubation the inhibition the zones of growth inhibition around each of the antibiotic disks against *E.coli* , *S.pyogen, P. aeruginosa* and *S.aureus* were measured to the nearest millimeter. Zone halo of growth produced by the synthesized biofilms were evaluated by measuring the diameter (mm) of the clear zone

around the disc against the test organisms using a ruler. The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium (Klančnik, Piskernik, Jeršek, & Možina, 2010).

CHAPTER FOUR

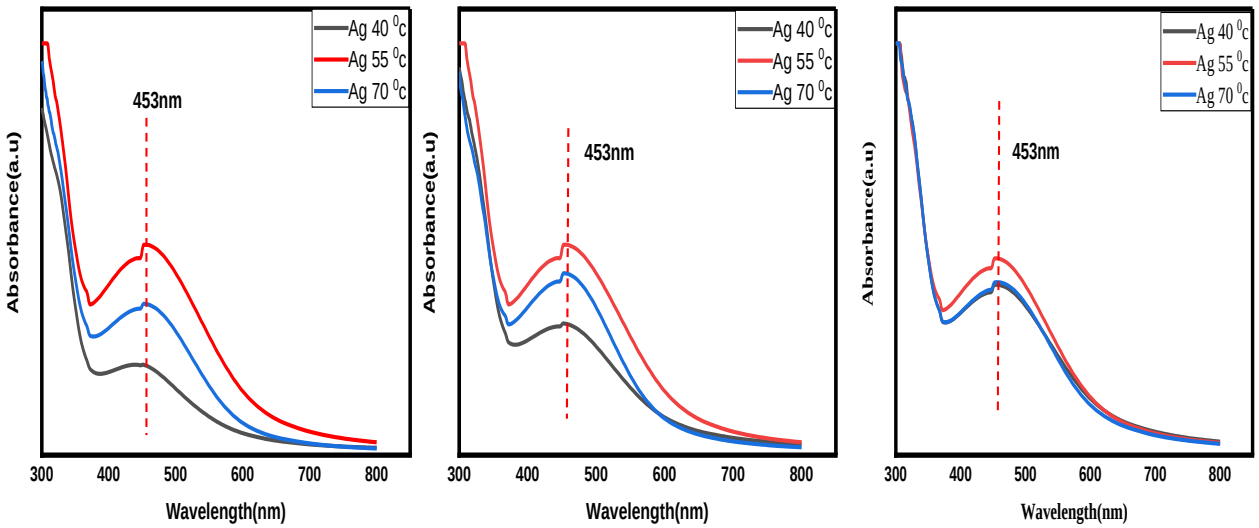
RESULTS and DISCUSSION

4.1. Preparation and characterization of *Dodonaea Angustifolia* (DA) leaves extract

4.1.1. DA extraction parameters optimization

4.1.1.1 UV-Vis analysis for extraction temperature selection

as shown in Figure (4.1) nine different experiments was conducted to select best oil extraction temperature of DA leaves powder by synthesizing AgNPs and conducting UV-Vis analysis for the NPs. The DA extraction time was fixed at (0.5, 1, and 1.5 hr) by varying the extraction temperature into (40 ^{0}c, 55 ^{0}c, and 70 ^{0}c) to synthesize AgNPs at 40 ^{0}c and 0.5 hr. Figure 4.1. shows that, as extraction temperature of DA increases from 40 ^{0}c to 55 ^{0}c the intensity of synthesized AgNPs in the solution increase but as temperature increase to 70 ^{0}c the intensity of AgNPs present in the solution starts to decrease. This may be due to the reason that, at higher temperature the photochemical components present on the extracted oil which have great role to reduce $AgNO_3$ into AgNPs started to degrade. This leads to decrease the formation of AgNPs because there is no enough reducing agent and this may lead to agglomeration of NPs. The same is true for figure b and c as as extraction time is fixed at 1 hr and 1.5 hr respectively but shifting of the peak from 453 nm to 443 nm was observed on Figure **a** for the DA extraction temperature of 40 ^{0}C .this result may confirms that at lower temperature the tendency to form agglomeration between AgNPs is low due to the presence of low reducing agents in the plant extract. This result was agreed with Green synthesis, characterization and antibacterial activity of silver nanoparticles by *Malus domestica* (Mariadoss et al., 2019).finally, according to the intensity of AgNPs shown, using DA extracted at temperature of 55^{0}c to synthesize AgNPs gives maximum amount of AgNPs. This indicated that using this optimum temperature to extract DA leaf gives muximum active phytochemical components.

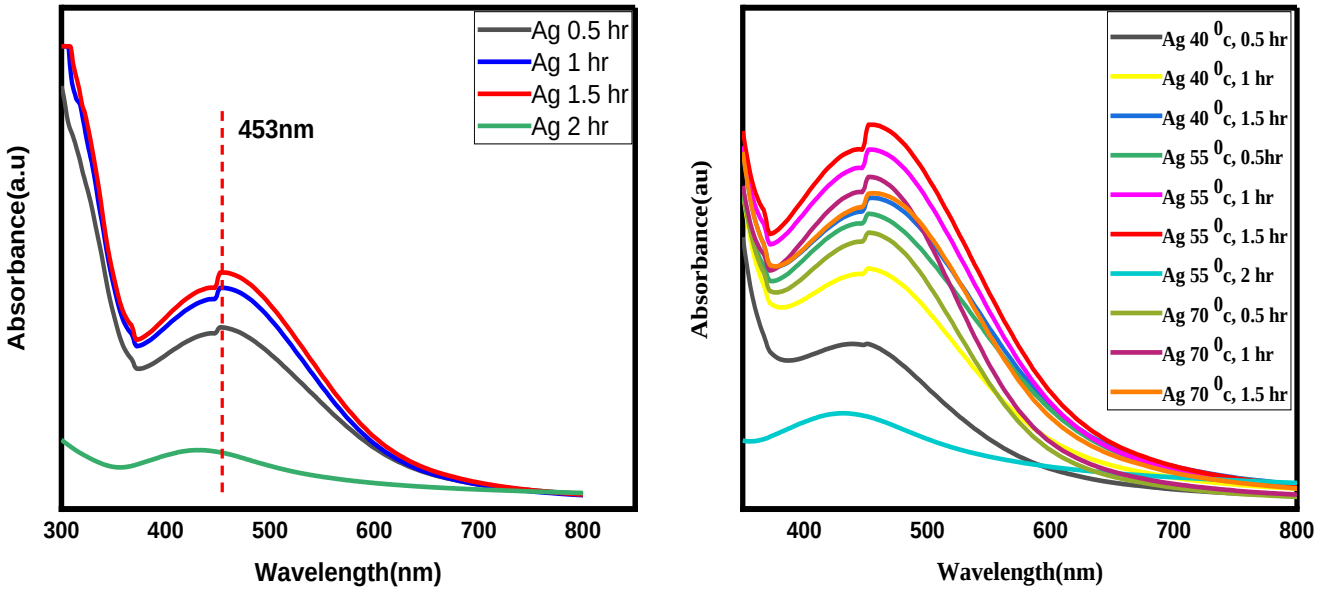


a **b** **c**

FIGURE 4.1 AgNPs synthesized by DA extracted at time of ; a)0.5 hr b)1 hr c)1.5 hr

4.1.1.2 UV-Vis Analysis for extraction time Selection

A different experiment was also conducted to select best oil extraction time of DA leaves powder as shown in figure 4.2.



a **b**

FIGURE 4.2 a) AgNPs synthesized by DA extracted at temperature of 55 ^{0}c and various times; b) combination effect of DA extraction temperature and time on synthesized AgNPs

DA extraction temperature was fixed at 55 ^{0}c and extraction time was varied as 0.5 hr, 1 hr, and 1.5 hr. using those parameters to extract DA AgNPs was synthesized and UV analysis was conducted. Figure 4.2 shows that as extraction time of DA increases from 0.5 hr to 1.5 hr the intensity of AgNPs in the solution increase. But as the DA extraction time increases to 2 hr the intensity of AgNPs decrease .this maybe, as contact time increases the active components present on the plant which are used to reduce $AgNO_3$ into AgNPs start to degrade. Depending on the result the DA extraction time which gives maximum concentration of AgNPs is 1.5 hr. Finally according Figure 4.2b maximum AgNPs was synthesized by using DA extracted at 55 ^{0}c and 1.5 hr.

4.1.2. Phytochemical analysis for *Dodonaea Angustifolia* plant extract

Phytochemical screening tests were done to determine the class of compounds present in the extract. Phytochemicals are large number of secondary metabolites or bioactive compounds found in plants. A phytochemical screening of Distilled water (DW) based extracts followed standard protocols were described as showed in Table 4.1.The Phytochemical Screening gives positive results with in ferric chloride test of phenols, frothing test, and alkaline test of flavonoids and Tannin preliminary phytochemical Test. This result also agrees with Phytochemical analysis results of *Dodonaea Angustifolia* plant extracts (Anode et al., 2018) .

Table 4.1 Phytochemical screening of distilled water extract of DA

Run	Phytochemical compound	Test performed	DW extract
1	phenol	Ferric chloride test	++
2	saponins	Frothing test	+
3	flavonoid	Alkaline test	++
4	alkaloid	Meyers reagent test	++
5	tannins	Ferric chloride test	++
6	terpenoids	Salkowiski test	+

Where ++ represents highly abundance of component and + represents weak abundance of components

4.1.3. Fourier transform infrared (FTIR) spectroscopy Analysis

FTIR is tool for identification for functional groups within organic compounds. The bio-active molecules present in (DW) based *DA* leaves extract is shown on the figure 4.3 using FT-IR spectra. The presence of different compounds was confirmed according to spectra showing the identity of DA extract. O-H stretching of intermolecular bonded alcohol is shown between 3547-3380 cm-1. A characteristic band at 2918 and 2082 indicates C-H stretching of alkanes and alkynes respectively. The C=C starching of ester takes place at 1600 cm^{-1}.the peak at 1382 cm^{-1} elucidates the occurrence of C-C stretching. The fingerprint region of the spectra accounts for the presence of C-O ester stretching at 1090 cm-1. The outcome of this result was confirmed as the presence of phytochemical components Phenol, Saponins, Flavonoids, alkaloids, terpenoids, and Tannins as indicated before(Oliveira et al., 2016);(Sahu & Saxena, 2013);(Ashokkumar & Ramaswamy, 2014).

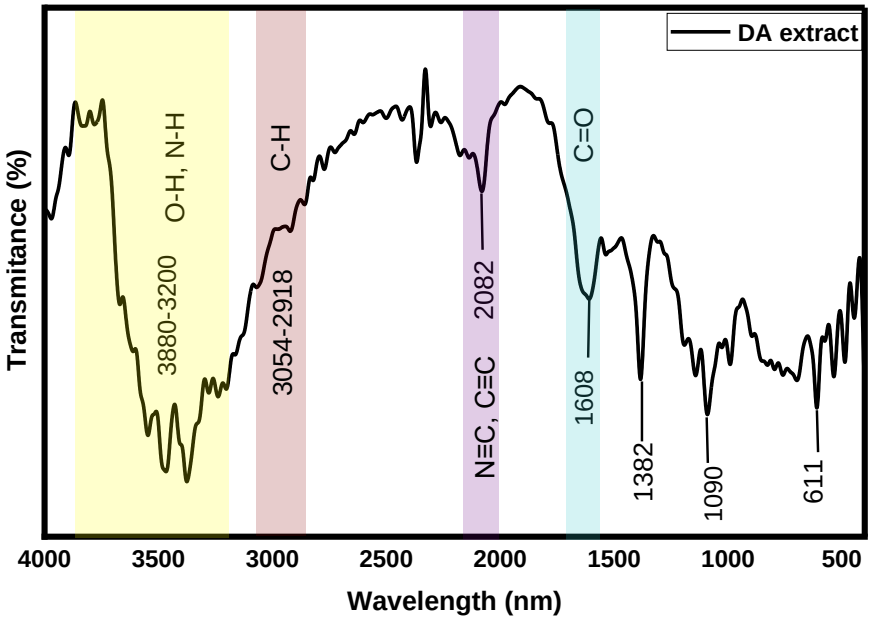


FIGURE 4.3 Functional group analysis of plant extract prepared with DW

4.1.3 Antimicrobial activity of *Dodonaea Angustifolia* leaf extract

Antimicrobial activity of extracted *Dodonaea Angustifolia* were tested against Gram negative bacteria (*E.coli* and *S. aureus*) and Gram positive bacteria species (*S. pyogen* and *p. aurugonosa*) using disk diffusion assay. The prepared *Dodonaea Angustifolia* leaf powder with different concentration of sample (100, 75, 50, and 25 µg/mL) and Ampicillin was used as reference drug

to test the antimicrobial activity. The zone of inhibition was indicated on the Table 4.2 and figuratively as shown on figure 4.4.DA extract bacteria test was Showed inhibition activities against Gram-negative *E.coli* with 10, 9, 7, 6.5 mm and *S. aureus* with11, 10, 8, 7 for concentration of 100, 75, 50, 25 µg/mL respectively. For *P. aeruginosa* bacteria DA extract also shows comparable inhibitions of 11, 9, 7.5, 6.5 mm and 11.5, 9, 8, 7 mm for *S. pyogen* bacteria for concentration of 100, 75, 50, 25 µg/mL respectively. Best spectrum of antimicrobial activity was found against Gram-positive bacteria. Generally when the concentration of DA extract increased the zone of inhibition produced also increased.

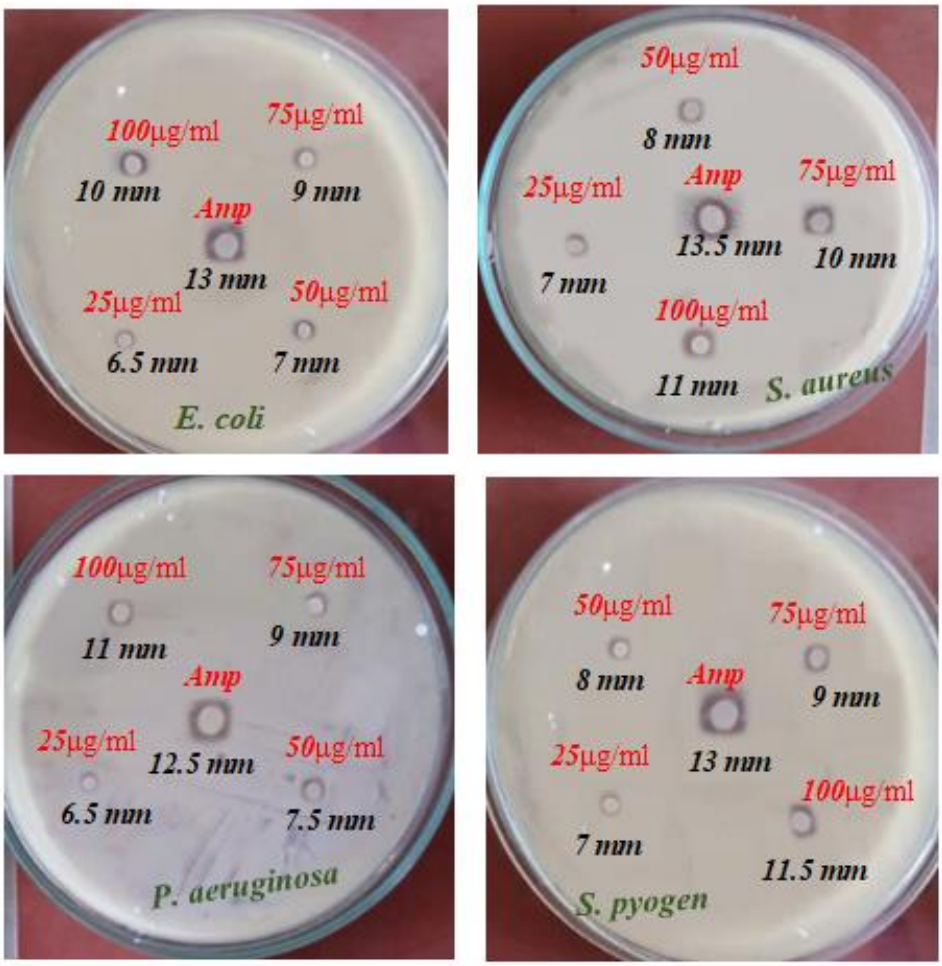


FIGURE 4.4 Inhibition zone of DA plant extract against four photogenes

Table 4.2 Inhibition zone (antibacterial activity) of DA plant extract

Bacteria Species and Zone of Inhibitions				
	Gram-negative		**Gram-positive**	
Concentration of DA	***E.coli* ATCC25922**	***S.aureus* ATCC2592**	***P.aeruginosa* ATCC27853**	***S.pyogen* ATCC19615**
25 µg/mL	6.5	7	6.5	7
50 µg/mL	7	8	7.5	8
75 µg/mL	9	10	9	9
100 µg/mL	10	11	11	11.5
Ampicillin (+ve control)	13	13.5	12.5	13

4.2. Study the effect of operational parameters on synthesis of AgNPs

4.2.1 Effect of temperature on synthesis of AgNPs

Reaction temperature was varied from 30 °C, 40 °C, and 50 °C using UV-Vis absorption spectra as shown in the Figure 4.5 effect of synthesis temperature was investigated. 5 mL of DA leaves extract was added in to 50 mL of AgNO3 containing Erlenmeyer flask. The reaction was performed at different temperature for 30 minute under stirring speed of 1200 rpm. When the reaction temperature increased from 30°C - 40°C the absorption peak was shifted towards a lower wavelength (453-438 nm) and increased in the intensity of the absorption Plasmon band. As the temperature increased to 50°C the absorbance peaks were decreased. The change in the absorption peak was caused by the AgNPs' surface Plasmon resonance.

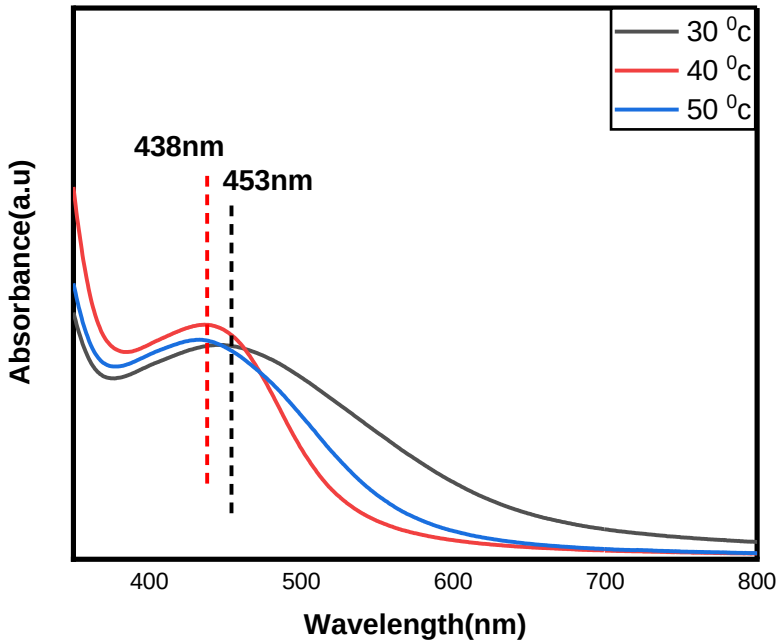


FIGURE 4.5 UV-Vis spectra of AgNPs synthesized at different Temperatures

4.2.2 Effect of time on synthesis of AgNPs

Effect of reaction time was investigated starting from the reactant is added in the flask to occur reaction. This was monitored plant extract added in to AgNO3 at 15 min, 30 min, 45 min, 60 min, and 75 min. varying the time taken for the formation of AgNPs was examined using UV-Vis spectrophotometer as shown on the figure 4.6. The synthesis of silver nanoparticles was at 40°C and other parameters were kept constant.

The intensity of the peak is a function of the contact time. At the early stage of contact time (15 min - 45 min) it shows excellent Plasmon band formation because a large amount of Ag+ has been converted to Ag^0. After the reaction is performed at 40°C for 15 min and 30 min the AgNPs obtained the UV-Vis absorption peaks showed at 445 nm. However, further increase in contact time from 45 min- 75 min leads to a noticeable decrease in absorption intensity. A decrease in absorbance peaks at 40°C with the reaction time increased indicates that the concentration of AgNPs decreased. This is due to the aggregation of AgNPs, which leads to an increase in particle size and settling of AgNPs that can‘t be detected through UV-Vis spectroscopy. This also confirmed the green synthesis of AgNPs using Tithonia diversifolia from 10 min to 90 min at room temperature. UV-Vis spectra showed rapid synthesis was obtained at a lower time. But excellent SPR was observed as the reaction time increased due to the large amount of Ag+ being

converted to Ag0 (Dada et al., 2018). Green synthesis of AgNPs using *E.japonica* leaf extract began to generate within 5 min after addition of AgNO3. As the reaction time was prolonged for 60 min, the intensity of the SPR peak observed at 435 nm was increased (Rao & Tang, 2017) .

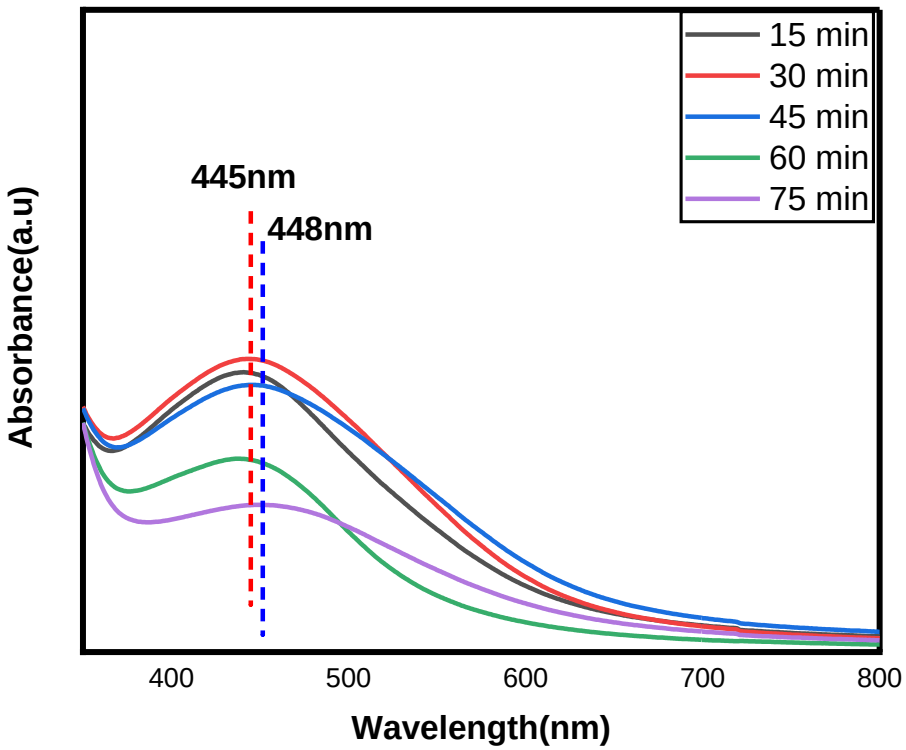


FIGURE 4.6 UV-Vis spectra of AgNPs synthesized at different times

4.2.3. Effect of DA extract volume on synthesis of AgNPs

The effect of 1mM of AgNO3 solution mixed with various extract (5 ml, 10 ml, 15 ml, 20 ml, and 25 ml) volumes was done. Other parameters were kept constant and volume of AgNO3 was fixed to 50 ml. The experiment was conducted using UV-Vis spectrophotometry as shown in the figure 4.7 in order to find out the highest composition of AgNPs.

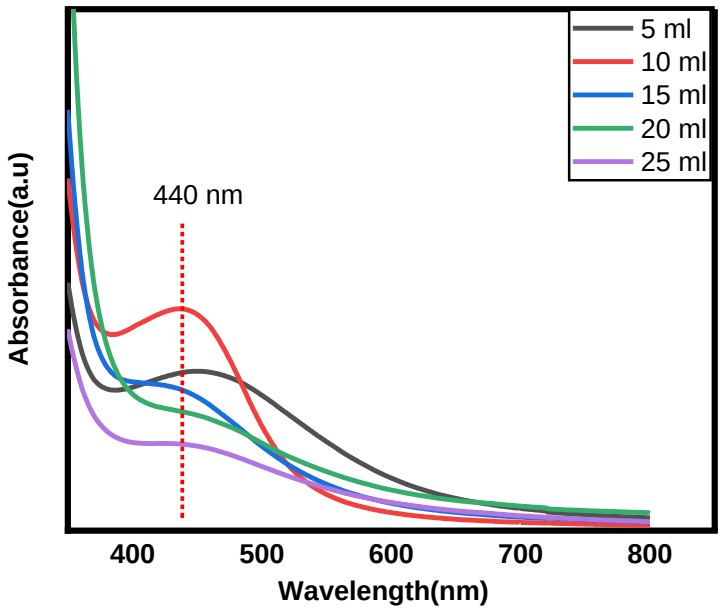


FIGURE 4.7 UV-Vis spectra of AgNPs synthesized at different volumes of plant extract

The effect of the volume of plant extract was performed at 5 different proportions. As demonstrated in the above figure, by increasing reducing agents the absorption peaks showed a decreasing intensity, Due to less amount of Ag+ concentration. In addition, the surface Plasmon peaks were shifted towards shorter wavelengths from 450 to 440 nm as volume of plant extract decreases. This result indicated a reduction in the mean diameter of AgNPs. Excellent SPR was recorded with 50 mL of 1 mM AgNO3 solution to 10 mL of plant extract (1:5 ratio) using UV-Vis spectra.

Similar results were also reported on the synthesis of AgNPs from *P. bracteosa* (1:1-1:10) v/v ratios with constant plant extract volume. This might result from the synthesis of AgNPs by speeding up the reduction of Ag ion. 1:10 ratios show high absorbance at 420 nm(Press, 2016). (Vivehananthan & De Silva, 2021) also confirmed that the effectiveness of the reaction ratio with the selected concentration was at the highest silver salt concentration to plant extract.

4.2.4 Effect of precursor concentration on synthesis of AgNPs

The Concentration of AgNO3 (precursor) or silver ion was majorly affects synthesis of AgNPs (Saxena et al., 2016). This parameter was investigated for identification amount of silver ion most suitable for generation of AgNPs. To investigate the effect of precursor concentration, 10

ml of DA extract was mixed with 50 mL of different molar concentration of AgNO3 solution. The Effect of concentration of silver salt (1mM, 3 mM, 5 mM, 7 mM, 9 mM and 11mM) was investigated and shown in figure 4.8 using UV-Vis spectra. The UV-Vis spectra help identifies a suitable concentration for synthesizing a number of AgNPs, which shows better surface Plasmon resonance.

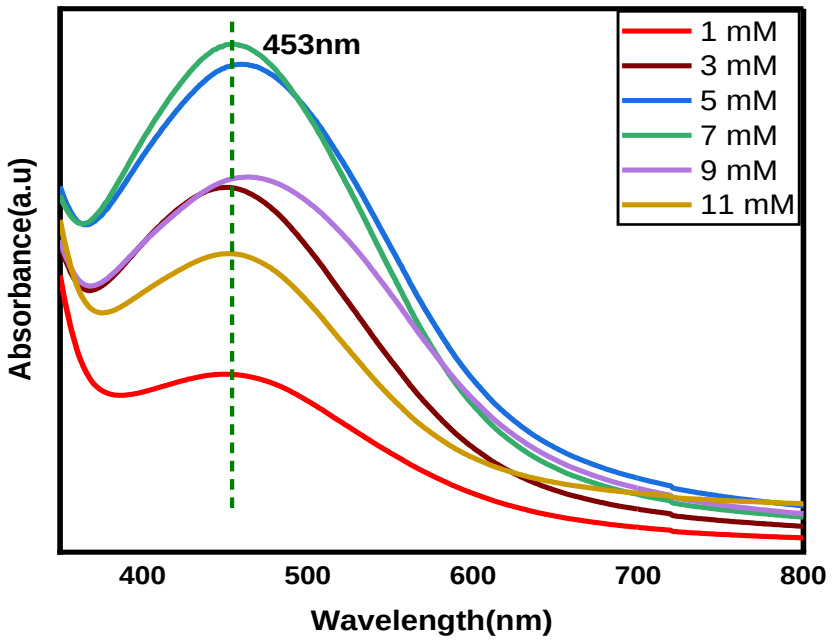


FIGURE 4.8 UV-Vis spectra of AgNPs synthesized at different precursor concentrations

At a low concentration of AgNO3 from (1mM – 3mM), the absorption peaks of AgNPs were broad and less intense. The absorption peaks were centered between 440 – 450 nm. However, the absorbance intensity increases gradually from 5mM – 7mM, the absorbance peak becomes sharper and more intense. At 7 mM the absorbance was maximum and. The AgNPs absorbance peaks get relatively smaller as the AgNO3 solution concentration increases to 9 mM and 11 mM. The increase in the intensities of SPR peaks from 1mM to 7mM might be due to an enhancement in the nuclei formation that resulted in the synthesis of a larger number of nanoparticles (Islam et al., 2019)**.** The decrease absorbance intensity of peaks at 9 mM and 11 mM may be caused due to the increase in the destabilization of AgNPs and increased agglomeration of Nanoparticles or not enough reducing agents.

This result was agreed with the green synthesis of AgNPs using spent 10 mL of black Tea Leaves Extract. Then the extract was mixed with 90 mL of AgNO3 solution of different

concentration (1, 3, 5, 7 & 9 mM) at room temperature shows increasing absorbance from 1mM - 7mM. However, with increasing the molarity to 9 mM the SPR peak is shifted toward the shorter wavelength with a decrease in the absorbance. Possibly this may cause by increasing in destabilization of the particles as indicated by the decreased absorbance due to increased particle aggregation and precipitation (Moosa, Ridha, & Al-Kaser, 2015). (Moosa et al., 2015) reported synthesized AgNPs using AgNO3 as a metallic salt and Aloe Vera Plant leaves extract as reluctant, Increasing AgNO3 concentration (1, 3, 5, 7 & 9 mM) resulted in a gradual increase of absorbance peak between 422 – 447 nm due to its surface Plasmon resonance absorption band. According to our study, 7mM of AgNO3 concentration was selected for further studies due to larger amount of AgNPs synthesized.

Finally among the operational parameters on synthesis of AgNps precursor concentration affects highly due to the reason that gives high absorption intensity of AgNps on the synthesizing process. The experiment was done by reacting 7 mM of 45 ml AgNO3 with using 5 mL of plant extract as reducing agent for 0.5 hr at 40 °C.

4.3. Synthesis of powder AgNPs

The yield of converted AgNPs was estimated by assuming that all silver atoms in the stack solution were converted to AgNPs using the extract as a reducing agent. Only 31% of ANPs were generated using this technique. This might be because a greater number of AgNPs were washed away with unconverted silver ions and contaminants.

4.4. Characterization of green synthesized powder AgNPs

4.4.1. X-RAY diffraction

The phase evaluation of produced AgNPs crystalline grain size was investigated from X-ray diffraction graph sown as figure 4.13. The XRD pattern showed four intense peaks in the whole spectrum of 2θ values ranging from 27^0 to 80^0 .A comparison of the obtained XRD spectrum with the standard confirmed that the silver particles formed in the four intense peaks appeared at 38.0946°, 44.4385° 64.494°, 77.349°. However, peaks at 27 °, 32°, 46°, 54°, 57°, shows for AgO nanoparticles. This AgNPs results were agreed with the green synthesis of AgNPs using *G. ofcinalisw* plant extract. The XRD peaks in degrees 2θ appear at 38.0946°, 41.4385° 64.494°, 77.349° this can be attributed to the planes (111) (200) (220) (311) and (222) sets of lattice planes consistent crystal structure of silver reported (Manosalva et al., 2019). The average crystal

size of Ag nanoparticles was calculated by using scherrer equation from the data gathered from the origin, as illustrated in Table 4.3. The crystalline size of Ag was 10.938 nm.

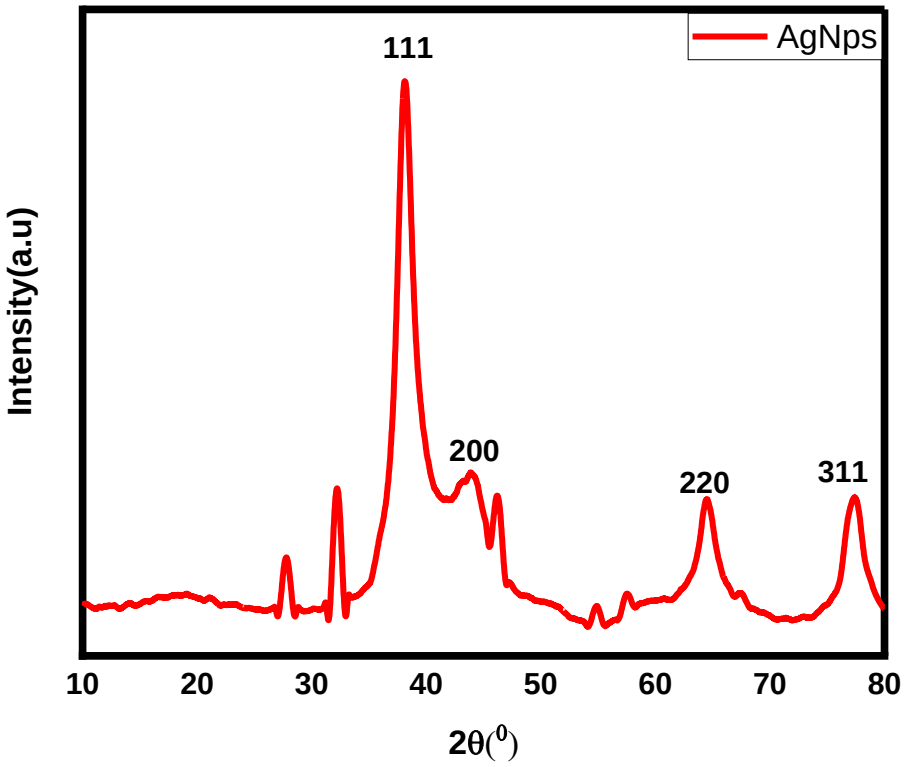


FIGURE 4.9 X-ray diffraction of synthesized powder AgNPs

Table 4.3 Crystalline size of AgNPs powder calculated from intense peaks

Peak position	FWHM	crystallite size D (nm)	D (nm) average
27.78784	0.298	27.46217722	
32.21791	0.28958	28.5550219	
38.14129	1.65188	5.088516942	
41.2815	9.53072	0.890721793	
46.2263	0.32525	26.55685874	10.938
47.02479	45.15274	0.191873061	
60.4267	13.90848	0.660971029	
64.56388	2.04494	4.595118297	
77.37746	2.28832	4.447701906	

The distance between two planes was calculated using Bragg‘s equation using the data illustrated in Table 4.2. The d-spacing of synthesized AgNPs was 0.263A°. The average crystal size of AgNPs was calculated by using scherrer equation from the data gathered from the origin, as illustrated in Table 4.3. The crystalline size of synthesized AgNPs was 10.938 nm.

Table 4.4 The d-spacing of synthesized AgNPs powder

2 Theta	Theta	l(nm)	d-spacing	Average d-spacing
27.78345	13.89173	0.15406	0.320841	
32.21367	16.10684	0.15406	0.277656	
38.13764	19.06882	0.15406	0.235779	0.263111 A^o
41.35157	20.67579	0.15406	0.218166	
64.52065	32.26033	0.15406	0.144314	
77.34766	38.67383	0.15406	0.12327	

4.4.2. Fourier transform infrared (FTIR) spectroscopy Radiation

FTIR is an important tool which enables us to understand the involvement of functional groups in the interactions between metal particles and biomolecules. In the present work, FTIR spectra were used for the identification of biomolecules responsible for capping and stabilizing the silver nanoparticles. The FTIR spectra of the *Dodonaea Angustifolia and* AgNPs is given in the Fig 2 The FTIR spectrum of AgNPs showed the intensity of the peaks observed from 3847-3255 cm−1 are attributed to O–H stretching vibrations (Oliveira et al., 2016). The absorption peaks located at 2913 cm- arising from C-H stretching of alkanes. Peak at 1714 cm-1 was assigned as C=O ester stretching. The peaks at 1606 cm-1 showed stretching of aromatic compounds of C=C (non-conjugated) Stretching. The existence of a C–C aromatic ring from the tannin molecule (bio-reducing agent) was detected at 2329 cm−1.The fingerprint region of the spectra accounts for the presence of C-O ester stretching at 1090 cm-1.

Therefore, from the results of FTIR analyses of extract mediated synthesized of silver nanoparticles it can be concluded that some of the biological molecules of leaf extract such as alkaloids, phenols, flavonoids, terpenoids, and tannins are responsible for the biotransformation of silver ions to silver nanoparticles and its stabilization in aqueous medium.This results were good agreement with the earlier reports (Amargeetha & Velavan, 2017; Manimegalai & Velavan, 2015) (Salama, Abedin, & Elwakeel, 2021).

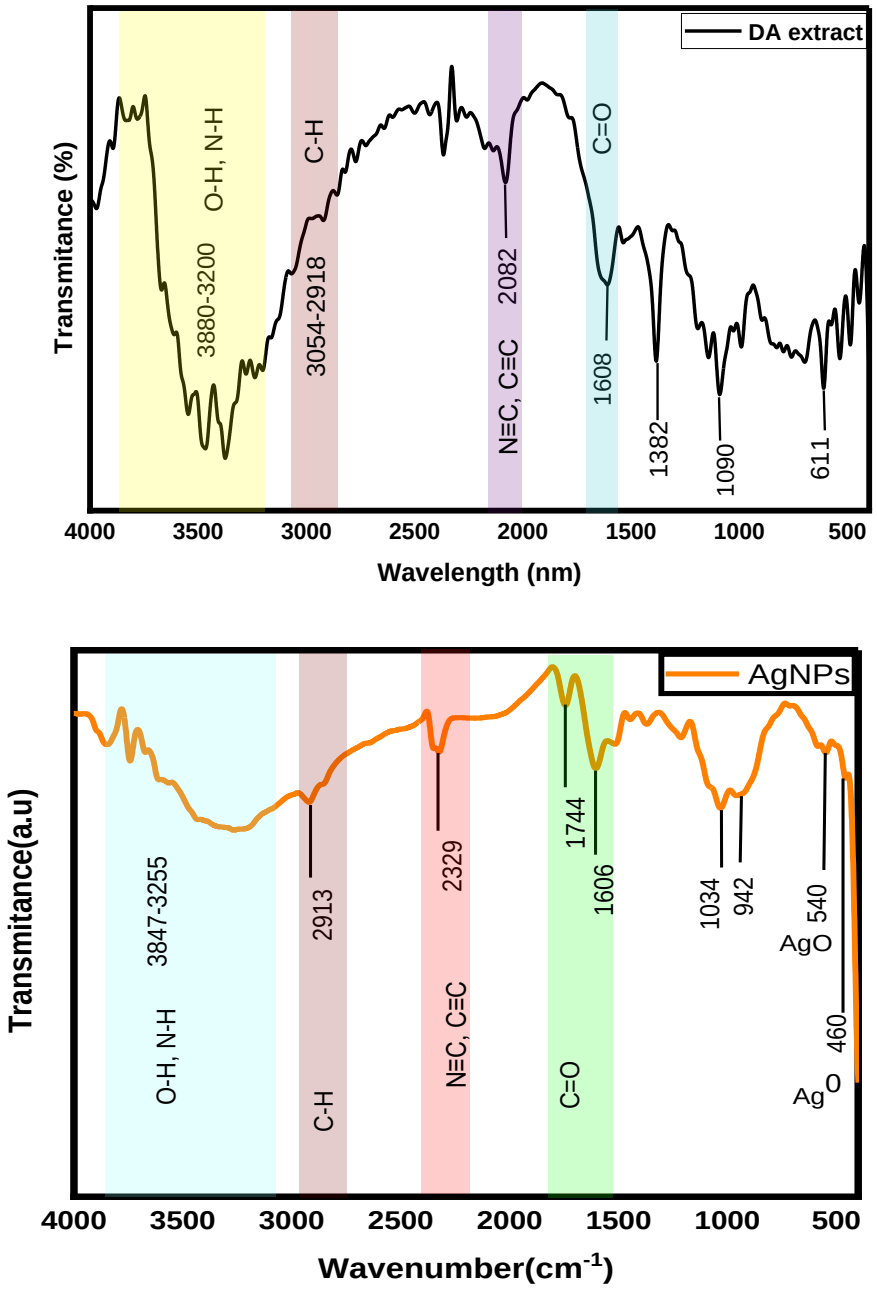


FIGURE 4.10 FTIR analyses of AgNPs and plant extract

4.4.3. Particle size analysis (PSA) of AgNPs

The size of AgNPs particles was determined by differential light scattering (DLS). Figure 4.11 shows the distribution of AgNPs particles size and The DLS result of AgNPs reveals that there are doublet peaks, the first peak is appeared at 28.61 nm contains intensity of 4.8% and the other

peak is appeared at 92.92 nm with 95.2 % intensity. Finally the result from DLS proves that the greenly synthesized AgNPs are in the range of nano size.

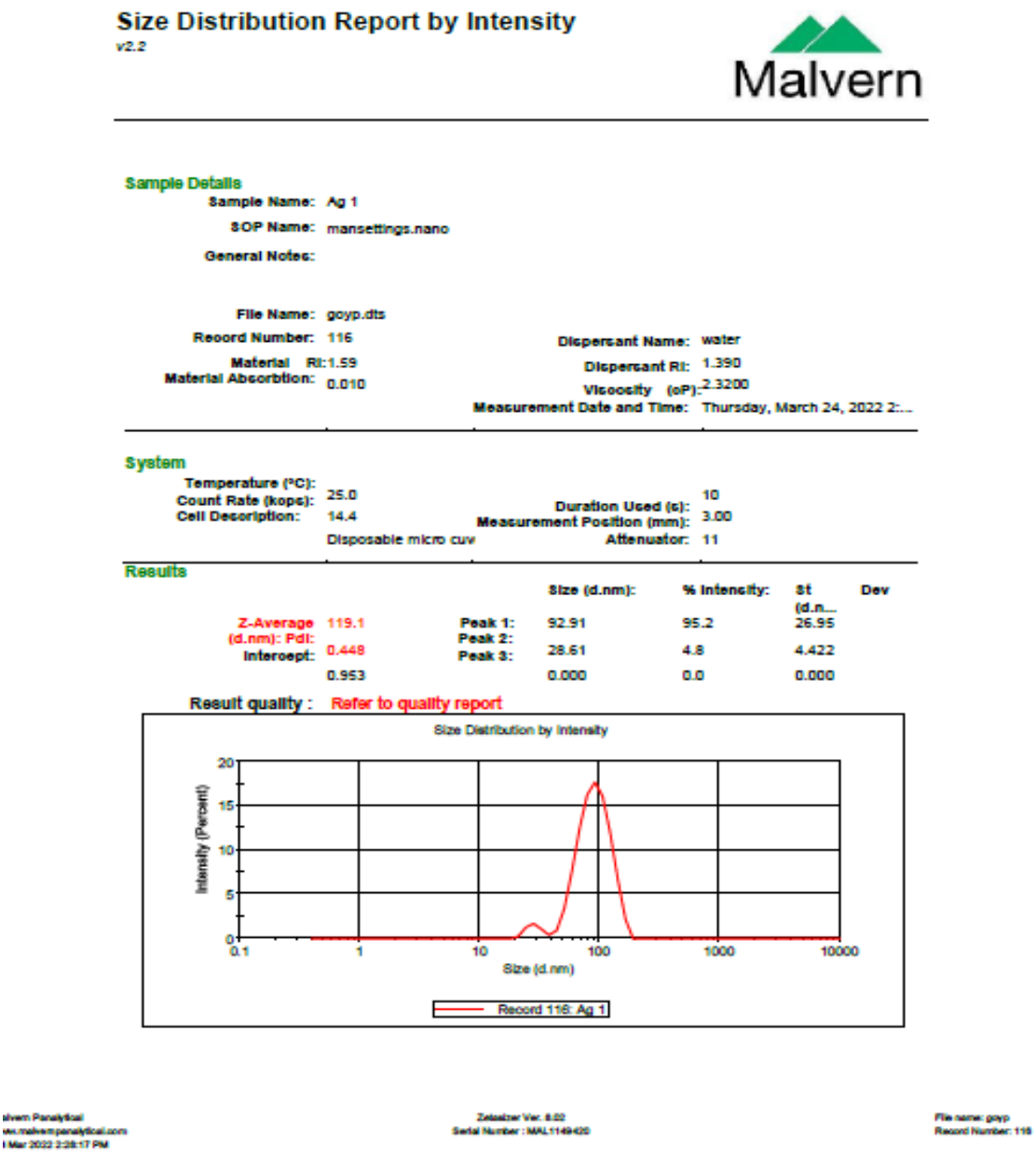


FIGURE 4.11 Particle size analyses of AgNPs

4.4.4. Antimicrobial Activity of AgNPs

Antimicrobial effect of greenly synthesized AgNPs using *Dodonaea angustifolia* were tested against Gram negative bacteria (*E.coli* and *S. aureus*) and Gram positive bacteria species (*S. pyogen* and *p. aurugonosa*)) using disk diffusion assay. Greenly synthesized AgNPs were

prepared at pH-7 and the prepared nanoparticles with different concentration of sample (100, 75, 50, and 25 µg/mL) and Ampicillin was used as reference drug to test the antimicrobial activity. The zone of inhibition was indicated on the Table 4.5 and figuratively as shown on figure 4.15 synthesized nanoparticle bacteria test was Showed inhibition activities against Gram-negative, *E.coli* with 11.5, 10, 9 ,7mm and *S. aureus* with12, 10.5, 9, 8 for concentration of 100, 75, 50, 25 µg/mL respectively. For *P. aeruginosa* bacteria AgNPs also shows comparable inhibitions of 11, 10, 8, 7mm and 12.5,11, 9,8 mm for *S. pyogen* bacteria for concentration of 100, 75, 50, 25 µg/mL respectively.

FIGURE 4.12 Zone of inhibition produced by AgNPs against four pathogens

Best spectrum antimicrobial activity was found against Gram-positive bacteria. Generally when the concentration of AgNPs increased the zone of inhabitation produced also increased.

Table 4.5 Anti-bacteria activity (inhibition zone) of AgNPs

Bacteria Species and Zone of Inhibitions				
	Gram-negative		**Gram-positive**	
Concentration of Ag	***E.coli*** **ATCC25922**	***S.aureus*** **ATCC2592**	***P.aeruginosa*** **ATCC27853**	***S.pyogen*** **ATCC19615**
25 µg/mL	7	8	7	8
50 µg/mL	9	9	8	9
75 µg/mL	10	10.5	10	11
100 µg/mL	11.5	12	11	12.5
Ampicillin (+ve control)	13	13.5	12.5	13

4.5. Extraction, optimization, and characterization of powder flaxseed hydrogel (FSH)

Factors which affect the extraction of FSH were optimized by design expert, box benkhen method.

4.5.1. Design experiment result of FSH analysis

Table 4.6.Experimental result of extracted FSH powder

Run	Factor 1 A:Temprature c	Factor 2 B:Time hr	Factor 3 C:Water Volume ml	Response 1 Yield of powder FSH %
1	100	4	900	12.3
2	100	3	525	14.93
3	90	3	900	8.56
4	100	2	150	5.03
5	90	3	150	4.6
6	110	3	900	13.73
7	90	4	525	13.23
8	110	4	525	13.43
9	90	2	525	8.7
10	110	2	525	12.0
11	110	3	150	4.1
12	100	2	900	14.93
13	100	3	525	14.93
14	100	3	525	14.93
15	100	3	525	14.93
16	100	4	150	4.2
17	100	3	525	14.93

Table 4.7 ANOVA result on yield of extracted FSH

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	22.75	9	2.53	788.25	< 0.0001	Significant
A-Temprature	0.9522	1	0.9522	296.90	< 0.0001	
B-Time	0.1741	1	0.1741	54.27	0.0002	
C-Water Volume	8.24	1	8.24	2569.83	< 0.0001	
AB	0.2025	1	0.2025	63.14	< 0.0001	
AC	0.7225	1	0.7225	225.28	< 0.0001	
BC	0.0256	1	0.0256	7.98	0.0256	
A^2	1.82	1	1.82	567.56	< 0.0001	
B^2	0.3480	1	0.3480	108.52	< 0.0001	
C^2	9.44	1	9.44	2944.09	< 0.0001	
Residual	0.0225	7	0.0032			
Lack of Fit	0.0225	3	0.0075	0.2544	0.856	
Pure Error	0.0000	4	0.0000			
Cor Total	22.77	16				

From the ANOVA (Analysis of Variance) Table, 4.8 results the higher F-Value and the lower P-value (<0.0001) indicate significance for the regression model. As the result demonstrates the model, the F-value of 788.25 implies it is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. The p-value less than 0.05 imply that the model is statistically significant. Table 4.8 gives clarity on interaction of the independent variables which is extremely important. The result showed that temperature (factor A) and water volume (Factor C) are the major important variable on extraction of FSH with P-value < 0.0001, and < 0.0001 respectively. in addition to that interaction effects of AB(temperature and time), and AC(temperature and water volume) affects highly to the extraction process, and From the second

polynomial model, A^2 ,B^2 , and C^2 are with P-values < 0.0001. The Lack of Fit of the F-value 0.2544 implies the Lack of Fit is not significant relative to the pure error. There is about 85.6% chances that a Lack of Fit F-value large could occur due to noise.

As shown from the ANOVA contact time indicates insignificant when compared with the other factors and this result confirms that its contribution on extraction of FSH was the lowest. Interaction effects of BC (contact time and water volume was also low comparing with remained interaction effects for extraction of FSH with p-value of 0.0256. Generally model terms of case A, B, C, AB, AC, BC, A^2, B^2, C^2 C, A^2 were significant factors with p values less than 0.05.Model equations from the ANOVA in terms of coded factors can be used to make predictions about the extraction of FSH for the given levels of each factor. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

The final coded equation for extraction of FSH

Yield of powder FSH = 14.93 + 0.345A + 0.1475B +1.015C - 0.2325AB + 0.425AC – 0.08BC- $0.6575A^2$ -$0.28B^2$ -$1.4075C^2$

Where A Represents Temperature B Time and C Represents water volume

4.5.1.1. Model Adequacy

The regression model was found to be significant with the correlation coefficients of determination of R-Squared, adjusted R-Squared, and predicted R-Squared having a value 0.9990, 0.9977, and 0.9842 respectively as shown from table 4.9. The goodness fitness of the model was measured by a coefficient of determination R^2, which measured how well the real experimental data points have been approximated together with the regression model. As the result in a table, 4.3 expressed the value of $R^2 = 0.990$ indicates the better fit of experimental data to that of predicted one. The value of larger Regression coefficient (R^2) approach to 1 illustrates, the validity of model in which it implies an acceptable agreement among the actual and predicted data. The Predicted R^2 of 0.9842 is in reasonable agreement with the Adjusted R^2 of 0.9977; i.e. the difference is less than 0.2. Therefore, this demonstrates that the measured data very well fitted with a quadratic model of the predicted value.

Table 4.8 the model adequacy measures

Std. Dev.	0.0566	**R^2**	0.9990
Mean	3.33	**Adjusted R^2**	0.9977
C.V. %	1.70	**Predicted R^2**	0.9842
		Adeq Precision	74.8253

4.5.1.2. Optimization of the responses

In order to find the optimum value of each independent variables, four statistical metrics such as the larger regression coefficient (R2), smaller lack of fit F-value, lower p-value and higher sum squares have been used. The response optimization was used to identify the combination of independent variables particularly temperature, Contact time, and water volume with response mass of FSH. The goal of the process was to obtain maximum mass of extracted FSH. According to the result obtained from optimizer Design-Expert version 11 software the maximum yield of FSH achieved 11.06 % by using 100 0c, 4 hr, and 290 ml of distilled water.

4.5.1.3. Plots of interaction effects

To analyze the combined effect of the independent variables on the mass of extracted powder of FSH, graphical representation of two-dimensional 2-D and three-dimensional 3-D surface plots were employed on (Fig.4.13, 4.14, 4.15).

RSM (BBD) analysis on figure 4.13 revealed that the mass of extracted powder FSH increase with increase in temperature and time. Maximum mass of extracted powder FSH was observed at around 100^oC and 3hr. But, a decrease in mass of powder FSH was shown at higher temperature. The reduction in mass of powder FSH can be attributed to the evaporation of water, leading to decrease in availability of water molecules. This simultaneous decrease in mass of extracted FSH at higher temperature indicated that optimum mass of extracted FSH reached.

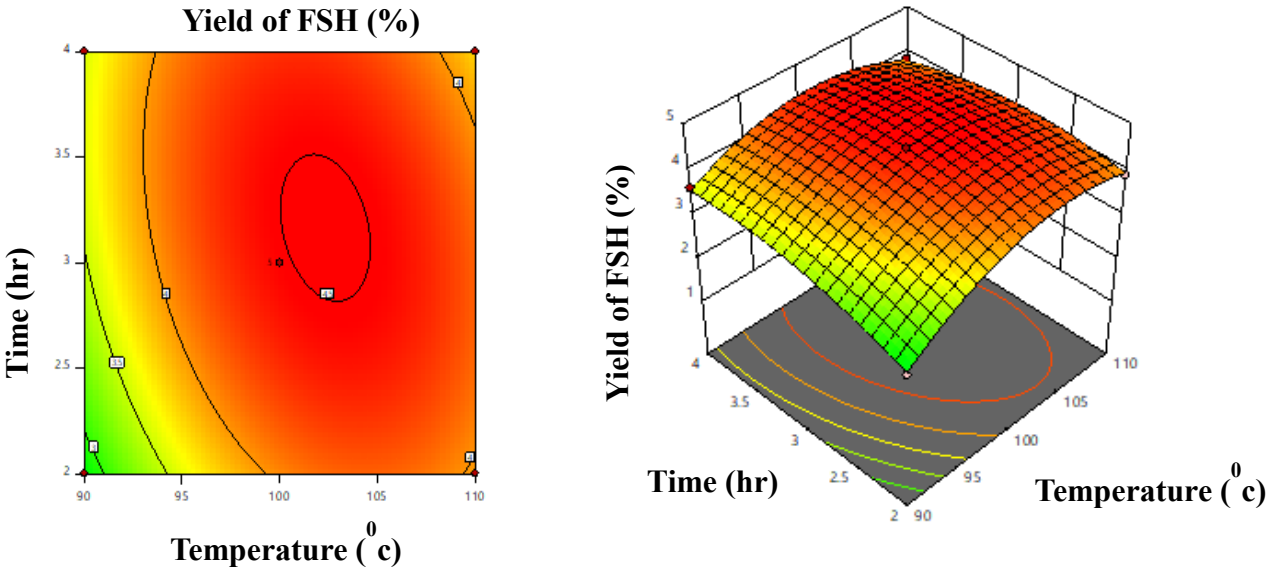


FIGURE 4. 13 Contour and 3-D plot for effect of temperature and time on yield of FSH

According to Figure 4.14 yield of extracted powder FSH increase with increase in water volume and temperature. But, a slight decrement in mass of powder FSH was observed at higher water volume and time. The reduction in mass of powder FSH is attributed due to the evaporation of water, leading to decrease in availability of water molecules. This simultaneous decrease in mass of extracted FSH at higher temperature indicated that maximum mass of extracted FSH.

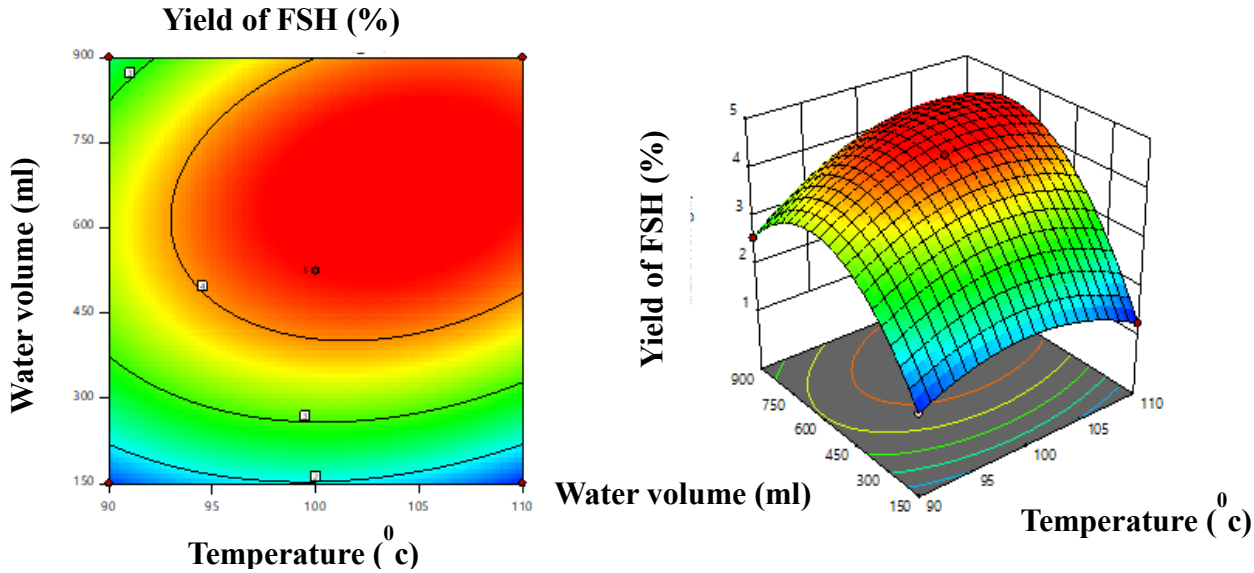


FIGURE 4.14 Contour and 3-D plot for effect of water volume and temperature on yield of FSH

Figure 4.15 shows the interaction effect of two variables (water volume and time) on yield of extracted FSH.

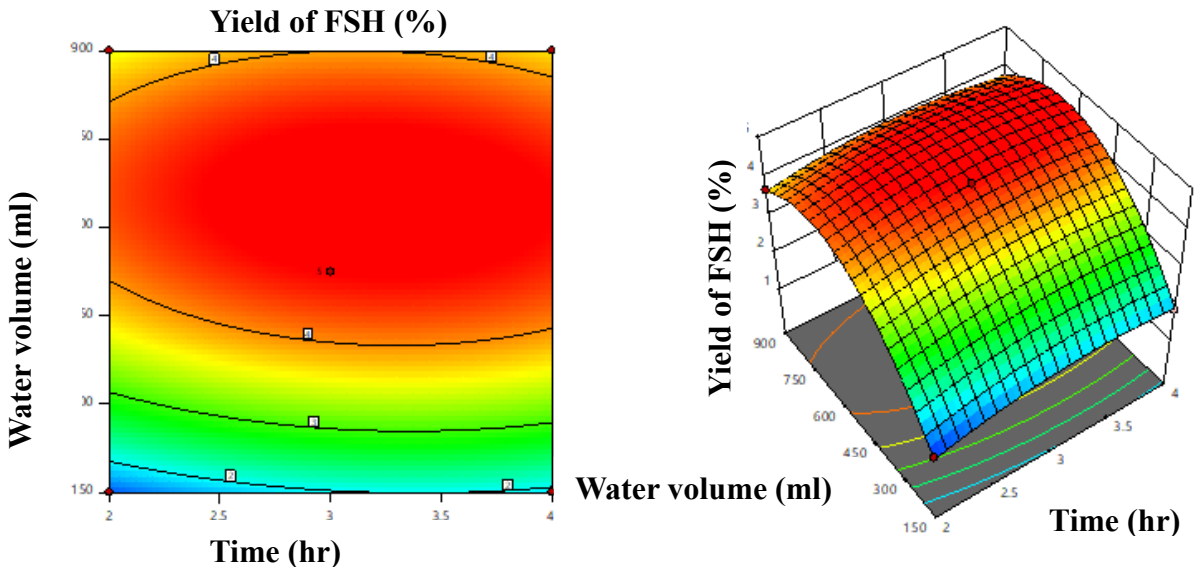


FIGURE 4.15 Contour and 3-D plot for effect of water volume and time on yield of FSH

Figur4.15 revealed that the yield of extracted powder FSH increase with increase in contact time. High mass of extracted powder FSH was observed at 290 ml and 4 hr. But, a decrease in mass of powder FSH was reported at higher water volume. The reduction in yield of powder FSH is attributed due to the bursting of hydrogel as it uptakes high amount water volume. This simultaneous decrease in yield of extracted FSH at higher volume indicated that optimum mass of extracted FSH reached.

4.5.2. Characterization of powder flaxseed hydrogel (FSH)

4.5.2.1. Fourier transform infrared radiation

Figure 4.16 illustrates the FTIR spectra of powder flaxseed hydrogel in the frequency region from 4000 to 500 cm^{-1}. In all spectra (Figure. 2), a wide range of absorbance and characteristic of the hydroxyl (O–H) stretch in the region of approximately 3433 cm^{-1} was observed. The peak appearing at 2079 is due to C=C stretching of alkenes (SP).The spectra of FSH shows a characteristics absorption band at 1388 cm-1 is due to C=C stretching of aldehyde and 1116 cm-1 due to stretching vibration of C-O in COH bands of carbohydrates (Mirbahoush, Chaibakhsh, & Moradi-Shoeili, 2019) (Hadad & Goli, 2018).

The peak appearing at 1633 cm^{-1} can be described as -C=C stretching of aromatic group. The peak appearing 618 cm^{-1} may be due to polymer backbone bending(Devi & Bhatia, 2019).

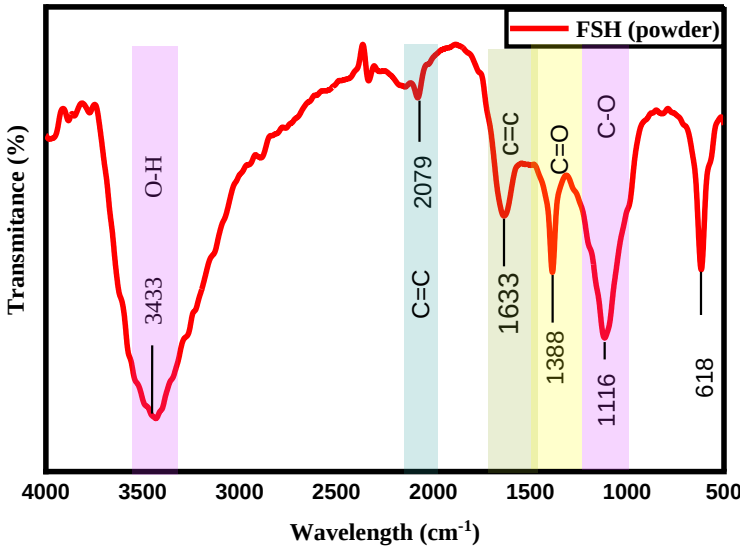


FIGURE 4.16 FTIR analyses of powder FSH

4.5.2.2. X-ray diffraction analysis (XRD)

The X-ray diffractometry was carried out for phase identification of samples. The XRD of the Powder FSH was carried out using an X-ray diffract meter and was scanned from 0^{o} to 80 ^{o} diffraction angle (2θ). Fig 4.17 shows that X-ray diffract gram of powder FSH (Mirbahoush et al., 2019). XRD patterns of FSH shows peaks at about 2θ≈19.75 degrees and broad peak at 2θ≈19.7 degrees indicated slight crystalline or amorphous structure of the FSH. It is probably attributed to irregular brunching polysaccharide structure and the presence of electrostatic repulsion between anionic chains (Hadad & Goli, 2018) (Mirbahoush et al., 2019).

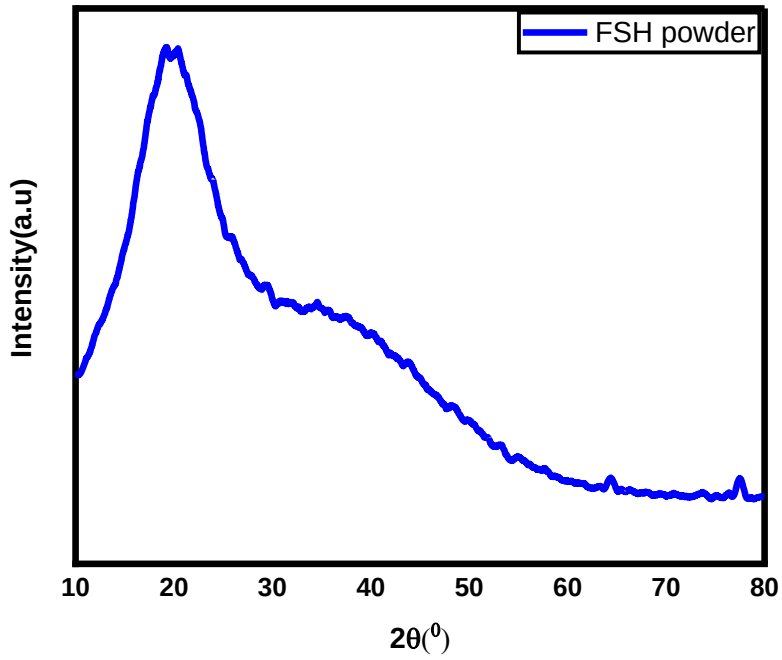


FIGURE 4.17 X-ray diffraction of extracted FSH powder

4.6. Characterization of plasticized flaxseed hydrogel (FSH)

4.6.1. Fourier transferred infrared radiation

Figure 4.18 illustrates the FTIR spectra of FSH-GLY film and shows the organic functional groups in the FSH-GLY structure.

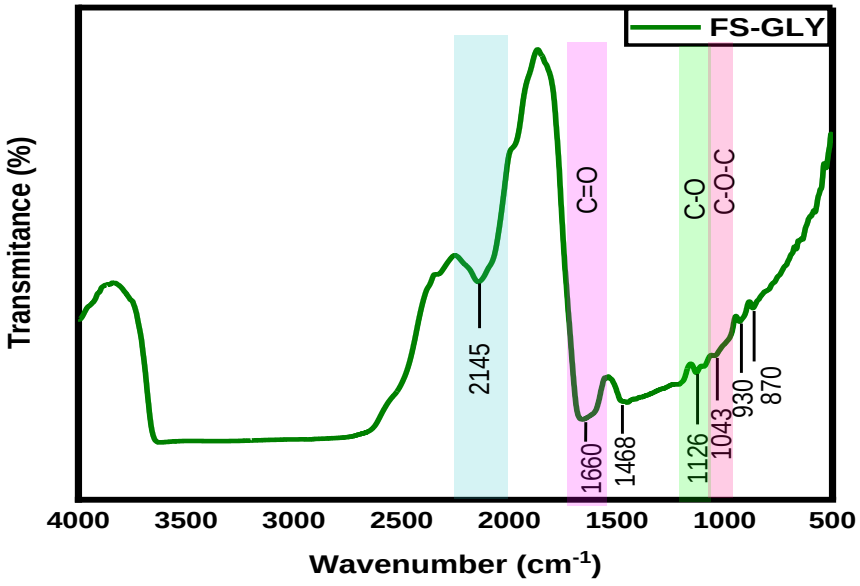


FIGURE 4.18 Functional group analysis for FS-GLY biofilm

As shown on figure 4.19 After addition of glycerol (5 wt%), Fourier transform infrared (FTIR) spectra shows an increase of intensity in the –OH stretching vibration region. Shifting of peaks was also experienced at band gap of 1644 and 1393 cm^{-1}. These changes may indicate possible interactions between the flaxseed hydrogel and glycerol (Tee, Tee, Daengprok, & Talib, 2017).

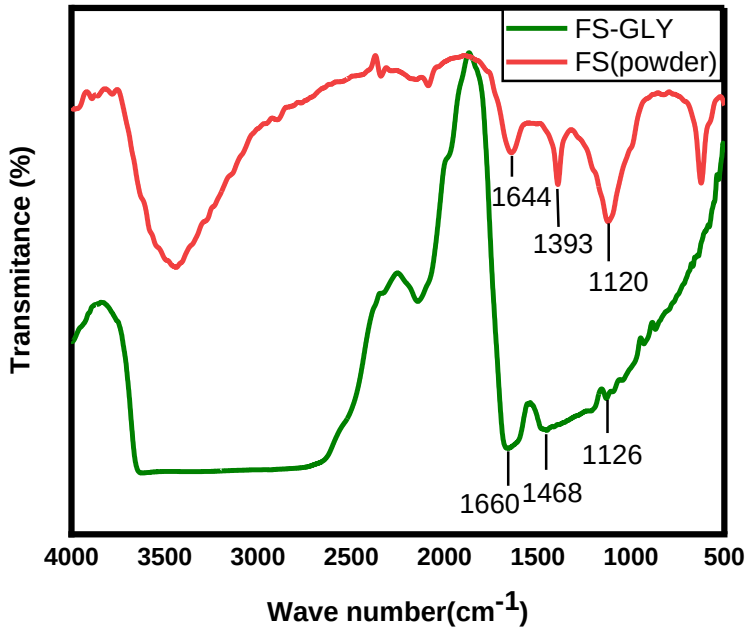


FIGURE 4.19 Functional group analyses for FS-GLY and FS (powder)

4.6.2. Antimicrobial activity

The antimicrobial activity of FS-GLY (5%wt) was observed on figure 4.24.according to the result FS-GLY exhibits antimicrobial activity against gram negative bacteria (***E.****coli)* ,(*S.aureus*) and gram positive bacteria *(P.aeruginosa)* ,(*S.pyogenes*) . the inhibition zone of FS-GLY (5%wt) was 6.5, 7, 7, 7.5 mm for bacteria of (***E.****coli),* (*S.aureus*), *(P.aeruginosa)* ,(*S.pyogenes*) respectively.

4.7. Characterization of DA induced flaxseed hydrogel (FSH) biofilm

4.7.1. Fourier transferred infrared radiation

The FTIR spectrum of FS-GLY-DA is shown on figure 4.20.According to the result sharp and intense peaks are shown at 923 and 850 cm^{-1}.the shifting of the peaks may be arise from interaction of functional group of DA extract with FSH.

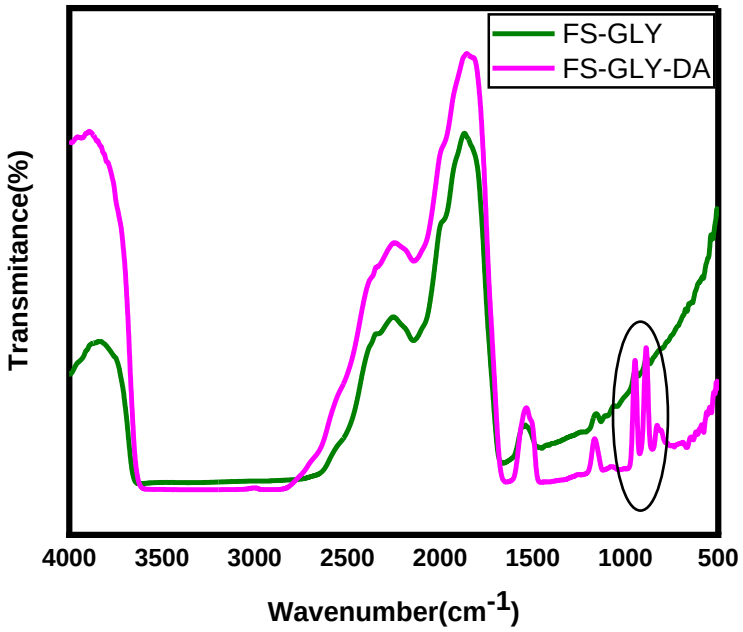


FIGURE 4.20 Functional group analyses for FS-GLY and FS-GLY-DA

4.7.2. Antimicrobial activity

Antimicrobial effect of *Dodonaea Angustifolia* extract induced FSH were tested against Gram negative bacteria (*E.coli* and *p. aurugonosa*) and Gram positive bacteria species (*S. pyogen* and *S. aureus*) using disk diffusion assay. The antimicrobial activities of prepared bio-film were investigated at different concentrations of DA extract. Figure 4.18 showed a clear zone of inhibition produced by using FS-DA biofilm with 5%wt of glycerol, after adding of 25, 50,and 75 ml of DA extract into FS-GLY biofilm. Ampicillin was used as a reference drug. Adding 25, 50,and 75 ml of DA extract into the FS-GLY-DA biofilm, shows the inhibition zone of 7, 8, 11.5 for (*E.coli)* bacteria. Also and for bacteria *S. pyogenes* Adding 25, 50,and 75 ml of DA extract into the FS-GLY-DA biofilm gives ,inhibition zone of 8, 9, 12 mm . for *(P.aeruginosa)* inhibition zone was 7, 8, 11 mm respectively and Inhibition zone for (*S.pyogenes*) bacteria after addition 25, 50,and 75 ml of DA extract into FS-GLY biofilm was 8, 10, 11.5, 12.5 mm. finally Increasing the concentration of dispersed DA extract into FS-GLY biofilm was increased the antibacterial activities also as shown in Table 4.5.

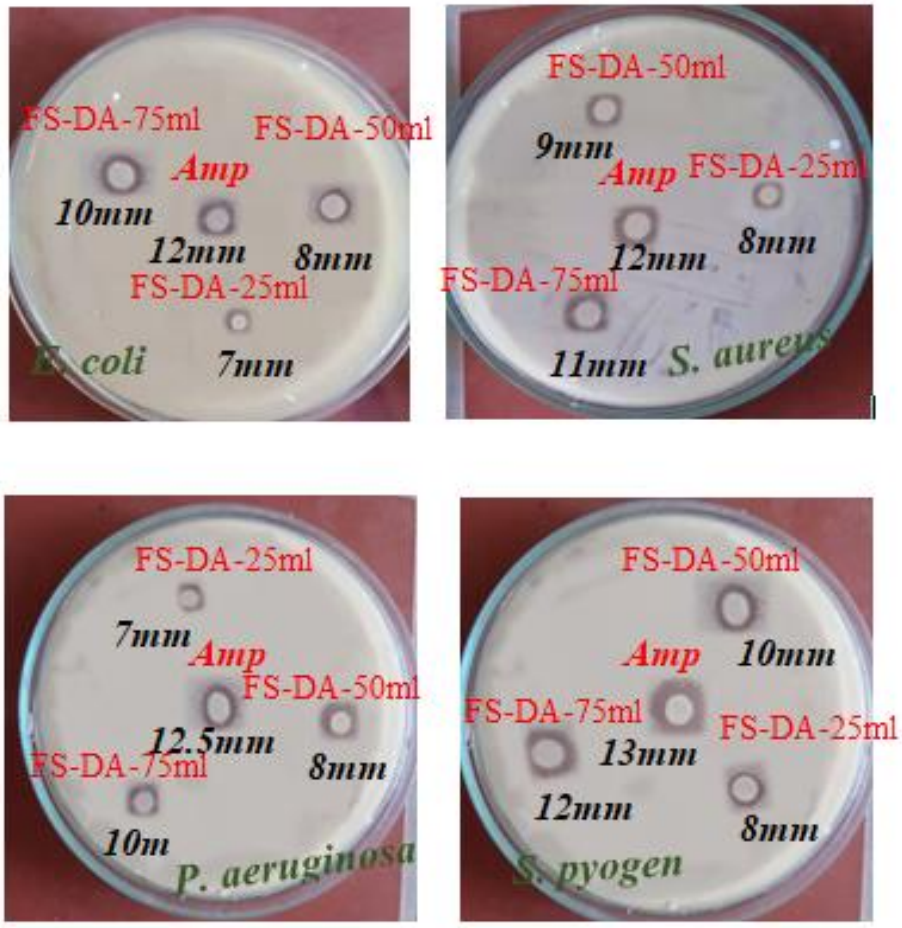


FIGURE 4.21 Zone of inhibition produced by FS based biofilm with different DA concentration

Table 4.9 Anti-bacteria activity (inhibition zone) of FS based biofilm with different concentration of DA

Bacteria Species and Zone of Inhibitions				
	Gram-negative		**Gram-positive**	
Concentration of DA	***E.coli*** **ATCC25922**	***S.aureus*** **ATCC2592**	***P.aeruginosa*** **ATCC27853**	***S.pyogen*** **ATCC19615**
FS-DA-25ml extract	7	8	7	8
FS-DA-50ml extract	8	9	8	10
FS-DA-75ml extract	10	11	10	12
Ampicillin (+ve control)	12	12	12.5	13

4.8. Characterization of AgNPs encapsulated DA induced flaxseed hydrogel (FSH) biofilm

4.8.1. Fourier transferred infrared radiation

The Fourier transform infrared (FTIR) result for the synthesized FS-GLY-DA and FS-GLY-DA-Ag 1% biofilm was analyzed on the figure 4.23. A purple color of FT-IR peak showed that 1% AgNPs incorporated in the FS-GLY-DA. After addition of AgNPs Shifted peaks was observed at1125 and 854 cm^{-1} for the C-O stretching on the hydroxyl group in glycerol due to presence of AgNPs. The formation of new peaks was also observed at 1040 and 995 cm^{-1}.this indicates the clear involvement of AgNPs on the FS based biofilm.

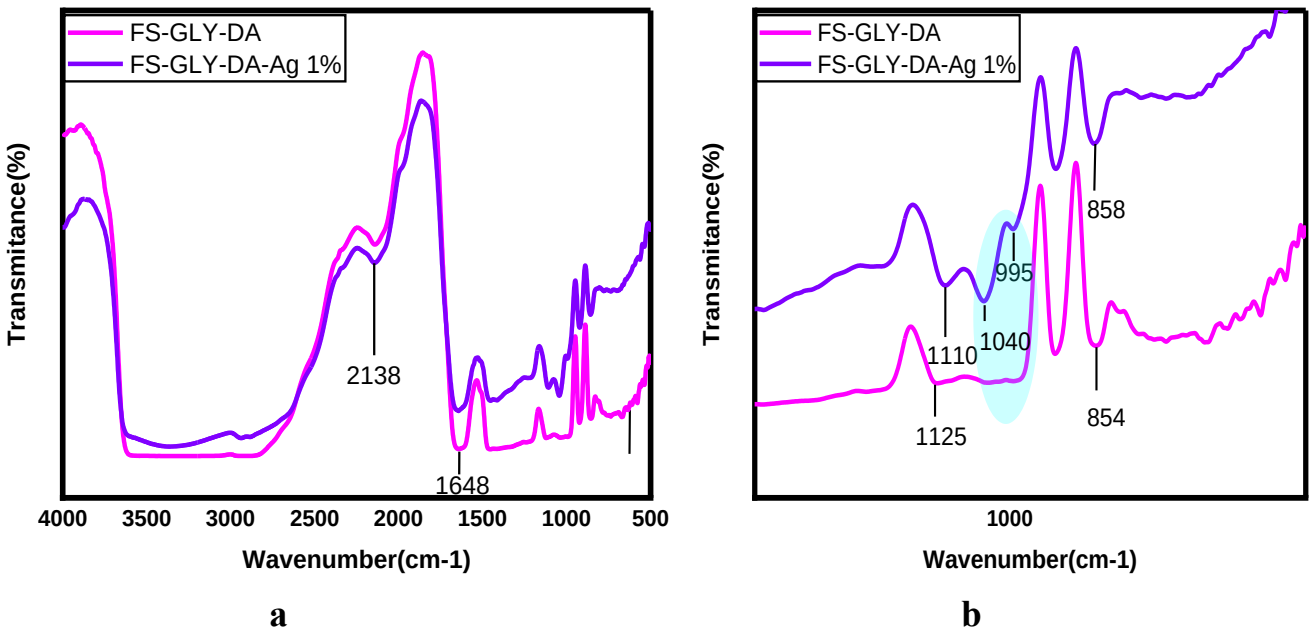


FIGURE 4.22 a) Functional group analyses for FS-GLY-DA and FS-GLY-DA-Ag 1%; b) enlarged part of graph a

4.8.2. Antimicrobial activity

The antimicrobial activities of prepared bio-film were investigated at different concentrations of AgNPs. The synthesized bio-film showed antimicrobial activities for both of Gram-positive *(P.aeruginosa)* ,(*S.pyogenes*) and Gram negative(*E.coli)* ,(*S.aureus*) bacteria by disk diffusion assay at different concentration.Figure 4.24 shows a clear zone of inhibition produced by using AgNPs/FS-DA with 5%wt of glycerol , where AgNPs dispersed with (1%, 1.5 %, and 3wt %). Hereafter ensuring stable nanoparticles dispersion in the biofilm, the antibacterial activity with the diameter zone of inhibition was observed for the film. Ampicillin was used as a reference drug. Net flaxseed with 5 wt% glycerol(FS) bio-film showed 6.5, 7, 7mm and 7.5 mm zone of inhibition against (*E.coli)* ,(*S.aureus*) ,*(P.aeruginosa)* ,(*S.pyogenes*) bacteria respectively. Adding 75 ml of DA plant extract into FSH with 5wt% glycerol(FS-GLY) increases the inhibition zone in to 8,9.5,9.and 10 mm respectively. However, after adding 1%, 1.5% and 3% AgNPs into the FS-GLY-DA biofilm, the inhibition zone was increased to 10, 11, 13 for (*E.coli)* bacteria. Also, for bacteria *S. pyogenes*, after adding 0.1%, 1% and 3% AgNPs the inhibition zone increases to 11, 12, 13.5 mm and for *(P.aeruginosa)* inhibition zone increases to 10, 11.5, 13 mm respectively. Inhibition zone of the biofilm also increases increase for (*S.pyogenes*)

bacteria after addition of 1%, 1.5% and 3% AgNPs in to 12, 11.5, 14 mm. Increasing the concentration of dispersed AgNPs on FS-GLY-DA biofilm increased the antibacterial activities compared to the biofilms without AgNPS also as shown in Table 4.10 .

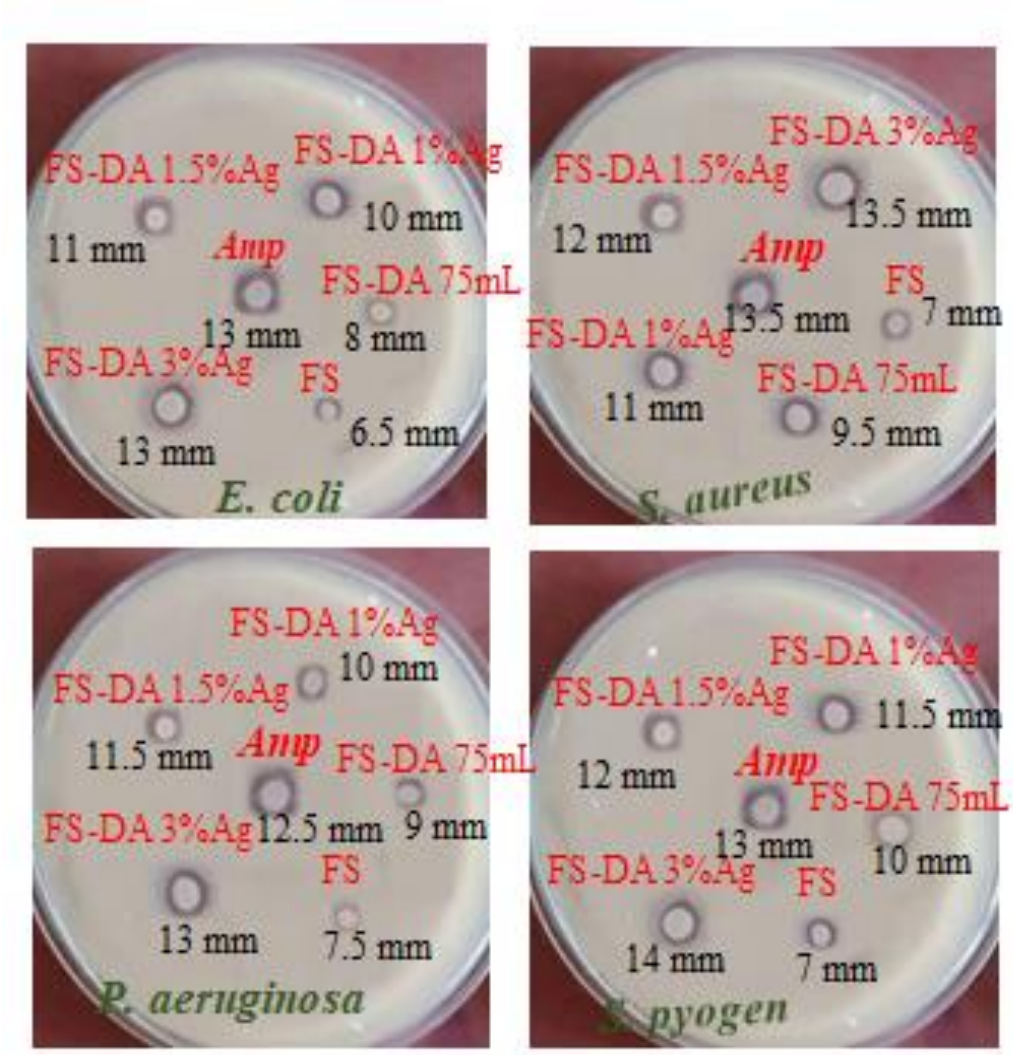


FIGURE 4.23 Zone of inhibition produced by FS based biofilm with different concentration of AgNPs

Table 4.10 Anti-bacteria activity (inhibition zone) of FS based biofilm with different concentration of AgNPs

Bacteria Species and Zone of Inhibitions				
	Gram-negative		**Gram-positive**	
Concentration of Ag	***E.coli*** **ATCC25922**	***S.aureus*** **ATCC2592**	***P.aeruginosa*** **ATCC27853**	***S.pyogen*** **ATCC19615**
FS-GLY 5wt%	6.5	7	7	7.5
FS-DA 75mL	8	9.5	9	10
FS-DA 1%Ag	10	11	10	11.5
FS-DA 1.5%Ag	11	12	11.5	12
FS-DA 3%Ag	13	13.5	13	14
Ampicillin (+ve control)	13	13.5	12.5	13

5. CONCLUSION and RECOMMENDATION

5.1. Conclusion

Dodonaea Angustifolia leaves extraction was takes place by using distilled water as solvent and the effect of operational factors such as extraction time and temperature was studied by fixing one parameter at time using indirect method (synthesizing AgNPs). The plant extract phytochemical components were characterized using the preliminary phytochemical screening method, FT-IR, UV-Vis, and antimicrobial activity.

Green synthesis method of silver nanoparticles (AgNPs) achieved using *Dodonaea Angustifolia* leaves extract as a reducing and stabilizing agent. The effect of different operational parameters like concentration of precursor, amount plant extract, Temperature, and were investigated.. Greenly synthesized and purified AgNPs characterized using UV-Vis, XRD, PSA and FT-IR. The preliminary photochemical screening of plant extract showed the presence of phenolic, saponins, terpenoids, alkaloid flavonoids and Tannins which are used as reducing and capping agents. This was also confirmed using FT-IR analysis. The UV-Vis spectra showed specific Surface Plasmon Resonance (SPR) absorption peaks for AgNPs between 438 nm- 453 nm for synthesized AgNPs at optimum conditions. The XRD peaks showed for AgNPs were around 38^0, 44^0, 64^0, $77^0$and the crystalline size of synthesized AgNPs was 10.84. The FT-IR results confirmed the presence of O-H, C-H, C=O, C=C bonds due to the presence of phytochemical components capping the AgNPs. The synthesized AgNPs showed antimicrobial activity with a clear zone of inhibition against Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and Gram-positive bacteria species (*S.pyogen* and *S. aureus*). The inhibition zone was increased when the concentration of AgNPs increased from 25-100 μg. The use of plant extracts as an effective reducing agent and stabilizing agent in AgNPs shows their potential values to use AgNPs for further applications, especially in medical applications. Effective way of operational parameters optimization also takes place for extraction of Flaxseed hydrogel (FSH) by using design expert RSM(BBD).the extracted FSH at the optimum condition(temperature of 100 ^{0}c ,time 4 hr, and water volume of 290 ml by fixing mass of FS at 30 gm) was used as matrix. The extracted *Dodonaea Angustifolia* and greenly synthesized AgNPs are applicable on the natural polymer matrixes (FSH) to enhance the antimicrobial activities of the matrix. The antimicrobial activity of FS-GLY biofilm was enhanced as concentration of DA extract increased (FS-GLY-DA 25

ml< FS-GLY-DA 50 ml < FS-GLY-DA 75 ml) and as AgNPs concentration increased (FS-GLY-DA 1 % < FS-GLY-DA 1.5 % < FS-GLY-DA 3 %) . Generally, the antibacterial activities of DA and AgNPs dispersed biofilms was found that more active against both gram-positive and gram negative bacteria comparing to their separate antimicrobial activities.

5.2. Recommendation

In this research work, *Dodonaea Angustifolia* was extracted and AgNPs were greenly synthesized using *Dodonaea Angustifolia* extract as a reducing and stabilizing agent. Then the different effect of operational parameters was investigated to get high concentration of AgNPs. Well dispersed DA extract and AgNPs used as fillers to enhance the antimicrobial activities of the flaxseed hydrogel based bio-films. The biocompatibility, Non-toxicity, and stability of Green methods and AgNPs high electrical conductivity, Thermal and optical properties make them a versatile application. Some recommendations for future work were opportunities are as follows:-

- Since operational parameters for the synthesis of AgNPs has effects on the Morphology and particle size of nanoparticles, the synthesized AgNPs may investigate using SEM (nm) and TEM.
- Due to the good antibacterial activities of DA extract and AgNPs in the films, it is good if the Anti-fungal activities may be investigated in detail.
- Due to the physical, chemical and higher conductivity properties of AgNPs in the biofilm Nano-composite. It may be applicable for bio-packaging, bio-sensor, and wound dressings applications. This is an area where further research might be done.
- Depending on the preliminary investigation the produced biofilm shows the tendency of extending shelf life of fruits (Tomato),due to this activity permeability test may be investigated in detail and the role of the biofilm plays on the inhibition of microbial that decrease the shelf life of tomato may be investigated.
- Further study can be explored on tensile strength, toxicity, the migration of AgNPs from the bio-film.

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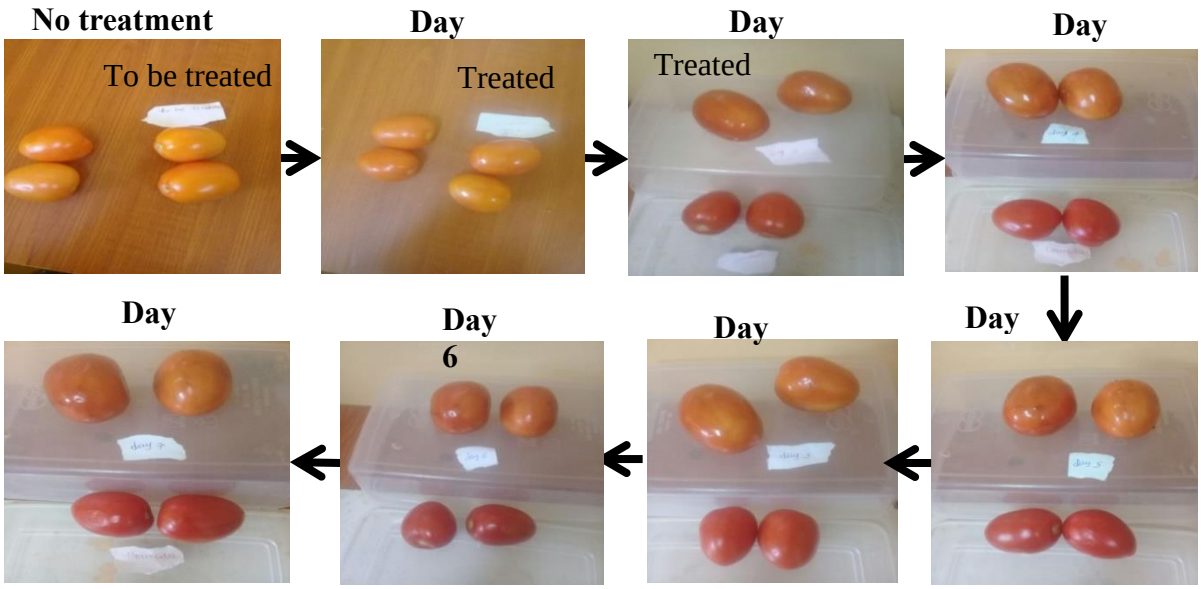
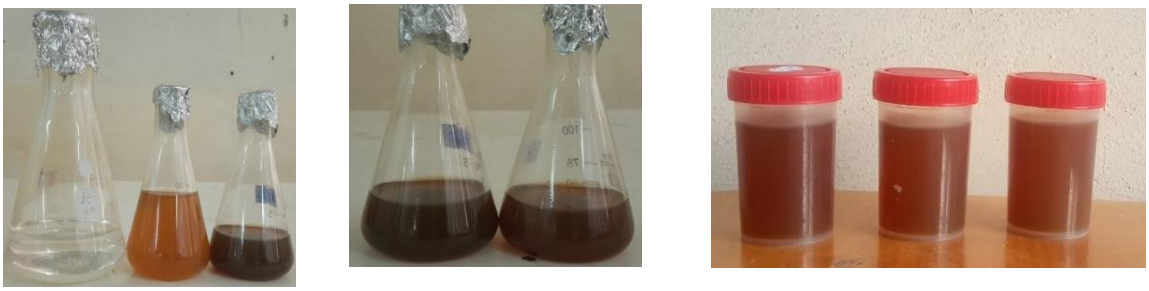
Appendix 1- Pictures of some laboratory works

Dodonaea Angustifolia



Preliminary investigation of *Dodonaea Angustifolia dispersed* flaxseed based hydrogel on extending shelf life of tomato



AgNPs synthesized with different precursor