Biological Properties Of Hops (Humulus Lupulus) Extract And Synthesis Of Hops Based Silver Nanoparticle And Silver Nano-Composite Film For Biomedical Application

A thesis submitted toward partial fulfilment of the requirements for the degree of

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In

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Jadavpur University

Submitted by

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CERTIFICATE OF RECOMMENDATION

We hereby recommend that the thesis entitled **"*Biological Evaluation of Hops(Humulus lupulus) extract and synthesis of Hops based silver nanoparticle and silver nanocomposite for biomedical application*."** carried out under my supervision by Pratik Das may be accepted in partial fulfilment of the requirement for awarding the Degree of Master in Biomedical Engineering of Jadavpur University. The project, in our opinion, is worthy for its acceptance.

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Certificate of Approval

The forgoing thesis is hereby approved as a creditable study of an engineering subject carried out and presented in a manner satisfactory to warrant its acceptance as a prerequisite to the degree for which it has been submitted. It is understood that by this approval the undersigned do not necessarily endorse or approve any statement made, opinion expressed or conclusion drawn therein but approve the thesis only for the purpose for which it is submitted.

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DECLARATION OF ORIGINALITY AND COMPLIANCE OF ACADEMIC ETHICS

I hereby declare that this thesis contains literature survey and original research work by the undersigned candidate, as part of his **Master of Engineering in Biomedical Engineering** studies during academic session 2017-2018.

All information in this document has been obtained and presented in accordance with academic rules and ethical conduct.

I also declare that, as required by this rules and conduct, I have fully cited and referred all material and results that are not original to this work.

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Date: ***Pratik Das***

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Dedicated to Ma, Baba , Bhaiya & Didun

for their love, endless support and encouragement...

ABSTRACT

Abstract

With the advent of research in the field of Contemporary and Alternative medicine all around the globe, natural products have gained a great importance in the last few years owing to its phytochemical content which in turn plays an important role in the field of medicine and biological sciences. Humulus lupulus (Hops) is an ancient plant used in various health problem throughout the world. Although the effectivity of the whole extract of Hops has not been evaluated so far. So this study aims to find different important phytochemicals present in Hallertauer Perle Hops extract and finally evaluating its anti-oxidant, anti-inflammatory and anti-cancer properties. The antioxidant and anti-inflammatory effectivity of the extract was compared with that of a standard and the results showed hops extract to be very proficient. The anti-cancer activity of Hops was tested at 3 time points to show its way of working and it effectiveness. The results showed time and dose dependent activity of extract on the HeLa cell line. Interestingly on Normal Cell i.e. PBMC cells the cytotoxicity was not be that high. Thus Hops extract showed good and selective toxicity towards cancer cell hence pointing its effectiveness as an alternative medicine in the field of biology.

Bacterial infections have been a constant threat to human health throughout the history. Bacterial colonization of biomedical devices and implants causes enormous problems for healthcare systems worldwide, costs and increases patient's suffering. Silver has been known, since the antiquity, by their antimicrobial properties and was used to produce reservoirs of food and with medical purposes. With the development of nanotechnology, silver nanoparticles have attracted the attention of different researchers due to their properties, as antimicrobial properties and high surface to volume ratio. However, these nanoparticles can form aggregates, which have toxic effects to the human cells. Recently, silver nanoparticles have been stabilized with several polymers and surfactants in order to avoid these problems.

Thus the present study deals with the in-situ production of gelatin-poly(vinyl alcohol)-silver nanocomposite films in view of their growing applications as antimicrobial packaging/container, wound dressing and antibacterial materials.. The use of silver nanoparticles is also significant, as several pathogenic bacteria have developed resistance against various antibiotics. A unique, nontoxic, simple, lucrative and ecofriendly technique was used to synthesize green silver nanoparticles (AgNPs). The AgNPs were synthesized using Humulus lupulus extract as a reducing agent for silver nitrate salt (AgNO3). The particle size distribution of AgNPs was examined by Dynamic Light Scattering (DLS) and the concentration was examined by UV-VIS Spectrophotometer. The nanoparticlre was also characterized with XRD, TEM and FTI.The stable dispersion of silver nanoparticles was added slowly in gelatin –PVA solution and was crosslinked using glutaraldehyde (cross-linker). The Gelatin-PVA Silver nanocomposite solution was casted in a petri dish and dried to form a film. The nanoparticles encapsulated within polymer chains were characterized by X-ray diffraction (XRD) and Scanning Electron Microscopy (SEM). The green AgNPs nanocomposite film exhibited significant antimicrobial activity against both Gram-negative bacteria, and Gram-positive bacteria. Therefore, the present study clearly provides an approach to develop novel antimicrobial films which are possibly useful in preventing/treating infections and can be used as antibacterial container or in food packaging.

KEYWORDS : Silver Nanoparticle, HOPS(Humulus lupulus), Green Synthesis, Gelatin/PVA Film, Antibacterial Film

CHAPTER 1:

INTRODUCTION

1.1Natural Products

1.2Hops(*Humulus lupulus*)

1.3Green Synthesis of Silver Nano-particle

1.4Mode of action of silver ions and its resistance towards bacteria

1.5Silver nanoparticle Composite with other materials

1.1 Natural Products

Natural products are now a prime center of attraction in the field of medicine and alternative medicine. Natural Products are combination of multiple organic chemicals and active compounds and in the meantime the efficacy of natural products as biological function transformers has also earned consideration worldwide. In this manner, they have been effectively utilized in the findings of new medications and have applied an expansive effect on the field of chemistry and biology [1-3]. Majorly in the past years the natural products were acknowledged for the point of view of physical science due to presence of wide variety of different structures. The biological potential of a natural product depends on the selectivity of the molecule or active compound present in the constituent of the natural product. To cite an example of novel drug development from natural products we can consider artemisinin [3] and its analogs which are being used uninterruptedly for the anti-malaria treatment till date. This demonstrates how research of natural products has made a noteworthy involvement in novel drug development [4, 5].

Natural products are troves of chemical diversity and their distinct, novel mechanism of action plays an essential role in drug development research. Due to this chemical diversity and unique mechanism of action natural products have played a very essential role in various drug invention and development programs as well as it has played a vital role in research programs as well. With the advent of time, research in the field of natural products; have experienced various experimental changes along with some significant advances. With increase in research natural products have become most vital dugs in the field of medicine [6-8]. During the year 2005 to 2007, sixty nine small molecules in the form of new drug got approved from which thirteen have its origin from natural product. Thus it points the importance of natural products in the field of medicine and drug discovery [9, 10].

In between the year's 1940 to 2002 more than fifty percent of the approved anticancer drugs are derived from natural products. For example, the Vinca Molecules alkaloids from Catharanthus roseus, and the terpene paclitaxel from Taxus baccata, are among successful anticancer drugs originally derived from plants [9, 11]. During the year 1991 to 2002 natural products gained a massive success in the field of novel drug discovery and in the field of medicine. Owing to the search of novel chemical structures natural products gained an important position in the field of research. Thus in the last twenty five years natural products have gained outmost importance and it is evident from the fact that in the case of antihypertensive more than 65 % of the newly discovered drug has their origin from natural products [9].

For the past fifty years, there has been an excessive use of natural products in the field of drug discovery and in the field of medicinal research using high-throughput screening methods and combinatorial chemistry; though, natural products and their active bio compounds are considered to be highly important parts of pharmacopoeias. Of the estimated 250,000–500,000 existing plant species only ten percent have been researched for its biological activities. [10]. Therefore, there are countless potential for upcoming discoveries from floras and other natural products which in terms offer enormous prospective in deriving beneficial information about new chemical structures and their novel types of action related to novel drug development [11].

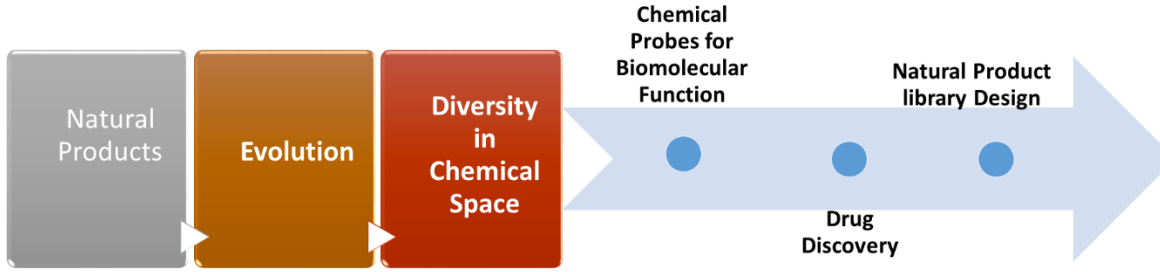

Figure 1.1: Natural product diversity (last circle has to modify)

Traditional Medicine (TM) is the primogenital form of health care around the globe and is used in the prevention, cure and treatment of physical and mental illnesses.TM is also known as complementary and alternative, or ethnic medicine, and it still plays a vital role in several countries today [12,13].

Natural products play a major source of the drugs used in TM. All drugs used as TM goes through clinical trial since the ancient time. Various advancement and changes have been made with respect to procedures of preparation, assortment of herbs, identification of medicinal materials, and the best time for procurement of various different plants for preparation of novel drug. Suitable handling and dose regulation are immediately required in TM to improve drug effectiveness and decrease drug toxicity.

Traditional medicine is an inseparable part of Chinese medical system. With the advent of time TM based on natural products have gained much popularity as complementary or alternative medicine in Western countries, too. Chinese herbal medicine, which is the utmost significant section of TM, is presently used in the health care of an assessed 1.5 billion people worldwide [14, 15].

1.2 HOPS (*Humulus lupulus*)

Hops (Humulus lupulus) were considered as a member of the Cannabinaceae family till it was replaced in the year 1972 as members of the Cannabaceae family (which also includes the genera Cannabis (hemp) and Celtis (blackberries)). The hop plant is a perennial climbing vine, which has separate male and female plants. The cones from the female plant are used in brewing. Before hops were introduced in the fifteenth century, ales were flavored with herbs and spices.

Hop is a perennial and dioecious climbing plant and only female infertile plants are nurtured to harvest hop cones. Hop cones comprise of a strig and bracts with glands that produce yellow lupulin. Hop cones in the present days are processed for pressed hops, hop pellets, and hop extract (extraction with ethanol or supercritical CO2) and other isomerized or fractionated products. Most hops currently are used in beer production. For brewers hop resins, hop polyphenols, and hop essential oils are the most important content of HOPS. The bitterness of the beer is due to the presence of isomerized products of hops resin. Hop polyphenols promote the precipitation of proteins, and hop essential oils give the beer a distinctive hops flavor.

Isomerized products of hop resins give beer bitterness, hop polyphenols promote the precipitation of proteins, and hop essential oils give the beer a distinctive hops flavor. Hops hold a wide range of secondary metabolites [16, 17]. Other than the variety, they are dependent on the growing habitat and the environmental climatic conditions [18].

Figure 1.2: HOPs a promising natural resource

Hops are rich in wide variety of active phytochemicals among which Xanthohumol (XN) gained the most popularity due its wide spread biological activities. There are numerous papers describing the biological activities of Xanthohumol (XN) [19-22]. Due to the presence of this component Hops is considered one of the most important natural sources for biological applications [22, 23].

Broadly the two major constituents of Hops are the α-Acids (AA) and the β-bitter acids (BA). α-Acids (AA) takes its origin from the luplin particle which is present inside the hops cones [18]. The three major correspondents of α-bitter acids that can be found in all variety of Hops are: adhumulone, cohumulone, and humulone [18, 19, 24]. Along with these two major

components there exist three minor components as well: posthumulone, prehumulone, and adprehumulone [24].

The β-bitter acids (BA) are also components of the soft resin of the Hops and like the α-Acids it also consists of similar kind of correspondents likely: adlupulone, colupulone, lupulone, postlupulone, and prelupulone [18, 19, and 24]. These substances have very low solubility in water hence it is not popular in brewing process. These substances showed an excellent antimicrobial activity especially for abolition of Helicobacter pylori [25]. Thus it plays a major role as an alternative to conventional antibiotics. Presently research are going on to explain the molecular structure that are formed by oxidation of α-Acids and β- acids [26-28]. These structures, contains β-carbonyl moieties, are shown in Figure 1.3. There are now various technologies for preparation of Hops extracts.

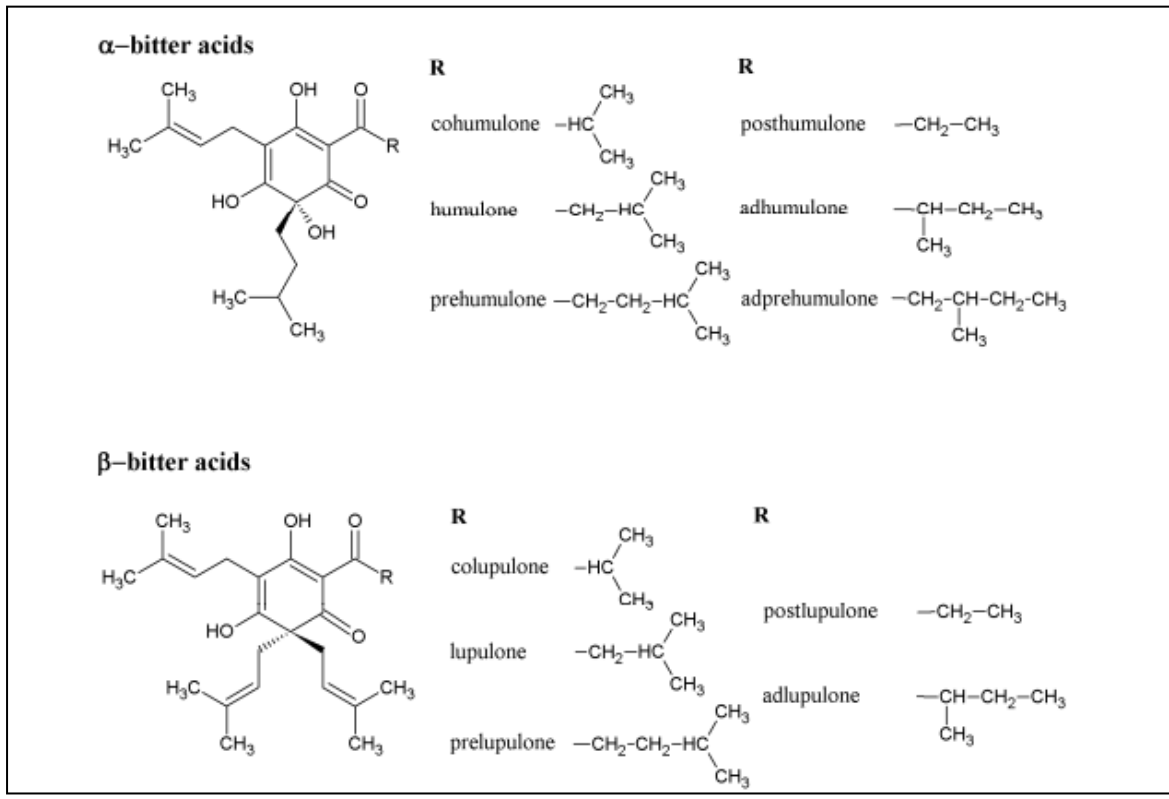

Figure 1.3: Chemical structures of main hop bitter acid components: α-bitter acids and β -bitter acids.

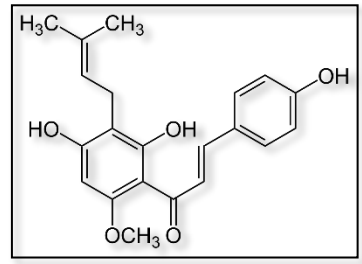

Figure 1.4: Xanthohumol

Hops are rich in polyphenols and flavonoids. Hops polyphenol signify a wide and broad group of secondary metabolites with very diverse chemical structures [18]. More than 1000

polyphenolic compounds are found in Hops. The polyphenols holds about 8% of the total dry weight of Hop cones. The green part of the cones mainly consists the polyphenols but the prenylflavonoids, hop resins and essential oils are present in lupulin granules [29]. Polyphenols majorly can be divided into two major groups in terms of Hops non-glycosylated and glycosylated polyphenols. A wide division of Hops polyphenol is described in the Figure 1.5.

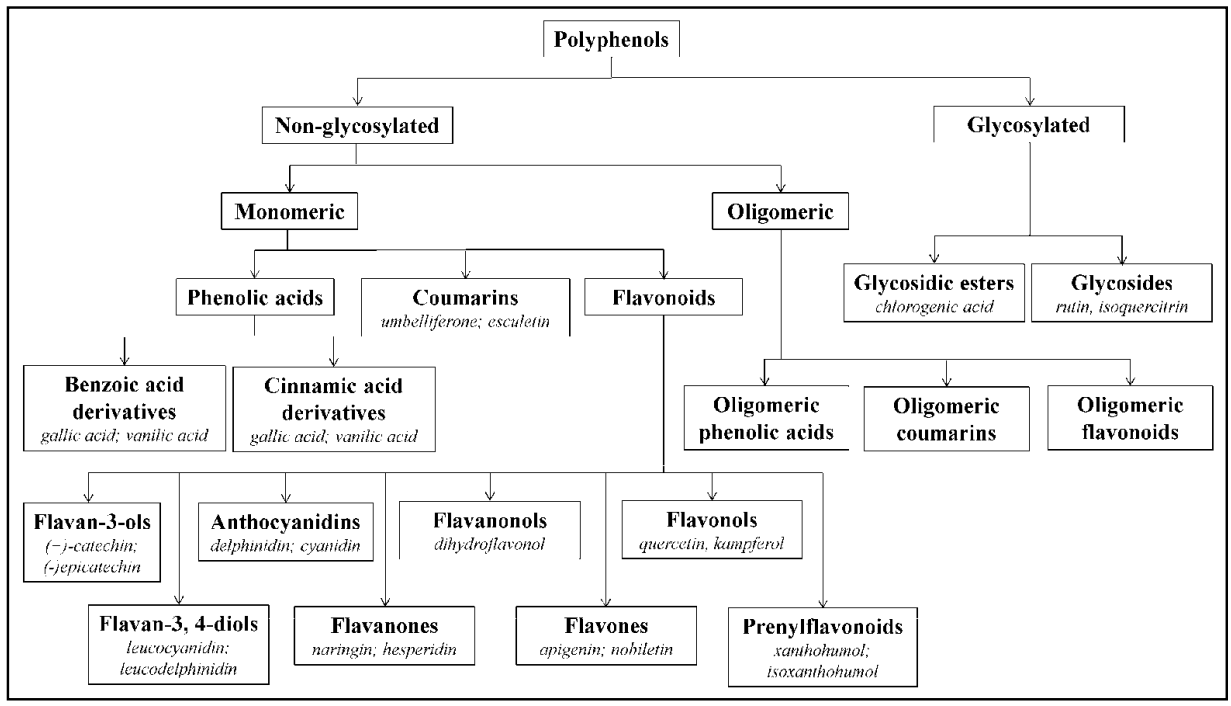

Figure 1.5: Overview of Hops Polyphenol

Major phytochemicals found in hops are: 2-methyl-3-buten-2-ol, lupulone, humulone, valerianic acid, quercetin, tannins, phytoestrogens, gallic acid. Due to the presence of such vital phytochemicals Hops are used in treatment of various diseases. Hop is a central nervous relaxant used in treatment of insomnia. Hops are frequently used to persuade sleep. Hop has been used to increase breast milk and calm irritable infants. Humulone and lupulone have anti-inflammatory and antibacterial activity and help to preserve beer. Hops are also used to treat upper digestive tract infection. Hops are also used in Crohn's disease. Hop contains phytoestrogens, which can help to ease menopausal symptoms.

1.3 Green Synthesis of Silver Nano-particle

Nanotechnology is the branch of science which deals with nanoparticle having a size of 1-100nm at least in one dimension. Currently, nanoparticles find its application commercially for an extensive variety of coating areas which includes electronics, energy contact actions, and

medicines. Apart from this Silver nanoparticle finds its application majorly in field of pharmaceuticals and other medical sciences (Figure1.6). Silver nanoparticle can be synthesized by various means like physical, chemical, biosynthesis etc. Among all the methods biosynthesis or green synthesis of silver nanoparticle has been accepted most in the field of biology for its superior biocompatibility. The practice of green chemistry including plant extract and microbes is eco-friendly, non-toxic, and cheap [30]. The present strategy is to improve the effectiveness of drugs is to conjugate them with silver nanoparticle and using them as an effective material for microbial infections [31]. In the field of biological removal process the interaction of Silver nanoparticle with heavy metal is still a mystery.

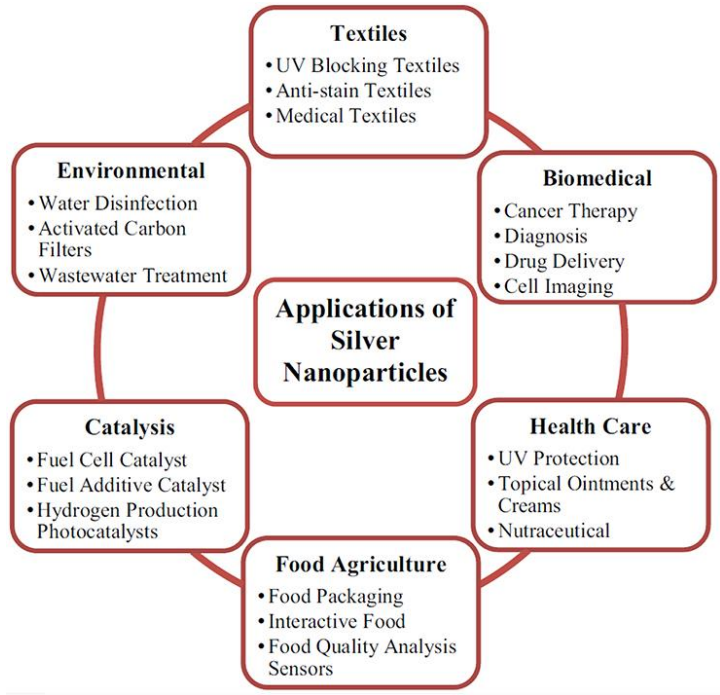

Figure 1.6: Various Application of Silver nanoparticle

Silver nanoparticle holds various unique and strong application antimicrobial, anticancer, larvicidal, catalytic, and wound healing activities [32]. For the last few years many attempt have been taken to develop greener and cheaper methods for the synthesis of silver nanoparticle [33]. The green synthesis of silver nanoparticle has been projected as an economical and eco-friendly alternative to chemical and physical methods [34]. Wide varieties of plant and microorganism have been identified which can be used for synthesis of Silver Nano-particle [35]. The purpose of this present study is to explore new standpoints of nanomaterial biosynthesis and the upcoming applications of synthesized nanoparticles as potential antimicrobial agents as well as anti-inflammatory agent.

Plants and their parts contain carbohydrates, fats, proteins, nucleic acids, pigments and several types of secondary metabolites which act as reducing agents as well as capping agent to produce stable nanoparticles from metal salts without producing any toxic by-product. The details have been provided in Table 1 [36].

Table 1.1: Plant-mediated synthesis of silver nanoparticles

Plant	**Plant part**	**Size and shape**	**Phytoconstituents responsible for reduction of silver nitrate**
Aloe vera	Leaf gel (removed skin)	5–50 nm; octahedron	Flavanones and terpenoids
	Leaf	70.7–192.02 nm; spherical (size varies through change of times and temperatures)	Lignin, hemicellulose and pectins
	Leaf	Size varies in accordance to different parameters; spherical	Flavonoids, terpenoids and phenols
Mangifera indica	Seed	14 nm; spherical and hexagonal	Phenolic compounds, gallotannins and tannin
Erigeron bonariensis	Leaf	13 nm; spherical	Flavonoids, steroids, glycosides, triterpenes, sugars and caffeoyl derivatives
Myristica fragrans	Bark and seeds	Spherical, polydispersed	Secondary metabolites
Momordica charantia	Leaf	11 nm; spherical	Momorcharins, momordenol, momordicius, momordin, momordolo, charantin, charine, cucuritanes, cucurbitns, goyaglycosides and goyasaponins
Carambola	Fruit	16, 13, 12 nm at pH 4, 7, 10 respectively	Polysaccharides, polyols and ascorbic acid
Rubus glaucus	Fruit	12–50 nm; spherical	Phenolic groups and flavonoids
Prunus serotina	Fruit	20–80 nm (blue LED) 40–100 nm (white solar); spherical	Chlorogenic acid, catechin, proanthocyanidin, and flavonol glycosides
Piper nigrum	Seeds	10–60 nm; rod shaped	Polysaccharides, amino acids, alkaloids, proteins and vitamins
Nigella sativa	Leaf	15 nm; spherical	Alkaloids, ascorbic acid, saponins, glycosides, amino acids, flavonoids like catechin, apigenin, gallic acid and benzoates especially vanillic acid
Calotropis gigantean	Flower	10–50 nm; spherical	–
Acmella oleracea	Flower	2–20 nm; spherical	–
Piper betle	Leaf	48–83 nm; spherical	Allylic benzenes, phenolic, amino acids, proteins, alcoholic

			compounds, terpenes and terpenoids
Morinda tinctoria	Leaf	80–100 nm; spherical and rod	Ascorbic acid, niacin, copper and iron
Trigonella foenum-graecum	Seeds	20–50 nm; spherical	Saponins and alkaloids
Picrasma quassioides	Bark	17.5–66.5 nm; spherical	–
Rosa 'Andeli'	Petals	0.5–1.4 nm; spherical	Polyphenols and flavonoids
Salvadora persica	Stem	1–6 nm; spherical	Phenolic compounds
Artemisia absinthium		5–20 nm; round shaped	Phenolic compounds and flavonoids
Chelidonium majus	Aerial parts	DLS-253.3 nm; spherical, quasi-spherical	Flavonoids and alkaloids
Calotropis procera	Flower	35 nm; face centered cubic	Tannins, triterpenes, flavonoids, steroids, alkaloids and cardiac glycosides
Sterculia acuminata	Fruit	~ 10 nm; spherical	Ascorbic acid, gallic acid, phenolic compounds, pyrogallol, methyl gallate and polyphenolic compounds
Terminalia cuneata	Bark	25–50 nm; spherical	Tannins, saponins, triterpenoids, flavonoids, gallic acid, ellagic acid and phytosterols
Cirsium japonicum	Plant	4–8 nm; spherical	Saponins, proteins and flavonoids
Isatis tinctoria	Plant	10–15 nm; spherical	Saponins and flavonoids
Aegle marmelos	Fruit	22.5 nm; spherical, hexagonal, roughly circular	Phytosterols, flavonoids, alkaloids, triterpenoids and amino acids
Trachyspermum ammi	Seeds	36 nm; cubic	Fatty acids, proteins, flavonoids and alkaloids
Eucalyptus globulus	Leaf	1.9–4.3 and 5–25 nm with and without microwave treatment respectively	Alkaloids and flavonoids
Cydonia oblonga	Seeds	38 nm; face-centered cubic	Flavonones, terpenoids, proteins and amino acids
Hydrocotyle asiatica	Leaf	21 nm; spherical	Flavonoids and glycosides
Lantana camara	Leaf	33.8 nm; spherical	Flavonoids, proteins, saccharides secondary metabolites like alkaloids, tannins, saponins, carbohydrates, steroids and triterpenoids

Nyctanthes arbor-tristis	Seeds	50–80 nm; spherical	Carbohydrates and phenolic compounds
Pennyroyal sp.	Leaf	19.14 ± 9.791 nm; spherical	–
Saraca indica	Leaf	23 ± 2 nm; spherical	Flavonoids and steroids
Terminalia chebula	Fruit	30 nm; distorted spherical	–
Euphorbia amygdaloides	Plant	7–20 nm; spherical	–
Pedalium murex	Leaf	50 nm; spherical	Flavonoids, alkaloids, steroids, rosins, saponins and proteins
Chelidonium majus	Root	15.42 nm; spherical	–
Salacia chinensis	Powdered plant	20–80 nm; spherical, rods, triangular, hexagonal	Flavonoids, saponins, proteins, carbohydrates and phenolics
Tamarindus indica	Seed coat	~ 12.73 nm	Flavonoids, tannin and saponins,
Parkia roxburghii	Leaf	5–25 nm; poly dispersped, quasi-spherical	Proteins
Aristolochia indica	Leaf	32–55 nm; spherical	–
Cerasus serrulata	Leaf	10–50 nm; spherical	Alcohol and phenolic compounds and proteins
Matricaria camomilia	Flower	8–35 nm; spherical	Terpenoids, flavones and polysaccharides
	Flower	~ 5.5 nm; spherical	Phenolics, carbonyl and amines or alcohol groups
	Fruits	~ 15.4 nm; spherical	Phenolics, flavonoids, terpenoids and vitamins
Alpinia calcarata	Root	5–15 nm; quasi-spherical	Proteins, flavonoids and polyphenols
Salvinia molesta	Leaf	12.46 nm; spherical	Alkaloids, flavonoids, tannins, phenols, sugars and proteins
Helicteres isora	Root	16–95 nm; spherical	Steroids, terpenoids, alkaloids, carbohydrates and phenolic compounds
Mukia maderaspatana	Leaf	158 nm; spherical	Phenolic compounds
*Ficus benghalensis*and *Azadirachta indica*	Bark	60 nm; spherical	Flavonoids, terpenoids and phenols

Azadirachta indica	Leaf	34 nm; spherical and irregular shape	Flavanoids and terpenoids
Adathoda vasica	Leaf	10–50 nm; spherical	Alkaloids compounds
Amaranthus gangeticus	Leaf	11–15 nm; globular and polycrystalline	Amino acids
Phlomis	Leaf	25 nm; spherical	Glycosides such as flavonoids, iridoids, diterpenoids, triterpenoids and other phenolic compounds
Syzygium alternifolium	Fruit	4–48 nm; spherical	Phenols and primary amines of proteins
Afzelia quanzensis	Bark	10–80 nm; spherical	Proteins
Allamanda cathartica	Flower	39 nm; spherical	(E,E)-geranyl linalool, n-pentacosane, 1,8-cineole and n-tricosane
Carica papaya	Peel	10–30 nm; spherical	Vitamins (C, K, E), amino acids, carbohydrates, β-carotene, lycopene and polyphenols
Vitis vinifera	Leaf	200 nm; spherical	Hydroxyl groups and phenolic compounds mainly myricetin, ellagic acid, kaempferol and gallic acid
Solanum indicum	Leaf	10–50 nm; spherical	Phenolic compounds
Tectona grandis	Leaf	26–28 nm; spherical	Phenols
Soymida febrifuga	Leaf	10–20 nm; spherical	Phenolic groups, amino acids, aliphatic and aromatic amines, amide-I and amide-II
Cardiospermum halicacabum	Leaf	SEM-less than 100 nm; spherical	Polyphenols and phenol
Ammannia baccifera		105–125 nm; spherical	Polyphenols, flavonoids and proteins
Diospyros paniculata	Root	17 nm (avg); spherical	Phenolics and proteins
Simarouba glauca	Leaf	33–50 nm; spherical	Amino groups and hydroxyl groups
Origanum majorana and *Citrus sinensis*	Leaf	40–70 nm; feather and 26–60 nm; spherical, cubical respectively	Proteins and phenolic compounds
Salmalia malabarica	Gum	7 ± 2 nm; spherical	Carbonyl and hydroxyl group
Psidium guajava	Leaf	10–90 nm; spherical	Leucocyanidin, flavonoids, tannins, saponins, carotenes, vitamin C, B6 and carbohydrates

Allium cepa	Bulb	–	–
Justicia glauca	Leaf	10–20 nm; spherical	Phenolic compounds
Skimmia laureola	Leaf	Irregular, spherical, hexagonal	Tritepenoids, skimmidiol and coumarins
Andrographis echioides	Leaf	~ 68.06 nm; cubic	Carbohydrates, tannins, saponins, flavonoids, alkaloids, quinones, glycosides, triterpenoids, phenols, steroids, phytosteroids and anthraquinones
Putranjiva roxburghii	Leaf	5.74 nm; spherical	Amino groups
Ixora coccinea	Flower	5–10 nm; spherical	Alkaloids, tannins, glycosides, flavonoids, saponins, terpenes and carbohydrates
Emblica officinalis	Fruit	10–70 nm; spherical	Alkaloids, phenolic compounds, amino acids and tannins
Hibiscus rosa-sinensis	Petals	~ 18.79 nm; spherical	Proteins
Bauhinia variegata	Leaf	32 nm; spherical, triangular, truncated triangles, decahedral	Reducing sugar, saponins, anthraquinone, alkaloids and terpenoids
Pteridium aquilinum	Leaf	SEM-35–65 nm; spherical	Phenols, alkaloids, tannins, flavonoids, proteins, carbohydrates, saponins. glycosides, steroids and triterpenoids
Aristolochia indica	Leaf	30–55 nm; spherical and cubical	Phenols
Cassia roxburghii	Leaf	~ 32 nm; spherical, triangular, truncated triangles, decahedral	–
Anisomeles indica	Leaf	TEM-18–35 nm; SEM-50–100 nm; spherical	Alcohols, phenols and carboxylic group
Hybanthus enneaspermus	Plant	16–26 nm; spherical, hexagonal, triangular	Proteins
Amaranthus dubius	Leaf, stem, root	Stem: 30–35 nm; Root: 18–21 nm; Leaf: 18–21 nm	Polyphenol compounds and aldehydes
Ziziphus jujuba	Fruit	25.75 nm; spherical	Alcohols and phenols
Chrysophyllum oliviforme	Leaf	25 nm; flower	Flavonoids, saponins, catechic tannins, traces of anthraquinones, reducing sugars and phenolic compounds

Plumeria alba	Flowers	~ 36.19 nm; spherical	Amino, carboxylic and sulfhydryls
Impatiens balsamina	Flowers	5–40 nm; spherical	Alkaloids, tannins, glycosides, flavonoids, saponins, terpenes and carbohydrates
Fraxinus excelsior	Leaf	25–40 nm; spherical and polydisperse	Flavonoids, alkaloids, glycosides, terpenoids, phenolic compounds, amino acid residues and peptides of proteins
Pongamia pinnata	Leaf	AFM-15–35 nm; spherical	Alkaloids, glycosides, flavonoids, saponins, carbohydrates, tannins, phenolic compounds and fat
Pongamia pinnata	Seed	5–30 nm; spherical	Pongaflavanol, tunicatachalcone, pongamol, galactoside and glybanchalcone
Areca catechu	Nut	18.2 and 24.3 nm; spherical	Polyphenols
Ficus talboti	Leaf	9–12 nm; spherical	Flavonoids, alkaloids, saponins, phenolic compounds, tannins, phytosterol and glycosides
Sida cordifolia	Leaf	10–30 nm; spherical, prism	Alkaloids, quinazolines, cryptoleptins, phytosterols, flavonoids and saponins
Clerodendrum phlomidis	Leaf	TEM 10–15 nm; SEM 23–42 nm; spherical	Phenolics, flavonoids, terpenoids and steroids
Theobroma cacao	Pod husk	4–32 nm; face-centered cubic	Proteins and phenolic compounds
Ficus carica	Fruit	20–80 nm (thermal approach), 10–30 nm (ultra sonication approach); spherical	–
*Parkia speciosa*Hassk	Pod	20–50 nm; predominantly spherical	–
Boerhaavia diffusa	Whole plants	25 nm; spherical	
Pelargonium endlicherianum	Roots	Different size; spherical	Gallic acid, apocynin and quercetin
Artocarpus heterophyllus	Seeds	10.78 nm; irregular	Lectin—a single major protein
Ceropegia thwaitesii	Leaf	100 nm; spherical	Triterpenoids; and methoxy groups of protein

Alternanthera sessilis	Leaf	30 nm; various shape	Alkaloid, tannins, ascorbic acid, carbohydrates and proteins
Dryopteris crassirhizoma	Rhizome	5–60 nm; almost spherical	Alcohol, amines, alkanes, carboxylic acid and or ester
Leptadenia reticulate	Leaf	50–70 nm; crystalline, face centered and spherical	Phenolics, terpenoids, polysaccharides and flavones
Ipomoea batatas	Root	TEM 30–120 nm; AFM 50–200 nm; polygonal	Glycoalkaloids, mucin, dioscin, choline, polyphenols and anthocyanins
Sambucus nigra	Fruit	26 nm; spherical	Polyphenols
Millettia pinnata	Flower	16–38 nm; spherical	Multi-functional aromatic gropus
Coptis chinensis	Plant extract	15 nm; spherical	Polyphenols
Lycium barbarum	Fruit	3–15 nm; spherical	Tannias, flavanoids, ascorbic acid and alkaloids
Embelia ribes	Seed	20–30 nm; crystalline, uniform and spherical	Alkaloids, quinones, proteins, reducing sugars and saponins
Zizyphus xylopyrus	Bark	60–70 nm; spherical	Reducing agents

Metal nanoparticles synthesis using plant (inactivated plant tissue, plant extracts and living plant) is a significant branch of biosynthesis processes. Various studies are there that plants have potential to reduce metal ions both on their surface and in various organs and tissues remote from the ion penetration site [37]. Biomolecules present in plant extracts comprising, enzymes, proteins, amino acids, vitamins, polysaccharides, and organic acids such as citrates are potentially able to reduce metal ions. In this regards, in vitro approaches have been successfully developed in recent years, in which plant extracts are used for the bio reduction of metal ions to form their nanoparticles. The extract of many parts of plants such as leaves, flowers, seeds, barks and roots have been useful for synthesis of AgNPs [38-40]. The plants extracts may work both as reducing and capping agents in AgNPs synthesis. These extracts have also been reported to have antibacterial, anti-diabetic, anti-inflammatory, antioxidant, anti HIV, snake venom neutralization, antifungal and larvicidal activities [41].

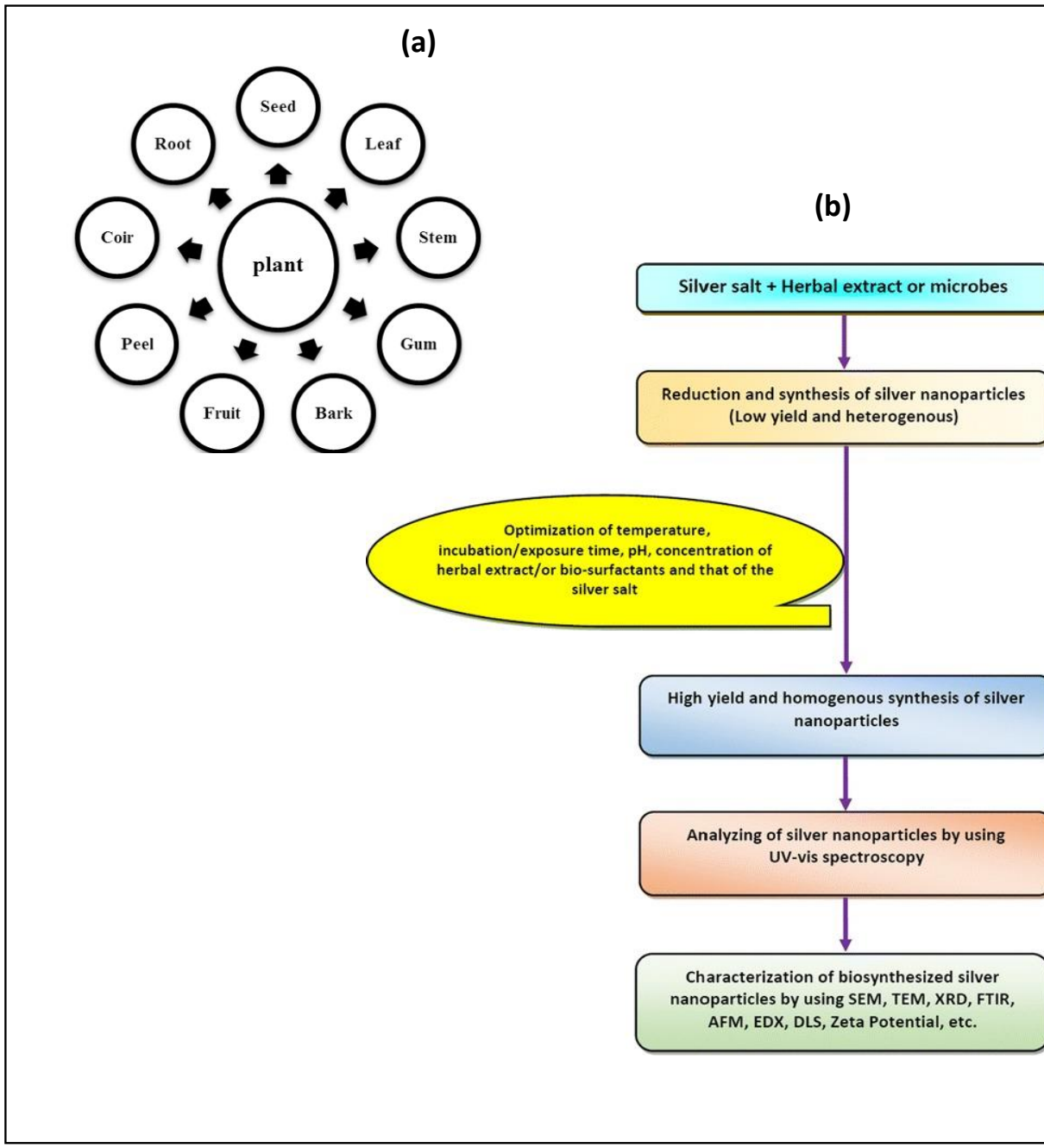

Figure 1.7: a) Plant parts used in Green synthesis of silver nanoparticle. b) Biosynthesis of silver nanoparticles and their optimization techniques

1.4 Mode of action of silver ions and its resistance towards bacteria

Multidrug resistance of various pathogenic bacteria to the antimicrobial medication has become a significant impairment toward fruitful identification and management of infectious diseases. Recent progresses in nanotechnology-based medicines have unlocked new horizons for battling multidrug resistance in bacteria. Specifically, the utilization of silver nanoparticles as a compelling antibacterial drug agent has received abundant attention. The primary essential physico-chemical factors that have an influence on the antibacterial potential of silver nanoparticle, are size, shape, surface charge, concentration and mixture state. AgNPs exhibits their antibacterial potential through varied mechanisms. AgNPs may adhere to bacterial cell wall, diffuse within the cells, generate atom through Reactive oxygen specis and intonate bacterial signal transduction pathways. These are documented as the most distinguished modes of antibacterial action of AgNPs. On the opposite facet, AgNPs exposure to human cells induces toxicity, genotoxicity, and inflammatory response in human cells in an exceedingly cell-type dependent manner. This has raised considerations relating to use of AgNPs in medical specialty and drug delivery.

The mode of action of silver ions against bacterium is wide studied. Silver ions will kill the bacterium by numerous ways. Silver ions react with negatron donors to make complexes with thiols, carboxylates, phosphates, hydroxyls, amines, imidazoles and indoles, therefore inactivating the enzymes. It had been according by Swanson et-al that metastasis chains of bacterium were stifled by silver ions at 2 sites one being between haemoprotein b and haemoprotein d and therefore the second being between the locations wherever the substrate enters the metastasis chain and flavoprotien in NADH and succinate dehydrogenase [42]. It had been shown that silver ions at 86μM were ready to inhibit the chemical reaction of carbohydrates like aldohexose, fumarate, succinate and glycerine in E. coli [43]. Reports showed that there was modification in catalyst conformation inside microorganism cells at concentrations between 0.001 to 1.00mM of Ag+ ions [44]. It was additionally shown that noble metal particle shaped complexes with macromolecule bases goes in deoxyribonucleic acid [45] Silver ions were additionally celebrated to make deformation of cell membranes as shown by Coward et al [46]. Despite the fact that silver may be a robust and speedy acting medication material, silver particle resistant microorganism strains are isolated from numerous places like clinic, silver mines also in laboratories [47]. The resistance of bacterium to silver ions is attributed to presence of resistant inclusion by body that brings the active outflow of silver ions from the cell, impounding of silver ions in periplasm, conversion of silver ions to mixture type and reduction of the outer membrane porins that brings regarding the intake of silver ions [48].

The influence of size on antimicrobial activity was also investigated by Baker *et al.* In this study, the antibacterial properties were related to the total surface area of the nanoparticles. Smaller particles with larger surface to volume ratios have greater antibacterial activity.

The synthesis of silver nanocrystals encapsulated in mesoporous silicon dioxide nanoparticles with a yolk/shell structure has been represented, and demonstrate their antimicrobial impact. An entire inhibition of microorganism growth was reached with one hundred mg/mL of the particles. These silver nanoparticles are also used as a promising vehicles for this antibacterial treatment.

The mechanism of the antimicrobial action of silver ions is closely associated with their interaction with thiol (sulfhydryl) groups [49-51] though different target sites which stay as an opening. Amino acids, and different compounds containing thiol teams, with atomic number 11 bisulfate, and sodium thiosulphate, were all unable to neutralize the activity of silver ions. Different findings including these point that the interface of silver ions with thiol teams in enzymes and proteins plays a necessary role in antimicrobial action, though different cellular elements, like element bonding, can also be concerned [50]. Silver was additionally planned to act as key binding material to enzymes site. Silver ions cause the discharge of K+ ions from bacteria; so, the microorganism plasma or cytoplasmic membrane that is related to several vital biological activities of bacteria [50, 53, 54].

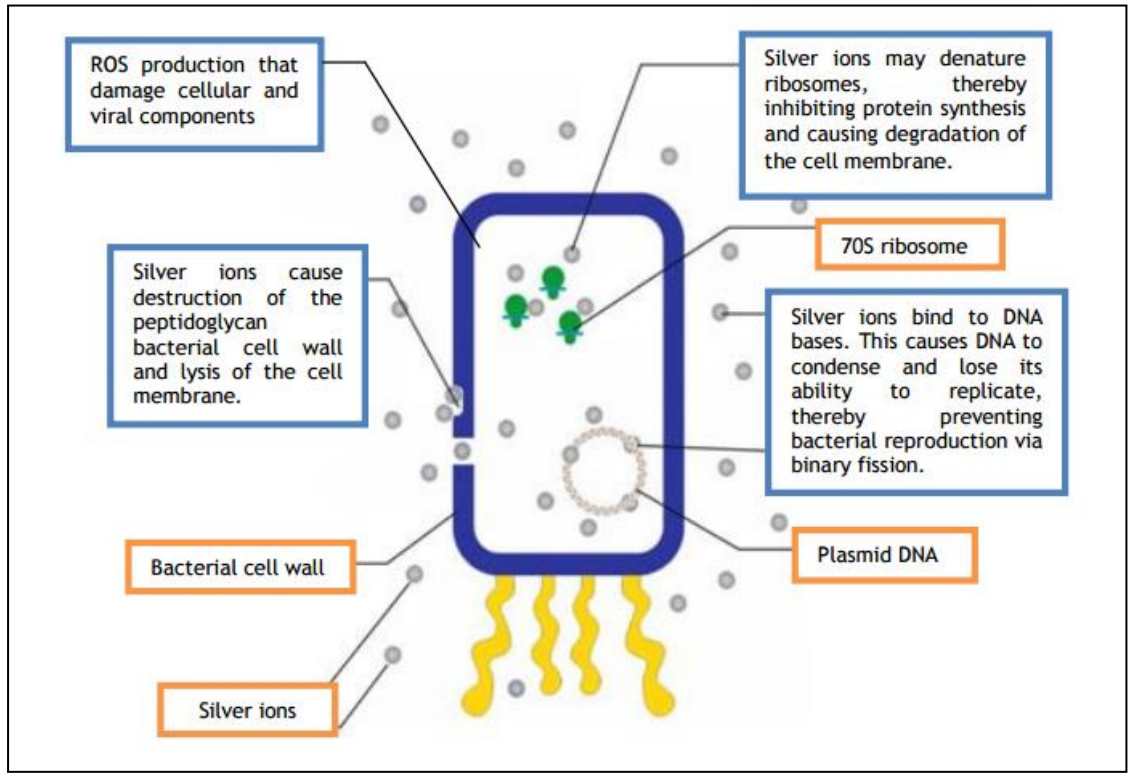

Figure1.8: Different mechanisms of action of AgNPs against bacteria. In general, these mechanisms include: photocatalytic production of ROS that damage cellular and viral components, compromising the bacterial cell wall/membrane, interruption of energy transduction, and inhibition of enzyme activity and DNA synthesis. Adapted from (Chaloupka et al. 2010; Huh et al. 2011).

1.5 Silver nanoparticle Composite with other materials

By explanation, nanocomposites are materials that comprise of dominions or inclusions which are of nanometer size scale [55]. Presently, nanocomposite materials that use the characteristics of silver at the nanoscale for biomedical uses are of growing interest (Figure 1.9), and might find its use in a variety of commercial products, including wound dressings and antiseptic creams.

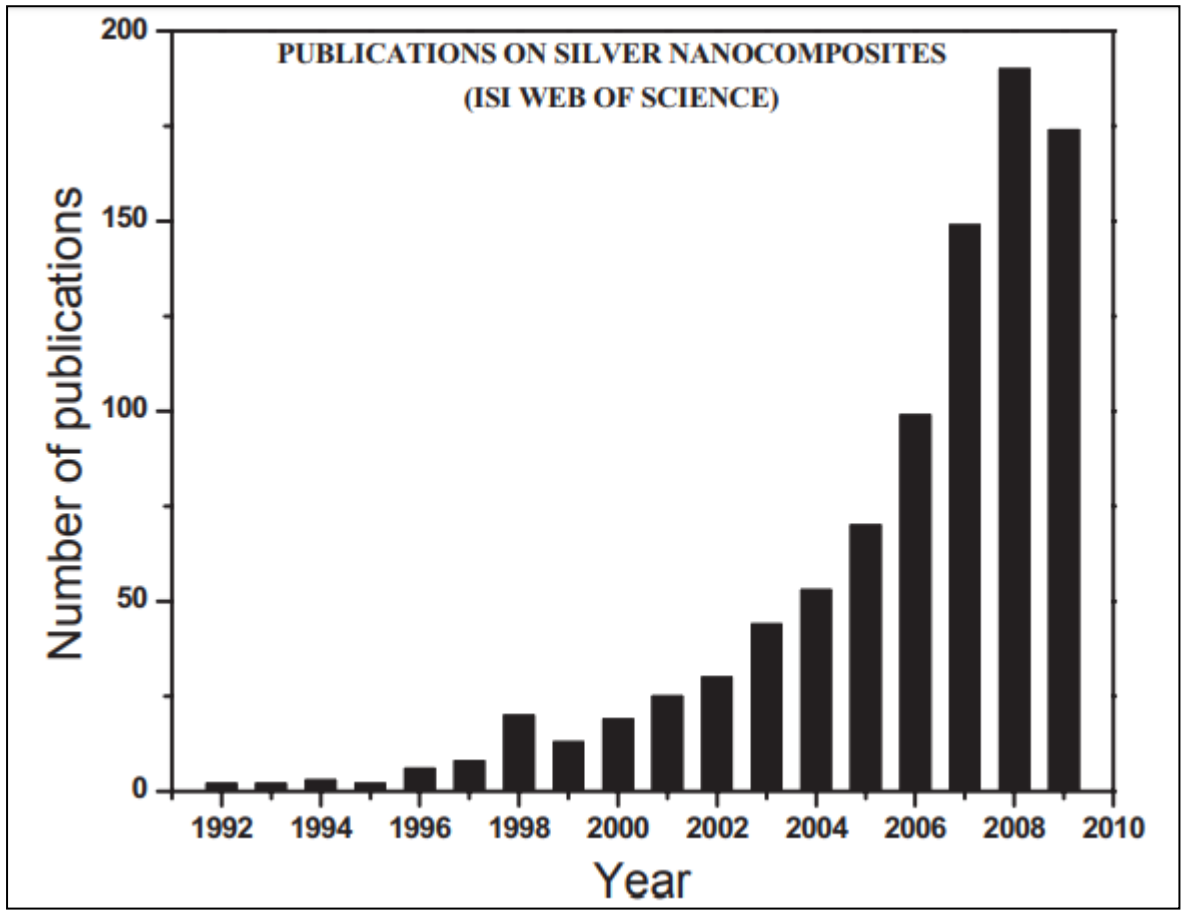

Figure 1.9 : Publication counts derived from the Thompson ISI Web of Science database on October 2009, using the key word "silver nanocomposites "

The idea behind these novel materials is that silver, at the nano level scale, displays distinctive properties which will be used for various functions, starting from antimicrobial to optical and chemical process applications.

Since silver nanocomposite constructs are meant to exploit the properties of silver at the nanoscale, much effort has been targeted at identifying efficient and reproducible routes for the production of silver nanoparticle s (AgNP s). Today, many potential uses for these novel materials are under investigation, ranging from antimicrobial therapies to molecular imaging. When preparing homogeneous nanocomposite materials containing AgNPs, one crucial issue is the tendency for nanoparticles to aggregate, which leads to loss of the specific properties associated with the nanoscale. For instance, in the area of antimicrobials, studies conducted by Lok et al. [56] revealed that non-stabilized AgNPs prepared via standard chemical methods (the reduction of silver salt solutions) tend to aggregate in culture media and in biological

buffers with a high salt content (chloride and phosphate are the most problematic anions). Such aggregation will lead to a reduction in the effective surface of the nanoparticles, or the degree to which they can associate to the bacteria.

To date, the preparation and stabilization of metal nanoparticles represent an open challenge, in order to produce new methods to prepare non-aggregated AgNPs for biomedical applications. These new methods mainly follow two approaches [57]:

- Wet chemical synthesis in the presence of a reducing agent and a stabilizing agent.
- Synthesis through physical processes.

Protective polymers will coordinate metal particles before reduction forming a polymer-metal complex in advance, which may then be reduced to make zero-valent metal colloids [58]. This method permits the assembly of nanoparticles with a narrower size distribution, than those obtained while not protecting polymers [59]. Once the reduction happens, particles are hooked up to the abundant larger protective polymers that cowl or encapsulate the metal particles and stabilizes them to be utilized in medicine field [60].

The polymers and surfactants, generally stabilize AgNPs to solve the cytotoxic issues [61] and might also result in synergistic antibacterial drug agents with new, improved optical, electrical and chemical change properties, out of stock within the individual elements themselves [62]. Thus, the employment of the helpful agents so as to avoid aggregation, conjointly offer a protecting surface barrier between the metal core and cells, that is particularly vital for preventing harm to the encompassing healthy tissues [63]. Moreover, it absolutely was incontestable that the incorporation of AgNPs into polymers produces protecting surface barriers that don't have an effect on the antibacterial drug properties of the nanoparticles and will increase them, as mentioned before. Of these blessings are wide utilized in an exceedingly huge variety of engineering and technical areas, particularly in medical field, to provide medicine devices with specific properties [64, 65].

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CHAPTER 2:

LITERATURE REVIEW

2.1 Natural Products and Hops

2.2 Synthesis and Characterization of Silver nanoparticle

2.3 Polymer/Silver Nanocomposites

2.1 Natural Products and Hops

The indispensable role of natural merchandise as a supply of medication in treatment of varied diseases can not be over-ruled because of its lesser facet effects and natural origin. Sources of natural product is each terrestrial and aquatic that embody plants and microorganisms. Therefore, Natural merchandise are utilized in fashionable medicines either alone or together with artificial medication [1-5]. The importance of natural merchandise to be utilized in medicines is as a result of their diversity in structures. several diseases are cured or prevented by them and their frequent usage [4,7]. Likewise, long artificial routes, high prices and low yield of artificial merchandise any increased the importance of natural products. additionally as they need synthesized in living systems, natural merchandise area unit more biological friendly than artificial ones [8]. Biological friendliness makes them ideal candidates for drug development [9]. medication introduced in market from last twenty five years present the good contribution of natural merchandise [10,11] suggesting that natural merchandise act as an honest beginning material for brand new medication development.

Medicinal and musky plants are reservoirs of curative parts employed by an outsized population within the treatment of varied diseases like protozoal infection, diabetes, mental disorders, cardiovascular disease, Human immunological disorder virus/Acquired immunological disorder syndrome and cancer. These healthful and aromatic plants are used on the idea of ethnobotanical evidences for being safer, acceptable, affordable, culturally compatible and appropriate for chronic treatment. Phytochemical screening of the plants unconcealed that they contain bioactive substances like Alkaloids, Flavonoids, Saponins with therapeutic potential [12].

The chemical constituents of healthful and aromatic plants may be found either within the roots, leaves, stems, flowers or bark which may be separated mistreatment associate applicable extraction technique. Plant choice for brand new drug discovery may be performed through phytochemical analysis, conductivity of bioassays on experimental model, ethnobotanical proofs and biological activities reported within the plant. Besides, the advantages obtained from plants, a number of them have few undesirable facet effects and complications which can flow from to overdosing and connected factors. this could typically lead to acute toxicities however once these issues square measure cautiously self-addressed, it'll assist to harness the therapeutic effectiveness of healthful and aromatic plants for future new drug development [13]

The value of hops within the beer-making method has been undisputed for centuries; the plant's medicative uses, on the opposite hand, area unit less wide acknowledged and have several aspects. The different aspects of Hops are described below:

2.1.1 Taxonomic classification

Kingdom : Plantae (Plants)

Subkingdom : Tracheobionta (Vascular plants)

Superdivision : Spermatophyta(Seed plants)

Division : Magnoliophyta (Flowering plants)

Class : Magnoliopsida (Dicotyledons)

Subclass : Hamamelididae

Order : Urticales

Family :Cannabaceae (Hemp family)

Genus : Humulus L. (hop P)

Species : Humulus lupulus L. (common hop P)

2.1.2 Brief on HOPS

Hop or hops (Humulus lupulus) may be a rise vascular plant happiness to the dicot genus within the family Cannabaceae, order Urticacales. Older taxonomists enclosed the genera genus Humulus within the family Moraceae (Moraceae).

The genus name genus Humulus has its origin within the Slavic term for hops, chmele, that was later Latinized. The species name lupulus may be a diminutive derived from lupus, the Latin word for wolf, supported the plant's habit of rise on different plants as a wolf will a sheep[14] Its common name comes from the Anglo-Saxon hoppan (to climb)[15]

Hops may be a dioecian perennial plant native to the hemisphere. It grows smartly from the top of Gregorian calendar month to the start of Gregorian calendar month within the temperate climate zone. It's found in shrubbery and at the sting of forests with access to ample water, and it reaches a height of up to 7–8 m (23–26 feet). Thus, below smart conditions, the expansion rate per day of the aerial elements will reach thirty cm (1 foot). The entire space coated by the leaves will reach twenty sq. meters (215 sq. feet), and therefore the total length of the roots will reach a hundred m (328 feet) in one season.

When the plant reaches a precise height, it starts to blossom. A second trigger for the arrival of florescence is that the length of the day. Too way south the times area unit too short for hops to blossom; too way north the climate is adverse. Hence, hops grows solely at sure latitudes (38º to 51º latitude). This explains why hops cultivation in North America is primarily wiped out areas like OR and Washington. Though Northern Golden State includes a history of hops cultivation (e.g., Hopland, CA), nowadays the three vital regions area unit American state with 100 percent, OR with V-J Day, and Washington with seventy fifth of the annual harvest. The National Hop Report, free in Gregorian calendar month 2009, found that production in those regions enhanced from sixty million pounds in 2007 to ninety five million pounds in 2009.[16]

In Europe, hops is cultivated in FRG, nice United Kingdom of Great Britain and Northern Ireland, Poland, and therefore the Czech Republic. In Asia, cultivation takes place in sure areas of China and to a restricted extent in Japan. For the hemisphere, cultivation of hops happens in Australia from 37º to 43º and in New Sjaelland from 41º to 42º latitude.

Only the feminine hop flowers area unit cultivated so as to forestall the ripening of fruits (nuts), that area unit significant and of no industrial worth. In breeding programs, male plants area unit essential.

Many feminine flowers type Associate in Nursing inflorescence, referred to as strobiles, that contains membranous stipules and bracts that area unit hooked up to a zigzag, hairy axis. Every little branch of the axis bears a husk, diagrammatical solely by its combine of stipules that subtends either four or six bracts, every enclosure a flower or fruit. The stipules and bracts check each other closely however are

literally various shining glands. Once separated, these represent the drug lupulin[17] The bracts and stipules of the hop contain polyphenols; the odor and style of the drug is due principally to the terribly advanced secretion contained within the lupulin glands.

The ideal time for harvest is August to Sep within the hemisphere, whereas it's Feb south of the equator. The complete aerial a part of the plant is cut and transported to a process unit, that separates the inflorescences from the remainder of the aerial elements. The roots and stalk stay within the ground, wherever they'll begin another vigorous season following year. The rootstock itself will reach Associate in Nursing age of fifty years.

After harvest, the inflorescences area unit dried forthwith to a water content of concerning 100 percent for stability reasons. Also, looking on the environmental conditions, hops is unbroken below constant refrigeration throughout some or all steps from harvest to final product. The bitter principles area unit renowned to interrupt down chop-chop throughout storage and, unless cold, their concentration decreases by fifty to seventieth in precisely six months[18] One study has shown that once nine months of storage, hops preserved solely around V-J Day of its original activity[19] The alpha and beta acids area unit sensitive to chemical element. The alpha acids endure the foremost intense degradation right once harvest, leveling off throughout storage. A rise of 10°C doubles the loss, however different factors just like the hops selection and even the environmental conditions throughout the season have an impression on alpha acid levels and its decrease throughout storage.[20]

Besides the restricted stability of the dried inflorescences, they're non-homogeneous and have a coffee bulk density. Consequently, nowadays solely five-hitter of the entire annual harvest is employed while not more process (raw). Concerning hour of the inflorescences area unit reborn into pellets. The hop flowers area unit cut, milled, homogenized, and ironed into granules. The granules area unit hold on and shipped in an exceedingly technique that protects them from air and lightweight, increasing their stability considerably. Such granulation conjointly will increase the density of the hops by an element of up to ten times, rising prices of transport.

Twenty-five percent of the harvested hop strobiles area unit extracted with plant product or critical greenhouse emission to get as several alphaacids as attainable. Since plant product and greenhouse emission area unit present throughout the production method, the employment of those solvents is of no concern.

When production isn't done consistent with the German purity law for brewage, the addition of changed hops is feasible at the top of the method. Throughout the brewage production method, compounds of hops endure transition. The substances are known, and it's renowned that these isomers is shaped by chemical action modification outside of the still. Concerning 100 percent of the annual hops reproductive structure harvest is employed for this purpose nowadays.

From the huge biomass production, the inflorescences (strobiles) area unit the sole a part of the hops plant that's used. Aside from some use of young shoots, ingested in salads, there's no human use for the stems, leaves, rhizomes, and roots. The above-ground (aerial) elements area unit composted and used for fertilization of the fields. As declared antecedently, the under-ground elements (roots, rhizomes) stay within the ground.

2.1.3 Historic Uses

Unlike alternative well-established healthful plants like flower (Valeriana officinalis, Valerianaceae), hops doesn't have a two, 1000-plus-year history of ancient healthful use at intervals European flavorer drugs. The historic use of hops is fascinating as its technical properties—as flavor and for the preservation of beer—were discovered within the middle ages, however reports of its healthful use from that point weren't terribly encouraging. Hildegard von Bingen, the noted German mother superior, herbalist, and author (1098–1179), wrote in Physica, a text on observations of nature and creatures and their virtues, that hops has very little use for humans, noting that it "increases melancholy in men." However, she notes that "its bitterness fends off decomposition of beverages and will increase period of time."[21]

This undisputed advantage of hops in production in all probability resulted in additional widespread recognition and distribution, that successively could have inflated the eye on hops for added (e.g., medicinal) uses. In keeping with Wiesner (1883), alleged herb-beers were created within the years 1300–1500, with varied herbs added for healthful functions[22] essentially, brewage was the bottom, that delivered the healthful properties of the added plants.

Paracelsus (1493–1541) used hops as an organic process aid, and Matthiolus (1501–1577) mentioned its drug and bile-increasing effects. These authors didn't specify plant elements used. Lager (1498–1554) and Lonicerus (1528–1586) praised the employment of the young hops shoots for improvement the blood, liver, and spleen.[14,22]

The use of hops flowers was represented by Hecker in 1814, United Nations agency mentioned its sturdy tonic options as a bitter (Amarum) and noted its calming properties (without having the results of a robust sleep aid). Clarus (1864) used the feminine flowers to treat eating disorder thanks to inflammation and temporary state. Alternative authors of that point, together with Osiander (1824), businessman and Churchill (1834), and Maton (1860), according on the sleep-promoting properties of the feminine inflorescences. [14]

One of the foremost outstanding patients treated with hops was King of England, King of the uk (1738–1820), United Nations agency was purportedly bedded on pillows stuffed with hops to calm him.11 The doctor Kahnt (1905), in his book on therapy, suggested the employment of hop pillows,* teas, or extracts for sleeping issues related to nervous disturbances.[23] it absolutely was believed that hops acted through its sturdy and serious odor, inflicting sleepiness.[24]

The supplement to the Edinburgh New Dispensatory (1829) states the observation that inhabitants from London were less subject to bladder stones since that they had been aware of adding hops to their brewage.[25].

In North yankee ancient medicines, the Cherokee used hops as a sedative, anti-rheumatic, analgesic, gynaecological aid for breast and female internal reproductive organ issues, and excretory organ and urinary aid for "gravel" and inflamed kidneys[26] The Delaware used hops against ache and ache. The Navajo used it for coughs and cold, and also the Dakota for wound healing and against gi disturbances.[28] For relaxation and as a sleep aid, it absolutely was utilized by the Delaware and also the Fox.[28]

In Indian-Ayurvedic drugs, hops has been suggested for restlessness related to nervous tension, headache, and indigestion;[29] its actions are according as sedative, hypnotic, and medication.[30]

According to a list of healthful plants employed in completely different countries, commissioned by the globe Health Organization (WHO) in 1978, the employment of hops was conjointly established in Asia in China, Japan, and Korea, additionally to the before-mentioned India [31].

2.1.4 Hops composition

Table 2.1 : Hops composition

Principle Components	**Concentration (%w/w)**
Cellulose + lignin	40.0 - 50.0
Protein	15.0
Alpha acids	2.0 - 17.0
Beta acids	2.0 - 10.0
Water	8.0 - 12.0
Minerals	8.0
Polyphenols and tannins	3.0 - 6.0
Lipids and fatty acids	1.0 - 5.0
Hop oil	0.5 - 3.0
Monosaccharides	2.0
Pectin	2.0

2.1.5 Potential Medicinal Uses of Hops

Considerable work has been done investigation the antibiotic, antiseptic, and tuberculocidal properties of hops and its constituents. Hop bitter acids have evidenced to be particularly effective against gram-positive microorganism. They work best at low pH in not unrelated type.[32-26] compared to phenol, alpha-acids (humulone) and beta-acids (lupulone) area unit regarding two hundred times and 700 times stronger, severally. Against gram-negative microorganism, identical substances area unit while not goodish result, and yeast and molds area unit minimally suppressed [24].

Lupulone was tested as a treatment for T.B., because it has the best in vitro result against mycobacteria of all the hops constituents[33] Enders (1950) reduced tubercular infection in mice treated with lupulone[However, Chin (1949) failed to see this result in his investigations.[34] once given to men, preliminary results were promising, though the treatment was related to GI disturbances[33], in a very run, treatment was impaired by facet effects of the only substance given in high doses.[35,36]

Statistics in European nation from the primary 1/2 the last century showed that mortality from T.B. in distillery employees was half-hour of the typical.[37,38] In province, within the same amount, the incidence rate of T.B. was four times lower in distillery employees than in alternative professions. However, the result might are attributable to general higher health of those employees.[38]. With the arrival of potent medicinal drug substances within the half of the last century, analysis about hops constituents for T.B. failed to progress additional.

The main prenylflavonoid in hops, xanthohumol, features a high scavenging capability against peroxyl radicals, that area unit among the foremost unremarkably reactive chemical element species within the body. Exploitation each hydrophilic and lipotropic chemical element radical absorbance capability tests, xanthohumol is stronger than vitamin C and tocopherol.[39] attributable to its robust inhibitor activity, variety of potential health edges area unit attributed to the substance. Principally in in vitro tests it's shown antiproliferative[40] anticarcinogenic,[41] antigenotoxic,[42] medicament effects,[43] and

reduce of plasma aldohexose, macromolecule levels, and weight of white animal tissue in diabetic mice[45]. Recent analysis has begun testing xanthohumol against sure viruses86 and also the protozoal infection protozoa (Plasmodium falciparum)[46,47].Special enriched xanthohumol extracts are developed for health edges in xanthohumol-enriched beers[48].

Hop derived compounds[49] have conjointly shown potential edges in treatment of polygenic disease[50-52].Following the invention that isohumulones cut back hypoglycaemic agent resistance, a double-blind, placebo-controlled pilot study showed that isohumulones (isomerized hop extract purchased from English Hop merchandise Co. Ltd, Kent, UK) considerably bated glucose and Hb A1c levels once eight weeks. Twenty volunteers with gentle kind a pair of polygenic disease were enclosed during this study. 10 men and ten ladies (ages 45-65 years) were randomised, receiving either placebo or a capsule containing one hundred mg of isohumulones doubly on a daily basis for twelve weeks. Results once eight weeks showed a big decrease for glucose, HbA1c, pulse vital sign, GPT, GOT and gamma-GPT versus baseline. Within the placebo cluster, solely glucose levels improved versus baseline [53].

In a follow-up study with ninety four subjects, intake of isohumulones (isomerized hop extract obtained from Botanix restricted, Kent, UK) had helpful effects in polygenic disease and fleshiness. The volunteers with prediabetes received either placebo, 16 mg, 32 mg, or forty eight mg of isohumulones for twelve weeks. Once treatment, abstinence glucose was bated within the thirty two mg and forty eight mg teams once four weeks however failed to modification within the placebo cluster. HbA1c was conjointly considerably bated once four weeks within the sixteen mg cluster and once eight weeks within the thirty two mg and forty eight mg teams. Body mass index was considerably bated within the forty eight mg cluster compared with the placebo cluster at twelve weeks. The decrease in total fat space was conjointly considerably larger within the forty eight mg cluster than within the placebo cluster at twelve weeks [54].

Other recent developments embrace analysis on changed hops extracts that have undergone conversion and chemical change. These alleged letter iso-alpha acids have medicament potential. in a very screening of natural merchandise, hop-derived substances known as MgRIAA obtained from Metagenics (Gig Harbor, WA), business hop materials from Betatech Hops merchandise (Washington DC), associate degree experimental extract from BetaTech, and META060 equipped by Hopsteiner (New royal line, NY) were found to be among the foremost active in terms of medicament potential [55,56]. These letter iso-alpha acids ameliorated joint injury as proven by vital reduction of the inflammatory disease index and microscopic anatomy score in a very murine model of collagen-induced inflammatory disease [57]. Bone and animal tissue degradation were reduced by META060[58]. In addition, clinical analysis on a proprietary mix of letter iso-alpha acids, rosemary, and oleanolic acid (Kaprex®, Metagenics Iraqi National Congress.) incontestable vital relief for folks diagnosed with degenerative arthritis, atrophic arthritis, or fibromalgia [59]. And letter iso-alpha acids from hops and proanthocyanidins from tree nilotica (Fabaceae), each equipped by Metagenics, Inc., are shown to modulate hypoglycaemic agent signal in vitro. in a very 12-week, double-blind, placebo controlled trial, completed by ninety one people, effects on body fluid aldohexose, insulin, and lipids were investigated with the mixture of the two ingredients. Daily supplementation with three hundred mg letter iso-alpha acids and 1500 mg proanthocyanidins, additionally to life-style modification as well as dietary alteration, reduced body fluid acylglycerol, triglyceride:HDL magnitude relation, and abstinence hypoglycaemic agent considerably quite diet and life-style modification alone in patients with options of metabolic syndrome [60].

2.2 Syntheses of Silver Nanoparticles

2.2.1 Physical Approach

In physical processes, metal nanoparticles area unit typically synthesized by evaporation condensation, that might be meted out employing a tube chamber at air pressure. The supply material at intervals a ship focused at the chamber is volatilized into a carrier gas. Nanoparticles of varied materials, such as Ag, Au, PbS and atomic number 6, have antecedently been made mistreatment the evaporation/condensation technique [61-63].However, the generation of AgNPs employing a tube chamber has many drawbacks, as a result of a tube chamber occupies an outsized house, consumes an excellent deal of energy whereas raising the environmental temperature round the supply material, and needs plenty of your time to realize thermal stability. A typical tube chamber needs power consumption of over many kilowatts and a preheating time of many tens of minutes to realize a stable operative temperature. moreover, silver nanoparticles are synthesized with optical maser ablation of aluminous bulk materials in answer [64-66]. One advantage of optical maser ablation compared to alternative standard methodology for making ready metal colloids is that the absence of chemical reagents in solutions. Therefore, pure colloids, which can be helpful for any applications, may be made by this methodology [67].

In summary, the physical synthesis of AgNPs typically utilizes the physical energies to supply AgNPs with nearly slender size distribution. The physical approach will allow manufacturing giant quantities of AgNPs samples during a single method. this can be conjointly the foremost helpful methodology to supply AgNPs powder. However, primary prices for investment of kit ought to be thought-about.

2.2.2. Photochemical Approach

The photo-induced artificial methods have conjointly been developed. As an example, Huang and principle synthesized AgNPs via photo reduction of AgNO3 in superimposed inorganic clay suspensions, that is stabilizing agent that stop nanoparticles from aggregation. Irradiation disintegrated the AgNPs into smaller size with one mode distribution till a comparatively stable size and diameter distribution were achieved [68]. However, during this methodology, the equipment with high value and experimental atmosphere area unit needed.

2.2.3. Biological Approach

Recently, synthesis strategies mistreatment naturally reducing agents like polysaccharides, biological being like bacterium and plant life or plants extract, i.e. inexperienced chemistry, have emerged as a straightforward and viable different to additional complicated chemical artificial procedures to get AgNPs. Bacterium are unit familiar to supply inorganic materials either intra- or extracellularly. This makes them potential bio factories for the synthesis of nanoparticles like gold and silver. Significantly, silver is acknowledge for its biotical properties. A. R. Vilchis-Nestor et al. used tea leaf (Camellia sinensis) extract as reducing and stabilizing agent to supply gold silver nanoparticles in solution at close conditions [69]. Moreover, K. Kalishwaralal et al. reportable the synthesis of AgNPs by reduction of liquid Ag+ ions with the culture supernatant of Bacilli licheniformis[70]. The synthesized AgNPs area unit extremely stable and this methodology has benefits over alternative strategies because the organism used here may be a nonpathogenic bacteria. The biological methodology provides a large vary of resources for the synthesis of AgNPs, Associate in Nursingd this methodology may be thought-about as a technique of nanoparticles synthesis with benefits over standard chemical routes of synthesis and as

an environmentally friendly approach furthermore as a coffee value technique. However, it's demanding to get an outsized amount of AgNPs by mistreatment biological synthesis.

2.2.4. Chemical Approach

Besides those approaches represented on top of, chemical reduction is that the commonest methodology owing to its convenience and easy instrumentality. Management over the expansion of metal nanoparticles is needed to get nanoparticles of little size with a spherical form and slender distribution in diameter. It's acknowledge that silver nanoparticles may be made by chemical process at low value and in high yield. During this review we have a tendency to describe sevorious chemical synthesis strategies to organize the silver nanoparticles principally. Generally, the chemical synthesis method of AgNPs in answer typically employs the subsequent 3 main components: (1) metal precursors, (2) reducing agents and (3) stabilizing /capping agents. The formation of mixture solutions from the reduction of silver salts involves 2 stages of nucleation and ensuant growth. It's conjointly unconcealed that the scale and also the form of synthesized AgNPs area unit powerfully obsessed on these stages. Moreover, for the synthesis of monodispersed AgNPs with uniform size distribution, all nuclei area unit needed to make at an equivalent time. During this case, all the nuclei area unit seemingly to possess an equivalent or similar size, so they'll have an equivalent growth. The initial nucleation and also the ensuant growth of initial nuclei may be controlled by adjusting the reaction parameters like reaction temperature, pH, precursors, reduction agents (i.e. NaBH4, antifreeze, glucose) and stabilizing agents (i.e. PVA, PVP, metallic element oleate) [71-73]

2.2.5 Characterization of AgNPs and Their Properties

Characterization of AgNPs is vital to grasp and management nanoparticles synthesis and applications. numerous techniques area unit used for determination of various parameters. The morphology of AgNPs is obtained mistreatment transmission and scanning microscopy (TEM, SEM). the scale distribution of AgNPs may be measured with a Zetasizer Nano Series instrument. Energy dispersive X-ray spectroscopic Associate used (EDS) measurements area unit used with an emission scanning microscope equipped with an EDS instrument. X-ray electron spectrum analysis (XPS), X-ray diffractometry (XRD), Fourier remodel infrared spectrum analysis (FTIR), and UV-Vis spectrum analysis are wont to characterize AgNPs. UV-Vis spectrum analysis is employed to verify AgNPs formation by showing the Plasmon resonance. Moreover, XRD is employed for the determination of crystallinity. The amount electrical phenomenon is measured with Loresta-GP MCP-T610 electrical phenomenon meter to gauge electrical conduction. As an example, in our recent studies, from the results of UVVis activity for the synthesized AgNPs, optical absorption peak of AgNPs attributable to surface Plasmon resonance is determined [74]. From EDS analysis, the extreme peak at around three keV is characteristic of AgNPs. No impurities area unit determined besides little amounts of carbon and atomic number 8, which indicates that the reagents used haven't remained. as a result of the chemicals utilized in this study area unit soluble, the ultimate product, that is insoluble in water, may be separated simply from the reaction mixture [75].XPS analysis is meted out to see the fundamental composition of AgNPs. The binding energies of silver (3d3/2) and silver (3d5/2) for AgNPs area unit determined at 374 heat unit and 368 heat unit, severally. Since the reportable energy of Ag0 (3d5/2) is 368.3 eV [76] these peaks prove the formation of AgNPs.

2.2.6 Surface Plasmon Resonance

Michael Faraday was the primary to indicate that metal particles repeatedly smaller than the wavelength lightweight|of sunshine} scatter and absorb light powerfully such even the dilute answer of the particles exhibit bright colours [77] The conducting electrons at intervals the metal particles together oscillate and therefore change scattering and absorption of sunshine at that specific frequency, giving the colour to the answer just in case of gold and silver nanoparticles. Surface Plasmon Resonance (SPR) may be a development within which there's a collective oscillation of electrons on the surface of a metal. per Wiley et al, the SPR peak changes per the scale and form of the silver nanocrystal .The characteristic SPR of silver nanoparticle is seen at wavelengths from 390nm-450nm betting on the scale of silver nanoparticle [78] The characteristic brownness color of silver nanoparticle is made owing to this SPR development.

2.3 Polymer/Silver Composites

Silver nanoparticles are the foremost used antimicrobial filler in chemical compound nanocomposites [79]. Particularly, in depth analysis has targeted on the event of gel composites wherever silver nanoparticles are in place synthesized as displayed in Figure 3a. In general, of these systems used silver-salt precursors, like nitrate, beside a chemical agent, in presence of the compound gel network acting as a nonreactor wherever the nanoparticles are shaped. As an example, swollen poly (acrylamide-co-acrylic acid) hydrogels is a medium for the formation of silver nanoparticles with sizes around 25–30 nm [80]. These nanocomposites incontestable glorious medicine activity looking on the nanoparticle size as this parameter changes the area that's in grips with the microorganism species. Superabsorbent hydrogel-silver nanocomposite supported poly(vinyl alcohol) and metal alginate has additionally been ready exploitation atom chemical change [81]. Extremely stable silver nanoparticles were in place synthesized within the gel networks by reduction of caustic with metal borohydride as a chemical agent. The silver nanocomposite gel showed excellent medicine activity on gram-positive and gram-negative microorganisms. Semi interpenetrating compound network (IPN) hydrogels, within which poly(vinyl pyrrolidone) chains were physically distributed throughout poly (acrylamide) gel networks, were synthesized and used as nanoreactors for manufacturing and stabilizing metal nanoparticles [82].Particularly, metal nanoparticles were entrapped throughout gel networks via polyvinylpyrrolidone chains with sizes around four nm. These semi-IPN hydrogel-silver nanocomposites bestowed medicine behaviors. Identical authors soon developed antimicrobial silver-based composites exploitation semi interpenetrating gel networks supported crosslinked poly(acrylamide) ready through a redox-solution chemical change within the presence of super molecule polymers [83]. During this case, 3 totally different polymers were studied: gum Arabic, carboxymethylcellulose, and starch. The strategy permits the synthesis of nanoparticles with sizes between five and twenty nm looking on the compound. All the nanocomposite hydrogels bestowed similar antimicrobial behavior. Gel networks supported N-isopropyl acrylamide and metal propenoate were additionally ready by redox-polymerization and accustomed synthesize extremely stable and uniformly distributed silver nanoparticles [84]. Almost like previous works, the gel network could be a carrier wherever the in place reduction of caustic within the presence of metal borohydride as a chemical agent occurred. These hybrid hydrogels with totally different sizes of silver nanoparticles is effectively utilized as medicine material.

The synthesis of silver nanoparticles also can be extended to non-hydrogel based mostly polymers. A straightforward technique supported the in place synthesis of silver nanoparticles by dissolving silver

one, 5-cyclooctadiene-hexafluoroacetylacetonate in amorphous vinylbenzene has been rumored [85]. The metal precursor will thermally decompose manufacturing silver atoms that diffuse into the compound and type lusters. These silver-doped polystyrenes give high medicine activity. An identical approach was accustomed add silver nanoparticles into polymer matrices manufacturing novel antimicrobial materials [86]. During this case, the nanoparticles were initial created in a very master batch by thermal reduction of silver acetate throughout soften process of the matrix. In a very second extrusion step, the master batch was diluted with pure olyamide. Notably, the antimicrobial behavior of those nanocomposites was compared with polyamide/silver micro composites. Polymer full of simply 0.06 wt % silver nanoparticles is in a position to eliminate the bacterium utterly at intervals twenty four h whereas micro composite containing 1.9 wt % maximize silver kills solely regarding eightieth of the bacterium throughout identical time. the speed of the silver particle unharness from the nanocomposites is regarding one order of magnitude higher as compared to the micro composites, owing to the abundant larger specific area of the nanoparticles [86]. Similar in place routes were any extended by these authors to polymer composites and compared with composites supported silver nanoparticles synthesized in invertible polyester before their incorporation within the matrix manufacturing smaller particles [87]. At a relentless weight proportion of silver within the compound, the composite with the ex situ silver nanoparticles exhibits a silver particle unharness that is regarding 2 orders of magnitude on top of the discharge from the composite with the in place silver nanoparticles [88]. This observation is explained by the upper specific area of the smaller particles and therefore the higher constant of diffusion for silver ions. The compound composite with an amount of solely 0.07 wt% maximize ex situ silver nanoparticles exhibits a high enough unharness of silver ions to realize antimicrobial properties.

There are different ways ready to manufacture antimicrobial polymer/silver nanocomposites. For example, natural Na alginate chemical compound acting as each reducing and helpful agent may also be wont to synthesis silver nanoparticles [89]. The nanoparticles capped with alginate displayed antimicrobial behavior and that they were additional integrated with varied amounts of chitosan forming electrolyte complexes that were casted into stable films. The integrated possess various medication activity. Silver nanoparticles may also be synthesized in water by reduction of silver salts within the presence of poly (acrylates) of various molecular weights [87]. These results clearly showed that the reduction technique and therefore the chemical compound chain length vie key roles within the accomplishment of few-nanometer-sized nanoparticles. The nanoparticle dispersions were then wont to functionalize cotton, wool, and polyester samples so as to get antimicrobial textiles for medical specialty applications. Addition of silver nanoparticles on chemical compound surfaces by plasma processes is another technique rumored for the assembly of antimicrobial materials [90,91]. Deposition of silver nanoparticles onto surface-functional porous poly(ethylene glycol dimethacrylate-co-acrylonitrile) microspheres has additionally been rumored with glorious biocide behaviors [92]. Antimicrobial coatings will additional be developed supported plasma polymerized polyacrylic acid (PPAA) deposited on a polythene terephthalate mesh [93]. This technique permits the denial of silver nanoparticle because the radical teams of PPAA will act because the anchor further as capping and helpful agents for silver nanoparticles synthesized by a discount technique [93]. different antimicrobial chemical compound coatings for a typical auriferous orthopedical substrate containing chitosan, Bioglass® microparticles, and silver nanoparticles, was recently invented employing a single-step natural action deposition (EPD) technique [94].These hydrogel coatings present swelling capabilities. Silver ion release was properly

tuned in order to assure antibacterial activity against the most common pathogens in implant infections while preserving osteoblasts response at the implant interface [94].

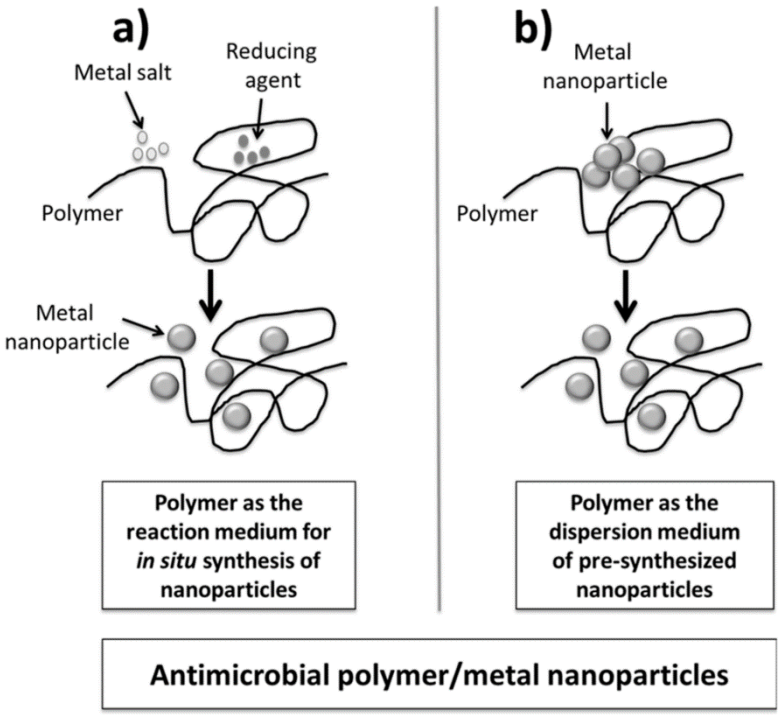

Figure 2.1: Two main routes producing antimicrobial polymer/metal nanocomposites: (a) Polymer as reaction mediun for in-situ synthesis of nanoparticles; and (b) Polymer as a dispersion mediun of pre-synthesized nanoparticles.

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CHAPTER 3:

OBJECTIVE & PROPOSED PLAN OF WORK

3.1 Objective

3.2 Proposed Plan

3.1 Objectives

Hops which was chosen as the natural product is one of the oldest herbs which are used in brewing industry for long time and it is also used in various medical aspects as well. Hops are rich in various phytochemicals and active compound [1] which contributes to its antioxidant, anticancer, anti-inflammatory property. Thus finding the efficacy of Hops extract as a potent antioxidant, anti-inflammatory and anticancer natural products is the primary objective and concern of this present work.

The Hops extract contains various phytochemical [2] which can be used to reduce silver to silver nanoparticle as well as it can used to cap the silver nanoparticle. Thus Hops finds its application in green synthesis of silver nanoparticle and finally it can be used as a potent antibacterial material. To explore the synergistic effect of Hops and Silver nanoparticle as a potent anti-inflammatory is also a major objective of this study.

Nanocomposite material and its biomedical application is one of the prime concerns of research around the globe [3]. Thus finding the application of nanocomposite prepared from PVA-Gelatin-silver nanoparticle through crosslinking by Glutaraldehyde, in the field of biomaterials; is a major objective of this study. Beside that exploring biocompatibility and antibacterial effect of the material will also be performed, so that it can used in wound healing for combating with bacterial infection.

Thus the major objective of the current study focuses on Hops extract, green synthesis of silver nanoparticle and finding its anti-bacterial effect and finally preparing a nanocomposite material for its application in the biomedical field.

3.2 Proposed Plan of Work

- Hops pellet collection.
- 40 % Ethanolic Extraction of Hops pellet.
- Phytochemical Screening of the Extract.
- Testing the efficacy of the extract as an anti-oxidant, anti-inflammatory and anticancer agent.
- Green Synthesis of Silver nanoparticle.
- Characterization of Silver Nanoparticle.
- Anti-bacterial and Anti –inflammatory effect of Silver nanoparticle.
- Synthesis of Silver Nanocomposite material.
- Characterization of the nanocomposite material.
- Evaluation of bio-compatibility and anti-bacterial effect of nanocomposite material.

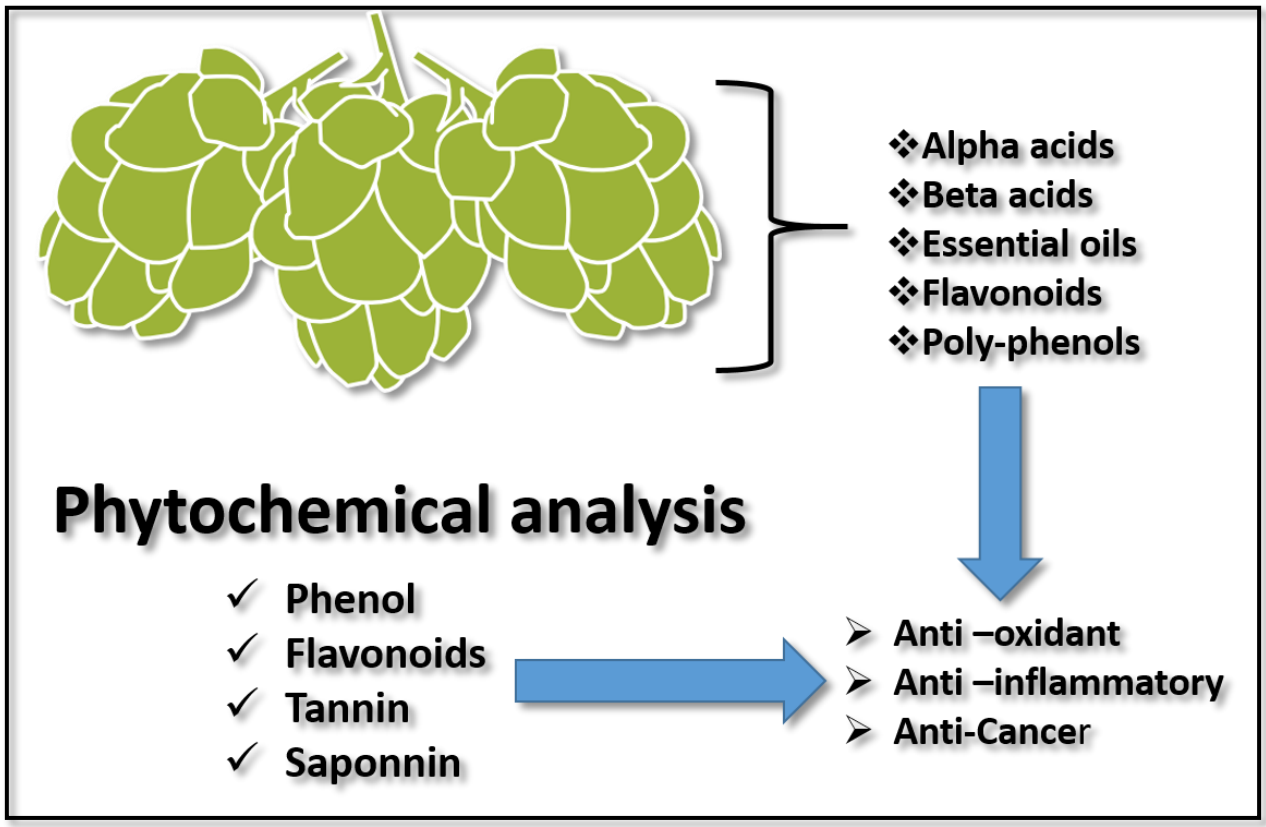

Figure 3.1 : Proposed work plan for Hops extarct and evaluation of biological efiicacy

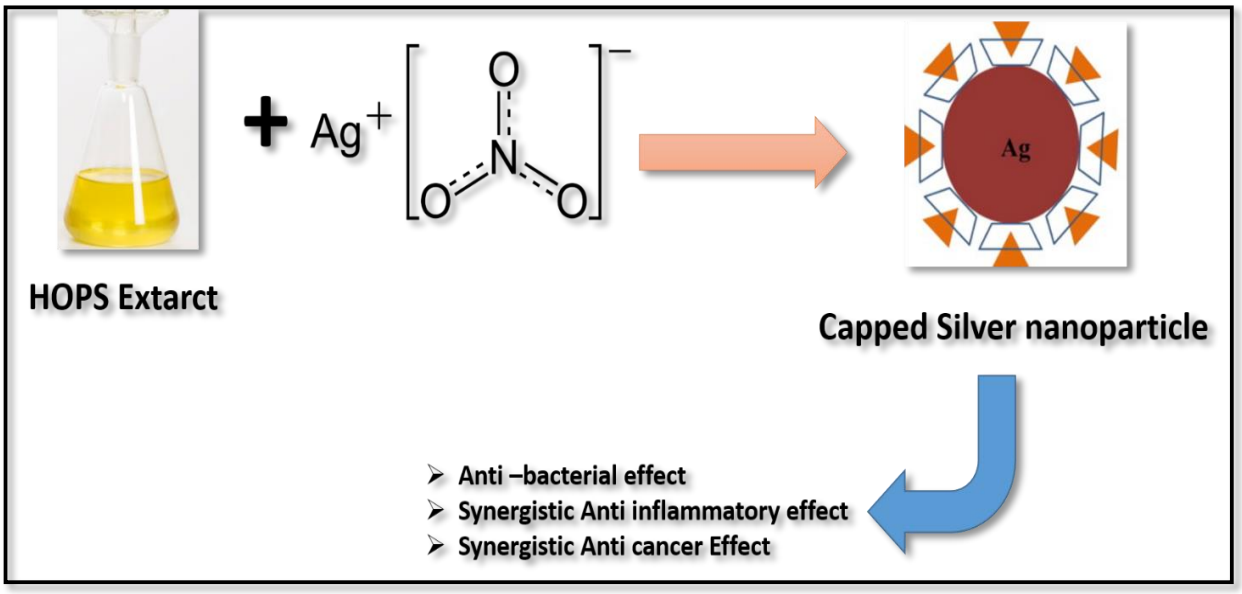

Figure 3.2 : Proposed work plan for green synthesis of silver nanoparticle

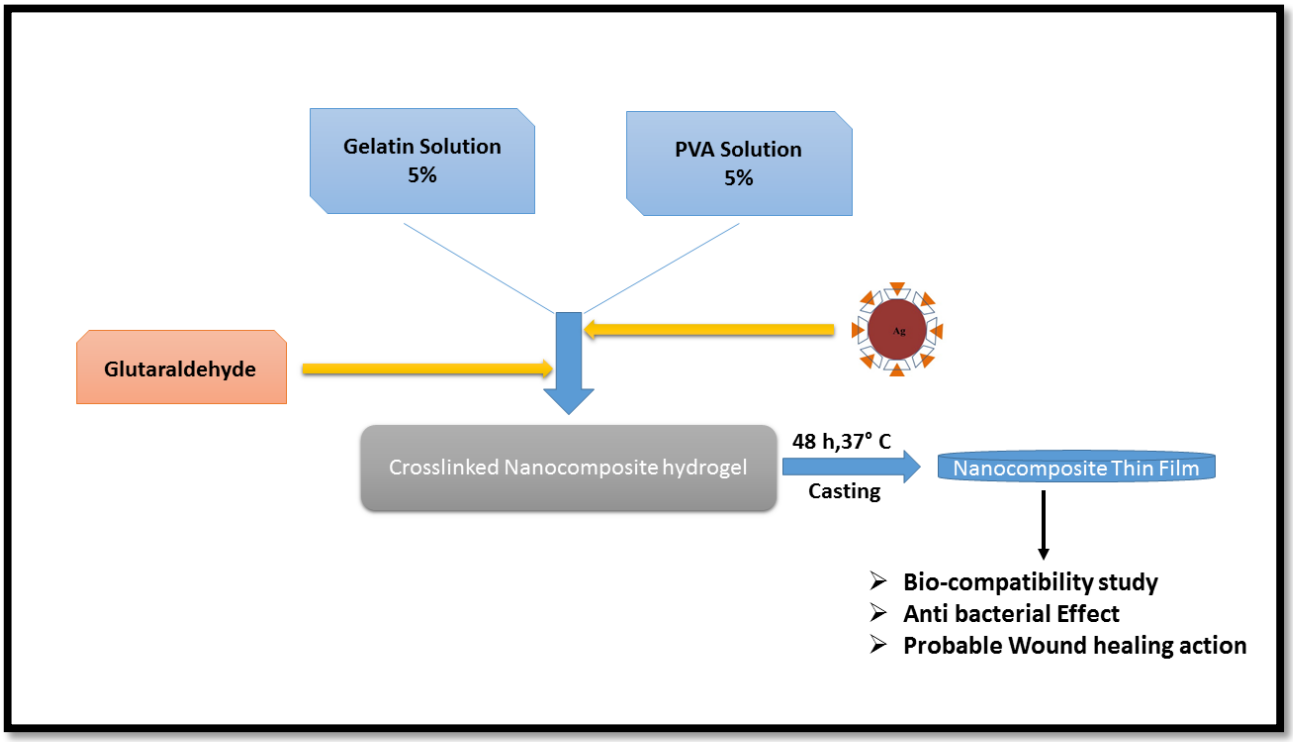

Figure 3.3 : Proposed work plan for Silver Nanocomposite Film.

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CHAPTER 4:

METHODOLOGIES

4.1 Hops Extract Preparation and finding its different Biological activity

4.2 Green Synthesis and Characterization of Silver Nanoparticle using Hops

4.3 Preparation and Characterization of Nanocomposite Films for biomedical use

4.1 Hops Extract Preparation and finding its different Biological activity

HALLERTAUER PERLE HOPS PELLET was obtained from BREWOF, leading suppliers of brewing agent in India. All chemical and solvents were procured from Merck. Standard drug Diclofenac sodium was procured from Novartis India Ltd.

4.1.1 Extract Preparation

100 mg portion of Hops HALLERTAUER Pallets were crushed into fine powder by a Mortar Pastel. It was then added to 100 mL (40% ethanol) solution and was stirred continuously for 1 h in a Magnetic stirrer and was kept at 4°C for 24 h for maceration. Finally the sample was filtered using a Buchner funnel to obtain the final extracted solution. The Mixture is then concentrated using a Rotary Evaporator to get rid of the solvent. The powder form of the extract which was obtained was used further for making drug dosage.

4.1.2 Phytochemical analysis

Phytochemical analysis for the presence of Phenols, Flavonoids, Tannin, Phlobatannins, Saponin, Alkaloids, Reducing sugars was done using standard protocols [1].

4.1.2.1 Qualitative Analysis of Phytochemicals

Phenols
In 2mL of the plant extract, a few drops of 5% ferric chloride solution was added. The appearance of a greenish blue, blue, green or violet colour, confirms the presence of phenol [2].

Flavonoids
1.5 mL of dilute ammonia was added to 1 mL aqueous filtrate of the plant extract. This was followed by addition of 0.5 mL concentrated Sulphuric acid (H_2SO_4). Yellow coloration in each extract showed the presence of flavonoids [2].

Tannins
A portion of the extract was taken and few drops of 10% ferric chloride solution was added to it. A blue or green color indicates the presence of tannins [3].

Phlobatannins
Plant extract was boiled with 1% aqueous HCL. Formation of red precipitate indicates the presence of phlobatannins [4].

Saponin
The extract was boiled. The mixture was then filtered and 5 mL of the filtrate was added to 10 mL of the distilled water in a test tube and the mixture was shaken well for about 30 seconds and observed for frothing [5].

Alkaloids
Plant sample (0.1 g) was added to 2 mL of hexane and was shaken well and filtered. This was followed by addition of 2 mL 2% HCL to the prepared extract. The solution was then heated

for some time and filtered. Next, a drop of picric acid was added to the filtrate to develop yellow precipitate indicating the presence of alkaloids [5,6].

Reducing Sugar

1mL of Fehling's solution A and B was taken in a test tube and was boiled and then poured in the aqueous ethanoic plant extract. Change in color reaction detected the presence of reducing sugars [7].

4.1.2.2 Quantitative Analysis of Phytochemicals

Total phenolic content

The total phenolic content of the extract was determined using Folin-Ciocalteu (FC) reagent [8]. 0.5 mL of the plant extract was mixed with 0.5 mL of FC reagent (2X diluted with distilled water) and the mixture was allowed to incubate for 5 min at 25°C and it was followed by addition of 2 mL of 20% Na_2CO_3. The mixture was further incubated at 25°C for 90 min and the absorbance was measured at 650 nm. The total phenolic content (µg /mL) was calculated using Gallic acid as reference.

Total flavonoid content

The total flavonoid content (µg/mL) was estimated using aluminum chloride ($AlCl_3$) method [9, 10]. 0.5 mL of the plant extract, 0.5 mL distilled water, and 0.3 mL of 5% $NaNO_2$ was mixed and incubated for 5 min at 25°C. Next, 0.3 mL of 10% $AlCl_3$ immediately 0.2mL of 1 M NaOH was then added to the reaction mixture and the absorbance was measured at 510 nm. Quercetin was used as a standard.

Total Tannin content

Total tannin contents (µg/mL) were estimated by the Folin -Ciocalteu method [11]. 0.1 mL of the sample extract was added to a test tube which contained 7.5 mL of distilled water and 0.5 mL of Folin-Ciocalteuphenol reagent, to this 1 mL of 35 % Na_2CO_3 solution was added and it was diluted to 10 mL with distilled water. The mixture was shaken well and kept at room temperature for 30 min. Finally, the absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The total phenolic content (µg /mL) was calculated using Gallic acid as a reference.

4.1.3 Antioxidant Activity

Total Antioxidant Activity Assay (TAC)

Total antioxidant activity was assessed by phosphomolybdenum assay [12]. 0.5 mL of extract was mixed with 5.0 mL reaction mixture (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM $(NH_4)_2MoO_4$). The final mixture was incubated for 90 mins at a temperature of 95° C in a water bath. After incubation, these tubes were cooled at room temperature for 20-30 min and the absorbance of the final solution was measured at 695 nm. TAC was expressed as the number of equivalents of ascorbic acid in µg.

Ferric Ion Reducing Antioxidant Power Assay (FRAP)

Ferric ions reducing power was assessed conferring to the technique of Oyaizu with few modification [13]. 0.5 mL of Hops *(Humulus lupulus)* extract of different concentration was mixed with 1.25 mL of phosphate buffer (0.2 M) and 1.25 mL of potassium ferricyanide (1%). Incubate the total mixture in an incubator at 50°C for 30 min. After the reaction the sample was cooled, to the cooled sampled 1.25 mL of (10%) trichloro acetic acid was added and mixed. Finally the mixture was centrifuged and the supernatant was mixed with equal volume of distilled water. To this mixture 0.5 mL of freshly prepared ferric chloride solution was mixed and the OD was taken at 700nm. Ascorbic acid was used as a standard antioxidant.

DPPH Radical Scavenging Assay:

The free radical scavenging activity (RSA) was performed using DPPH method [14]. Different concentration of the Hops *(Humulus lupulus)* extracts (10, 50, 100, 250, 500 µg/mL) were used. A volume of 2.5 mL of 0.04 % DPPH solution was mixed with 0.5 mL of all concentrations of extracts separately. After 30 min incubation at room temperature in dark, the absorbance was taken at 517 nm in triplicates for each concentration. Ascorbic acid was used as a standard antioxidant. Water was used as a control. The percent inhibition of free radical formation was calculated as follows:

$$RSA(\%) = \left(\frac{\{Abs\ Control - Abs\ Sample\}}{Abs\ Control}\right) \times 100$$

4.1.4 In-Vitro Anti-Inflammatory Activity

Inhibition of Albumin Denaturation Assay:

The assay was done by following the method of Muzushima and Kabayashi with certain modifications.0.05mL of plant extract of different dosage was added to 0.45 mL bovine serum albumin (1% aqueous solution) and the pH of the mixture was adjusted to 6.3 using a small amount of 1N hydrochloric acid. These samples were incubated at 37ºC for 20 min and then heated at 60ºC in a water bath for 30 min. After cooling the samples, the absorbance was measured spectrophotometrically at 660 nm. Diclofenac Sodium was taken as the standard drug. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows [15]:

$$Percentage\ Inhibition\ = \left\{\frac{Abs\ Control - Abs\ Sample}{Abs\ Control}\right\} \times 100$$

The Membrane Stabilization Method:

HRBC membrane stabilization method:

The anti-inflammatory effect of the extract was assessed by in vitro HRBC membrane stabilization method. Blood was collected from healthy volunteers at their own will. The blood

was mixed with equal proportion of Alsever solution (dextrose 2%, sodium citrate 0.8%, citric acid 0.05%, sodium chloride 0.42%, and distilled water 100 mL) and centrifuged and washed with iso-saline (0.90% NaCl). To 0.5 mL of HRBC suspension, same volume of test drug in five different concentrations 10, 50,100, 250 and 500 µg/mL, was added. To this mixture 1mL of phosphate buffer and 2 mL of hypo saline was added. All the assay mixtures were incubated at 37°C for 30 minutes and centrifuged. The hemoglobin present in the supernatant solution was estimated by using spectrophotometer at 560 nm [16]. The percentage of hemolysis was calculated then by the formula as given below:

$$Hemolysis\ (\%) = \left(\frac{Abs\ Sample}{Abs\ Control}\right) \times\ 100$$

Hence the percentage of protection can be determined from the below given equation:

$$Protection\ (\%) = \ 100 - \left[\left(\frac{Abs\ Sample}{AbsControl}\right) \times\ 100\right]$$

Here, the control (negative control) used was Alsever's solution with blood in it and it contained no Diclofenac Sodium or plant extract in it.

4.1.5 Isolation of PBMC Cells from Blood

PBMCs: Human peripheral blood mononuclear cells (PBMCs) are isolated from peripheral blood and identified as any blood cell with a round nucleus (i.e. lymphocytes, monocytes, natural killer cells (NK cells) or dendritic cells)[17]. The cell fraction conforming to red blood cells and granulocytes (neutrophils, basophils and eosinophils) is removed from whole blood by density gradient centrifugation. A gradient medium with a density of 1.077 g/mL separates whole blood into two fractions; PBMCs makes up the population of cells that remain in the low density fraction (upper fraction), whilst red blood cells and PMNs have a higher density and are found in the lower fraction (Figure. 4.1)

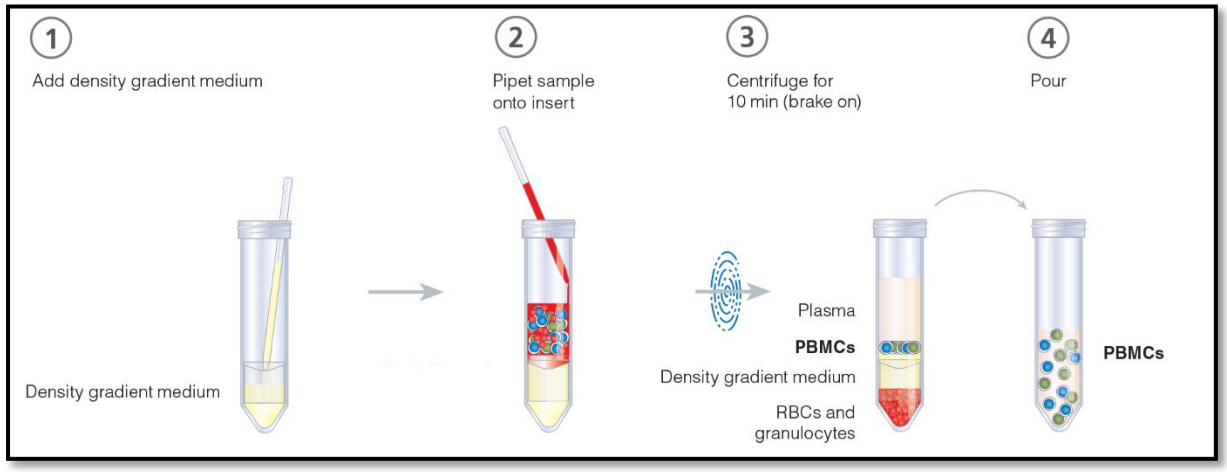

Figure 4.1: PBMC separation process

Separation of Peripheral Blood Mononuclear Lymphocyte cell:

1. Fresh blood (6mL) was taken in EDTA-coated vials and stored in room temperature and processed within a maximum of 2 h.

2. 0.9% saline (0.9g of NaCl in 100ml Millipore water) was prepared, autoclaved and cooled.

3. A 1:3 dilution of the whole blood was made with saline and care was taken so as to prevent the formation of any blood clots.

4. 5 ml of HiSep LSM 1077(Himedia) was taken in a 15mL centrifuge tube and 10 mL of diluted blood was layered over it avoiding any mix. Care was taken to ensure there is no mixing of the two layers and there is a sharp interphase between them.

5. Centrifugation was done at room temperature for 15 mins.

6. The blood gets separated into 4 layers post-centrifugation. The top most clear pale yellow layer of plasma thrombocyte layer was aspirated using a Pasteur pipette and discarded. The following whitish ring-like layer is that of the lymphocytes. This white ring layer together with half of the HiSep layer was taken in another 15ml tube. The lowermost red pellet of RBC's was discarded.

7. The lymphocytes and HiSep mixture was added to an equal volume of serum free RPMI 1640 medium, mixed well and centrifuged at 800 rpm for 10 minutes.

8. The same process of washing was repeated to remove excess platelets, HiSep and plasma.

9. The cell pellet obtained was suspended in complete RPMI 1640 medium with 10% Fetal Bovine Serum and 2% Penicillin-Streptomycin and maintained at 37^0C in a humidified incubator with 5% CO2.

4.1.6 Cytotoxic Assay on PBMC Normal Cell Line:

In the present study, we investigated the cytotoxic effects of the extract on PMBC normal cell line. PBMC cell line was isolated from the blood using standard protocol [17] and the extracted cell was used for further study. The cytotoxic effect of the Hops*(Humulus lupulus)* extract was evaluated by performing MTT assay[18] on the isolated cell line with PBS as control. The Study was done for 12, 24, and 48 h to observe the gradual effect of the extract on the Cells.

4.1.7 Cytotoxic Assay on HeLa cell Line:

The human cervical cancer cell line (*HeLa*) was procured from National Centre for Cell Science, Pune, and grown in complete Media (88 % DMEM, 2% Antibiotic Solution, 10 % FBS). All the cells were retained at 37°C, 5% CO_2, 95% air and 100% relative humidity.

For the treatment 200 µL/well of cell suspension was seeded into three 96-well plates at plating concentration of 10,000 -15,000 cells/well and incubated to at 37°C, 5% CO_2, 95% air and 100% relative humidity for the cell attachment. After 24 h the cells were treated with serial concentrations of the extracts (50, 100, 250, 500 µg/ml) . The three different plates was incubated for different time period 12, 24 and 48h to check the gradual effect of Extract on the

cells. 20 µL of MTT (5 mg/mL) in phosphate buffered saline was added to each well and incubated at 37°C for 4 h. The medium with MTT was then struck off and 100 µl of DMSO was added to solubilize the formed formazan and finally the absorbance was measured at 570 nm using micro plate reader ((BIO-RAD microplate reader-550[19,20]

The % cell viability was determined using the following formula.

$$\%\, Cell\ viability\ = \frac{\text{Abs (sample)}}{\text{Abs (control}} \times 100$$

4.2 Green Synthesis and Characterization of Silver Nanoparticle using Hops

Silver nitrate was purchased from Merck India Pvt Ltd. Diclofenac sodium was purchased from Novartis India Ltd. Streptomycin was procured from Advacarepharma.

4.2.1 Synthesis of Silver Nanoparticles: Different concentration of 20mM silver nitrate solution in D.D water was mixed with 10x diluted Hops Extarct so the final concentration is1mM, 2.5 mM, 5mM and 10mM. The reaction mixture was kept in normal temperature for 2 hr. The colour of the solution changed from pale yellow to red wine colour indicating the formation of silver nanoparticles.

4.2.2 Characterization:

The nanoparticles were scanned from 300 to 800 nm in the UV-VIS spectroscopy (Carry 60).

Dynamic light scattering (DLS) was used to measure the average hydrodynamic diameters and polydispersity indexes (PDIs) (Malvern Zetasizer Nano-ZS, Malvern Instruments, UK). We analyzed each sample in triplicate at 20 ℃ at a scattering angle of 173°. Pure water was used as a reference-dispersing medium.

The zeta potential was also observed through electrophoretic light scattering at 25 ℃, 150 V, in triplicate for the sample (Malvern Zetasizer Nano-ZS, Malvern Instruments, UK) in D.D water.

In order to analyze the crystalline phases of Silver nanoparticles the sample, X-ray diffraction (XRD) analysis was conducted by Panalytical, X'Pert at 40 kV and 30 mA at a 2θ angle pattern. The scanning was done in the region of 20º–80º.

The synthesized silver nanoparticles were examined using FTIR analysis to conclude the functional groups capping the nanoparticles. Analysis was completed by using KBr pellet (FTIR grade) method and spectrum was recorded in Bruker alpha.

TEM examination was done to conclude the morphology, size and shape of the silver nanoparticles. TEM measurements were done by JEM -2100. The TEM grid was prepared by placing a drop of the diluted solution on a carbon-coated copper grid and later drying it for 48 hrs.

EDAX analysis of silver nanoparticles was performed on a using EDAX detector attached with the TEM Instrument (JEOL 2100 UHR-TEM instrument).

4.2.3Antimicrobial Activity

The antimicrobial activity of the developed nanoparticles were tested by disc diffusion method and MIC method using E.coli (gram negative) and S.aureus (gram positive).

For disc diffusion method, filter papers were cut into a disc shape with 5 mm diameter, sterilized by autoclaving for 30 min at 120^{o}C, and placed on different cultured agar plates and

AgNP solution was applied to it. The plates were incubated overnight at 37°C in an incubator and the inhibition zone was then measured in mm[21,22].

The MIC is defined as the minimal concentration of AgNPs that produces inhibition of bacterial growth. MIC of the prepared Silver Nanoparticles was done by standard protocols previously published[23,24]. Different micro molar concentration of Silver nanoparticles concentration [10,20,40,60,80,100 µM]was used for this purpose. Streptomycin was used as standard drug. The percentage of Bacterial inhibition was calculated using the below formula:

$$Bacterial\ Inhibition\ = \frac{\{\ (Abs\ Control - \ Abs\ Sample)\}}{Abs\ Control} \times 100$$

4.2.4 In-vitro anti-inflammatory Assay

The assay was done by following the method of Muzushima and Kabayashi with certain modifications.0.05mL of plant extract of different dosage was added to 0.45 ml bovine serum albumin (1% aqueous solution) and the pH of the mixture was adjusted to 6.3 using a small amount of 1N hydrochloric acid. These samples were incubated at 37°C for 20 min and then heated at 60°C in a water bath for 30 min. After cooling the samples, the absorbance was measured spectrophotometrically at 660 nm. Diclofenac Sodium was taken as the standard drug. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows[25]:

$$Percentage\ Inhibition\ = \frac{\{\ (Abs\ Control - \ Abs\ Sample)\}}{Abs\ Control} \times 100$$

4.3 Preparation and Characterization of Nanocomposite Films for biomedical use

4.3.1 Preparation of AgNp Nanocomposite Film

18mL of 0.1mM AgNO3 solution was added to 2mL of Hops Extract and was mixed thoroughly for 6h until silver Nano particles are formed. The formation of silver nanoparticle was confirmed from the change of the color of the solution from pale yellow to dark reddish brown. From the solution 10mL solution is taken and mixed thoroughly with 5mL of 5 % Gelatin solution and 10 mL of 5% PVA solution and the solution was stirred in a Magnetic Stirring Plate (REMI) at a temp of 40-60 ^{o}C for 30min. To the homogenized solution, 1mL of 2 % Glutaraldehyde was poured dropwise and the solution was again stirred for another 30mins at a speed of 650 rpm. The final solution was then solvent casted at 40^{o}C for 24 h to obtain the desired membrane. A Gelatin-PVA film was also prepared in similar manner without the nanoparticle.

4.3.2 Characterization

Morphology of the films and nanoparticles are observed by SEM (Inspect F50 SEM). To image the film samples (surface or cross-sections) were coated with a thin layer of palladium gold alloy after mounting on a double sided carbon tape.

The FTIR spectra of the Nanocomposite films are recorded on Bruker Alpha-Eco ATR. To record the FTIR spectra of films, the samples were completely dried in an oven at 60 ^{o}C for 12 h. These samples were read between 500 and 4000 cm^{-1} using ATR mode.

Thermal studies of the films were carried out using Perkin Elmer TGA 4000 System, 100-240V/50-60Hz at a heating rate of 10^{o}C/min and the temperature range was 40-450 ^{o} C

The wetting property was analyzed by measuring the contact angle of nanocomposite film with respect to Glycerin.

The Shore D hardness of the nanocomposite material was measured using a SHORE-D meter.

Finally the Mechanical properties of the film was measured using a UTM machine (Scientico 125 N load cell)

The membrane was immersed directly in buffers of pH 4, 7, or 9 (prepared as per Indian Pharmacopoeia 1996, Ministry of Health and Social Welfare, New Delhi, India) at room temperature for 72 hours; after that, the swollen product was dried at 37-C under vacuum to a constant weight.The equilibrium percentage of swelling (% swelling) of the product was calculated as follows:

$$Swelling\ (\%) = \frac{We - Wd}{Wd}$$

Where We is the weight of the product after hydration for 72 h, and Wd is the weight of the dried product.

4.3.3 Hemocompatibilty Test

Estimation of Hemocompatibility of the Composites is performed through Hemolysis Studies using fresh human blood, collected in a EDTA tube was diluted with normal saline solution (2 mL blood + 2.5mL normal saline). A standard sample without sharp edges was kept in a centrifuge tube containing 10 mL of normal saline and was kept in an incubator at 37 °C for 30 min. To this was added 0.2 mL of the diluted blood which was then mixed gently and incubated for 60 min. For the positive control, 0.2 mL of diluted blood was taken in 10 mL of 0.1% sodium carbonate solution and for negative control; 0.2 mL of diluted blood was taken in 10 mL of normal saline solution and incubated for 60 min at 37 °C. In a similar way, sample material was incubated for 60 min at 37 °C. After 60 min of incubation, all the test tubes were centrifuged for 5 min at 4000 rpm and the supernatant was carefully removed and transferred to the cuvette for readings at 545 nm wavelength and percentage hemolysis was calculated [26]. Percentage hemolysis is calculated based on average of thee replicates.

$$\%\text{Hemolysis} = \frac{\text{ODtest} - \text{ODnegative}}{\text{ODpositive} - \text{ODnegative}} \times 100$$

1. Highly hemocompatible (<5% hemolysis)
2. Hemocompatible (within 10% hemolysis)
3. Non Hemocompatible (>20% hemolysis)

4.3.4 Antimicrobial Activity

The antimicrobial activity of the developed nanocomposite films were tested by disc diffusion method using E. coli (gram negative) and S. aureus (gram positive).

For disc diffusion method, the films were cut into a disc shape with 5 mm diameter, sterilized by autoclaving for 30 min at 120 ^{o}C, and placed on different cultured agar plates. The plates were incubated overnight at 37 ^{o}C in an incubator and the inhibition zone was then measured.

4.3.5 Cytotoxicity experiment

MTT assay was applied to determine the cytotoxicity of prepared sample film on PBMC normal Cell line. The PBMC cells were isolated from blood of a normal human who donated the blood voluntary. Briefly, PBMC cells were dispensed in 24-well culture plates incubated at 37 °C. After 24 h, the nanocomposite film and PBS was introduced to the grown cell. The medium containing only PBS was used as a control. Each group was analyzed in triplicate. After 24 h, 50 µl MTT (5 mg/mL) was added to the medium which was then incubated for another 4 h at 37 °C. The formazan crystals in the cells were solubilized with stock DMSO

solution (100 µl/well). The optical density (OD) value was then measured at 590 nm using spectrophotometer. The % viability of is calculated by the following equation [27]:

$$\%\ \text{Vaible cel} = \frac{\text{OD value of samples}}{\text{OD value of negative control}} \times\ 100$$

And cytotoxicity was assessed according to cytotoxicity grading criteria as indicated in Table 4.1.

Cell Vaibilty (%)	**Cytotoxicity grading criteria**
100	0 (non-poisonous, qualification)
75–99	1 (light poisonous, qualification)
50–74	2 (moderate poisonous, disqualification)
25–49	3 (severe poisonous, disqualification)
1–24	4 (disqualification)
0	5 (disqualification)

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CHAPTER 5:

RESULTS & DISCUSSION

5.1 Result and Discussion for phytochemical analysis and biological activity Hops Extract

5.2 Result and Discussion for Silver Nanoparticle characterization and antibacterial and anti-inflammatory activity.

5.3 Result and Discussion for Characterization and biomedical use of Silver nanocomposite Film

5.1 Result and Discussion for phytochemical analysis and biological activity Hops Extract

5.1.1 Qualitative Analysis of Phytochemicals

The results of phytochemical analysis of all the extract is given in Table 1. The result shows presence of major phytochemical which includes Phenol, Flavonoid, Tannin , Saponin , Alkaloid and Reducing sugar . Although the extract didn't show any presence of Phlobatannins .

SL No.	Phytochemicals	Results
1	Phenols	+
2	Flavonoids	+
3	Tannin	+
4	Phlobatannins	-
5	Saponin	+
6	Alkaloids	+
7	Reducing sugars	+

Table 5.1: Phytochemical Analysis of Hops Extract

The presence of different phytochemicals indicates that Hops*(Humulus lupulus)* extract can be potentially used for various medical purposes. Based on the initial phytochemical study quantitative estimation of phytochemicals was carried out.

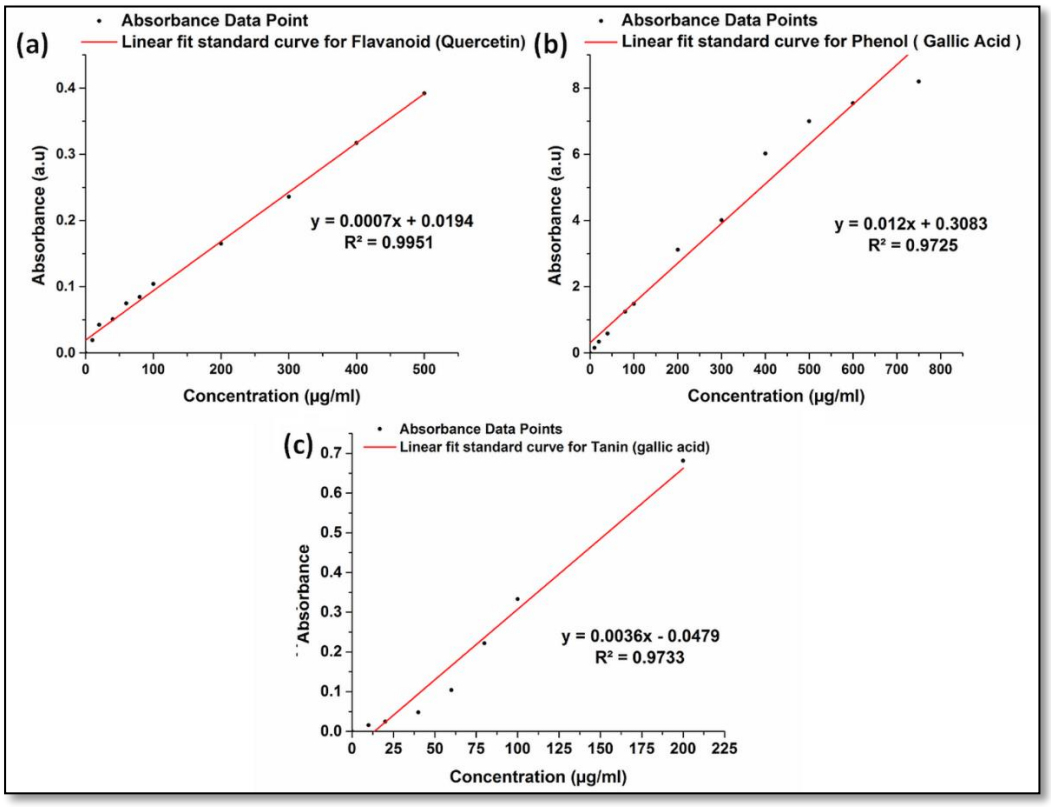


Figure5.1- (a) Standard curve for phenol (b) standard curve for Quercetin, (c) Standard curve for Tanin

A standard Curve of Gallic Acid and Quercetin (Figure 1(a), 1(b)) was prepared for estimation of Phenol and Flavonoid respectively whereas standard curve of Gallic acid (Figure 1(c)) was prepared for estimation of Tannin.

5.1.2 Quantitative Analysis of Phytochemicals

Total phenolic content of Hops*(Humulus lupulus)* extract was found to be 580µg/mL Gallic acid equivalent hence reflecting the effectiveness of the plant in scavenging free radicals. Phenols are very significant plant constituents. There is a good relationship between total phenols and antioxidant activity of many plant species, as the hydroxyl group present have scavenging activity[1]. Various sources reveals that antioxidant activity of plant extract is primarily due to existence of phenolic compounds, which have antioxidant properties as free radical scavengers, as a hydrogen donating sources or as singlet oxygen quenchers and metal ion chelators.

Flavonoids are considered as one of most spread groups of natural constituents found in plants. The flavonoids content of Hops*(Humulus lupulus)* extract was 231 µg/mL Quercetin equivalent. It has been discovered that flavonoids actively contribute in antioxidant scavenging and the mechanism of action of flavonoids is through scavenging or chelation procedure which finally terminates free radical. These are very significant phytoconstituents for their hydroxyl groups confer scavenging capability[2]

The entire tannins content of Hops*(Humulus lupulus)* extract was found to be 194 µg/mL Gallic acid equivalent (GAE) at concentration of 1 mg/mL of plant extract. Tannins and tannins like constituents are extensive in nature and are possibly present in all plant materials. Tannins possibly will affect the inflammatory response via their radical scavenging actions[3].

5.1.3 Antioxidant Effect

Phosphomolybdenum assay is primarily based on the reduction of Phosphate-Mo (VI) to Phosphate Mo (V) by the natural product extract and successive formation of a bluish green colored phosphate/Mo (V) compound at acid pH. The phosphomolybdenum technique is regularly practiced in the laboratory to assess the total antioxidant capacity of many plant extracts[4].

Phosphomolybdenum assay evaluates the reduction degree of Mo (VI) to Mo (V). Phosphomolybdenum assay is a quantifiable technique to inspect the reduction response rate amongst oxidant, antioxidant and molybdenum ligand. It includes thermally producing auto-oxidation during sustained incubation period at higher temperature .Thus it gives an undeviating approximation of reducing ability of antioxidant. The Total Antioxidant capacity of the Hops *(Humulus lupulus)*Extract was estimated using ascorbic acid (a common anti-oxidant) as the standard. The total anti-oxidant capacity of Hops*(Humulus lupulus)* extract was found out to be 132.57 µg/mL ascorbic acid equivalent.

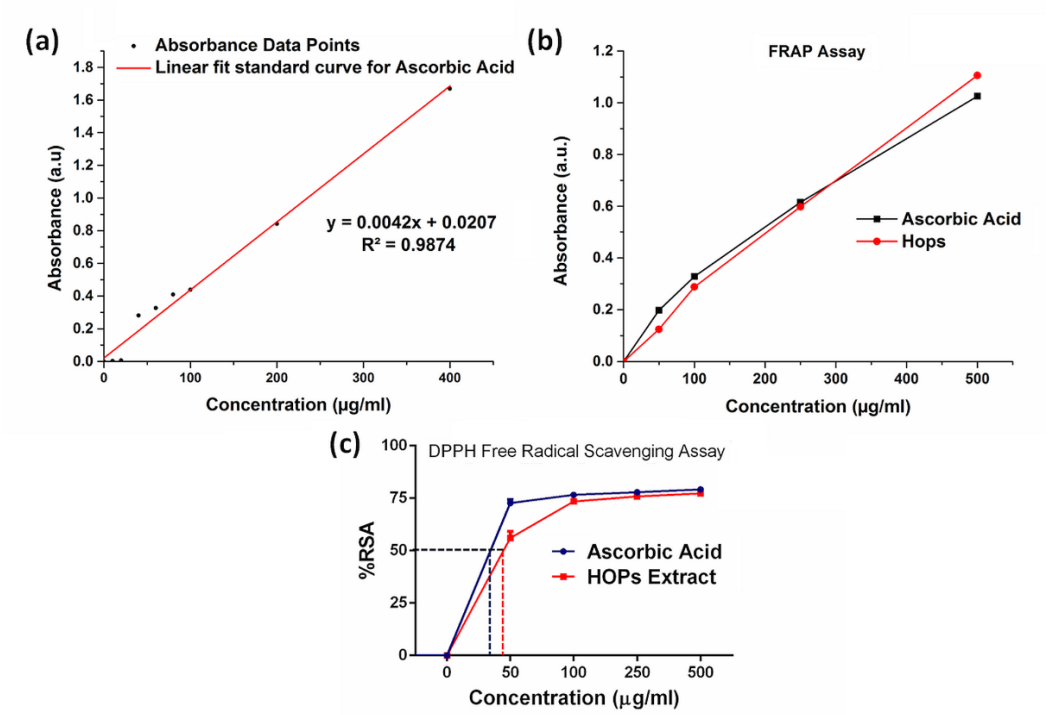


Figure 5.2:(a) Standard Curve for Ascorbic Acid (b) FRAP Assay (c) DPPH Free Radical Scavenging Assay.

The FRAP assay was used to confirm the reducing and antioxidant capacity of the Hops *(Humulus lupulus)*extract. The Reducing capacity of the Hops *(Humulus lupulus)* extract is shown in the Figure 2(b) .Increase in the absorbance value corresponds to the increased reducing capacity and hence increased reducing antioxidant capacity[5]. As observed in the assay the test solution which was yellow in color changed to blue color mixture with the addition of the extract and ascorbic acid. The change in color of the sample from yellow to blue is due to the reducers present in the compounds which reduces Fe^{3+} ferricyanide complex to its ferrous form. Thus the absorbance reading at 700nm actually measures the Fe^{2+} concentration[6]. The intensity of the blue color depends on the reducing capacity of the compound. It was observed that with the increasing concentration of both the compounds the reducing capacity is increased, although the reducing capacity of the Hops *(Humulus lupulus) extract* is not as significant as Ascorbic acid in very low concentration but at higher concentration it is almost equivalent to Ascorbic acid which is a standard antioxidant material.

The free radical scavenging activity of different concentration of Hops *(Humulus lupulus)* extract on the DPPH free radical was compared with standard anti-oxidant, Ascorbic acid. The results were expressed as % Radical Scavenging activity (%RSA) shown in Figure 2(c) The extract exhibited dose dependent response in scavenging free radical. The EC_{50} value for both the compound i.e. Standard Ascorbic acid and Hops *(Humulus lupulus)*extract was calculated from the data and the estimated EC50 value for Ascorbic Acid is 34 µg/mL and for Hops *(Humulus lupulus)* extract it is 47 µg/mL in linear scale.

The chemistry behind DPPH method is that the antioxidants (present in the extract) react with the deep blue color, stable and free radical α-diphenyl-β-picrylhydrazyl and convert it to pale yellow color or colorless α,α-diphenyl-β-picrylhydrazine. The presence of phenolic compound in the extract may have contributed to this antioxidant property and have scavenged this stable and free radical. DPPH is a free and free radical

only at room temperature and it exhibits a violet color. When reacted with an antioxidant it can accept an electron or hydrogen from the antioxidant and its reduction ability can be estimated by measuring the absorbance value at 517 nm.

The DPPH radical scavenging assay is an accepted and widely used technique for evaluating in-vitro antioxidant activity of various natural compounds [7].

The Radical scavenging activity of Hops *(Humulus lupulus)* extract was compared with a well-known antioxidant i.e. ascorbic acid. The antioxidant capacity of ascorbic acid was found to be better than Hops *(Humulus lupulus)* extract in lower concentration but as the concentration increased both ascorbic acid and Hops *(Humulus lupulus)* extract showed almost the same scavenging activity. The free radical scavenging activity of Hops *(Humulus lupulus)* extract can contribute to the way in which plant extract can be used as an alternative medicine. Thus Hops extract can be modified and can be used as a future medicine in inhibiting oxidative stress and hence can be used in curing degenerative diseases[8].

5.1.4 Anti-inflammatory effect:

The in-vitro anti-inflammatory effect of Hops *(Humulus lupulus)* extract based on Inhibition of albumin denaturation assay is indicated in Figure 3(a).

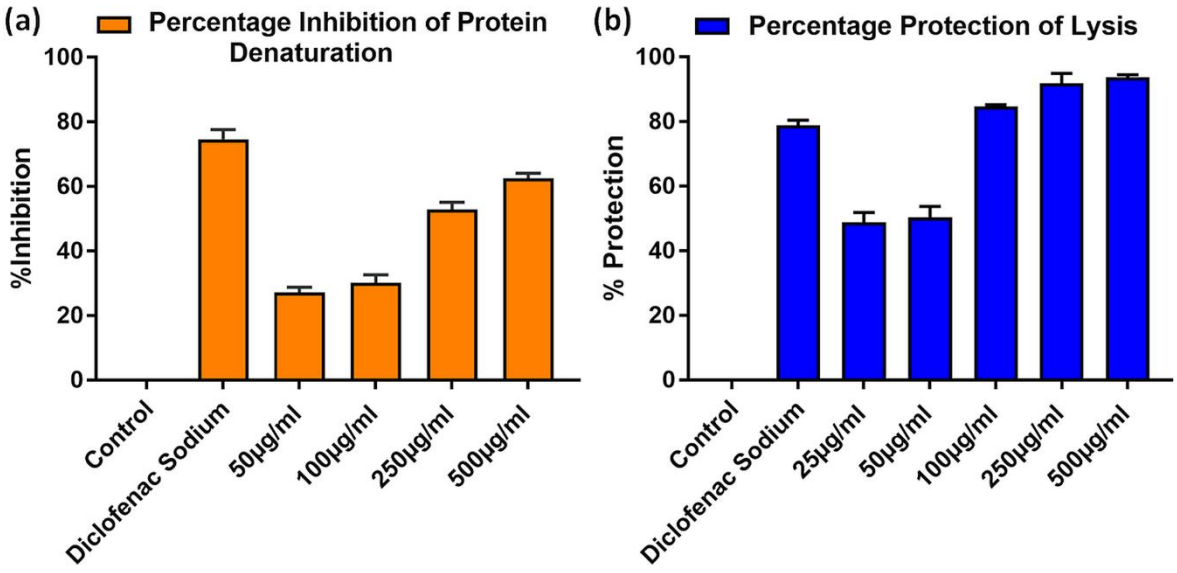


Figure 5.3:(a) Inhibition of Protein Denaturation (BSA) (b) Membrane Stabilization Assay(HRBC)

The Hops *(Humulus lupulus)* Extract (50-500 µg/mL) showed anti-inflammatory effect and protection of denaturation of bovine serum albumin in a dose dependent manner. The maximum protection was found to be (62.5 ± 1.331224) % at a concentration of 500 µg/mL. Although the protein denaturation protection in Diclofenac sodium was found to be much better which is (73.5 ± 2.453189) at a concentration of 200 µg/mL. The protection percentage of Hops *(Humulus lupulus)* extract increased with the increase in concentration and at a concentration of 250 and 500 µg/mL the extract showed significant protection with respect to negative control where no drug was used. The percentage inhibition of denaturation of protein with respect to different concentration is given below.

Table 5.2: Percentage of Inhibition of Protein Denaturation

Concentration	% Inhibition
50 µg/mL	27.17391 ± 1.34504
100 µg/mL	30.16304 ± 2.00609
250 µg/mL	52.85326 ± 1.83298
500 µg/mL	62.5 ± 1.331244
D.S (200 µg/mL)	74.59239 ± 2.45318

Protein denaturation is reported to be one the cause of inflammation. During inflammation, auto antigens are produced and the probable cause for this may be in-vivo protein denaturation. Alternation in boding (electrostatic , hydrophobic , disulphide and hydrogen) may contribute to the mechanism of this denaturation[9]. From the results it can be concluded that higher concentration of Hops *(Humulus lupulus)* extract can control the generation of auto antigen and can hence protect the denaturation of protein.

The Hops *(Humulus lupulus)* extract also inhibited hypotonicity induced lysis of erythrocyte membrane and hence it can be stated that it can stabilize erythrocyte membrane. The percentage stabilization of Hops *(Humulus lupulus)* extract was compared with standard drug diclofenac sodium (200 µg/mL) and is indicated in Figure 3 (b) . The Hops *(Humulus lupulus)* extract showed significant percentage of stabilization /protection in lysis of erythrocyte membrane. The maximum protection was found to be (93.82 ± .59811) % at a concentration of 500 µg/mL, which is highly significant in terms of stabilization of membrane. The % protection of lysis for the standard was found to be (78.90436 ± 1.2483230) %. The Hops *(Humulus lupulus)* extract showed an increase of % protection with the increase in concentration hence it showed a dose dependent activity. The presence of phenolic compound and the extract having good anti-oxidant property may contribute to this excellent stabilizing property which in terms contributes to a better anti-inflammatory property.

Stabilization of Lysosomal membrane is a significant factor in preventing inflammatory action by thwarting the release of several constitutes like proteases and bactericidal membrane which are part of activated neutrophil in the lysosomal membrane[10]. In the above experiment HRBC sell suspension is used and the extract is treated on the erythrocyte membrane which is comparable to the lysosomal membrane and the stabilization of the erythrocyte membrane in turns implies that it can also stabilize or protect the lysosomal membrane. The lysosomal membrane stabilization plays an important role in anti-inflammatory effect. Proper expiation for this membrane stabilization by the Hops *(Humulus lupulus)* extract is still a point of question but the results obtained are highly significant. During hypo tonicity induced hemolysis in HRBC the cell lysis is due to the cell shrinkage which in turn is due to the osmotic loss of the intracellular cellular component i.e. electrolyte and fluids or may be due to interaction with the proteins of the membrane[11,12] . The extract may stabilize the system by replenishing those intracellular components of cell membrane. Thus it can be concluded that Hops (*Humulus Lupulus*) extract can be potentially used to stabilize membrane and can be hence used as an anti-inflammatory agent.

Nonsteroidal anti-inflammatory drugs has been used for years as anti-inflammatory drugs and pain-killers due to their ability in inhibiting protein denaturation and protecting lysosomal membrane. Thus compounds or material with similar type of properties can be hence used as anti-inflammatory drug production. Even these natural products like Hops (*Humulus Lupulus*) can be used as an alternative medicine in treatment of inflammatory or arthritis.

5.1.5 Cytotoxicity Effect:

The Cytotoxic effect of Hops (*Humulus Lupulus*) extract on PBMC cell line has been indicated in Figure 4 .The extract was treated for 3 different time points i.e. 12hrs, 24hrs and 48hrs. The data showed dose dependent toxicity.

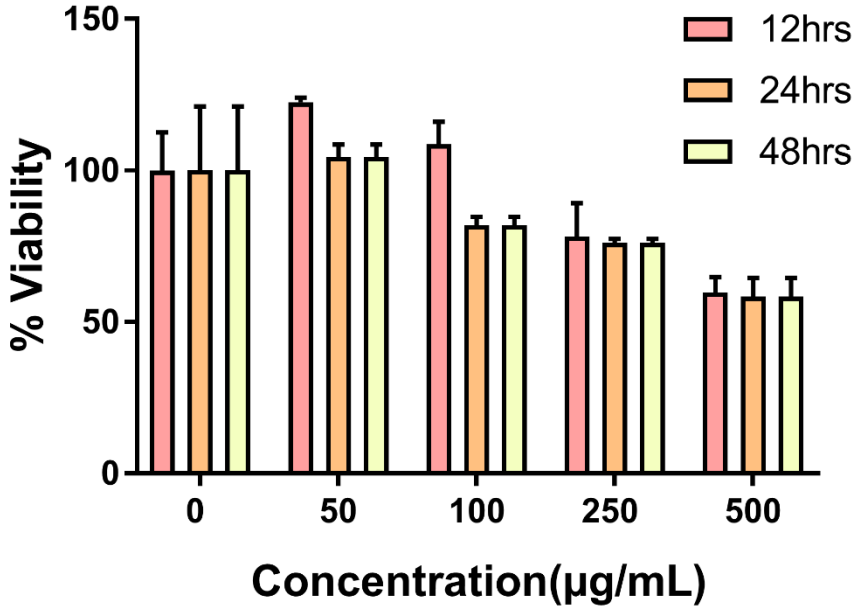


Figure 5.4: Cytotoxicity of Extract on HeLa Cell Line at 3 different time points

Though the toxicity increased with increase in concentration of the extract but the toxic levels are not that significant till the concentration of 250 µg/mL. At a concentration 500 µg/mL the toxicity level went down 50 % level after 48 hr. thus showing signs of toxicity.

The MTT assay revealed the ethanolic extract showed toxicity towards HeLa cell line. The results are indicated in Figure 5, Figure 6 and Figure 7 indicating the effect of extract on HeLa cell line at different time points.

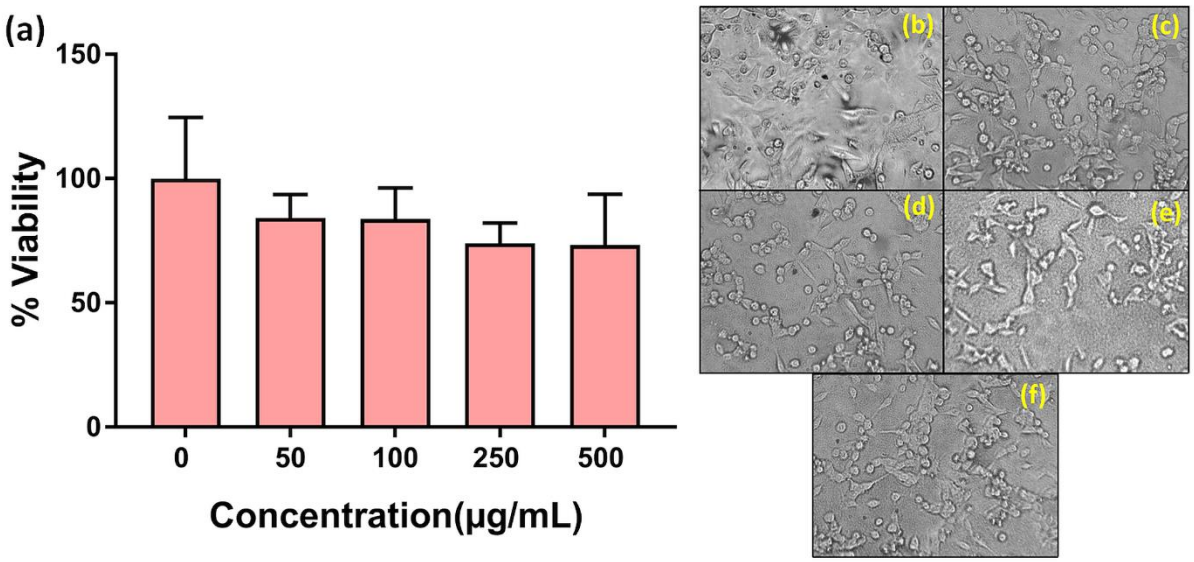


Figure 5.5: Cytotoxicity assay on HeLa Cell Line at a time point of 12 hrs.

This result also indicted a dose and time dependent response. with the increase in time there is an increase in the toxicity level and similar trend was followed in the increasing dose .In the 12 hr study the extract didn't showed any significant toxicity but with increase in time the toxicity level increased i.e. in 24 hr the toxicity level went below 50 % for 250 µg/mL and in 500 µg/mL it went below 20 %.

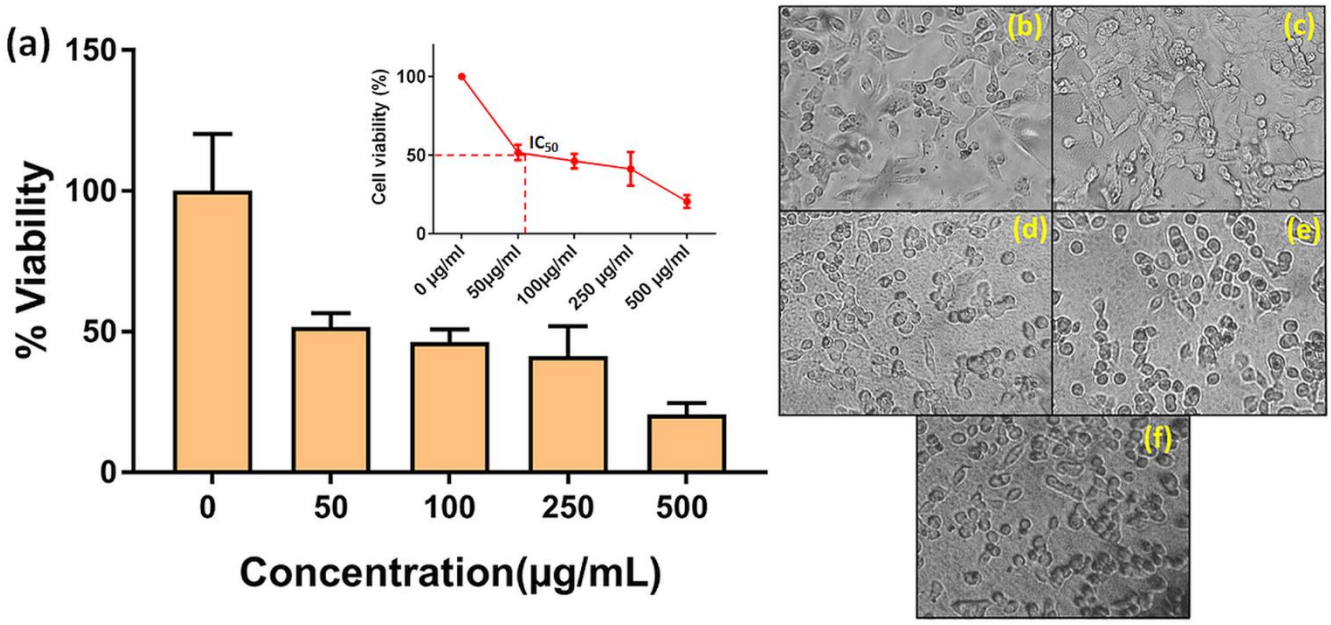


Figure 5.6:- Cytotoxicity assay on HeLa Cell Line at a time point of 24 hrs.

In the 48 hr study, the results indicated that the cell viability percentage went below 50 % at 50 µg/mL and for the other concentration it was around 15 %. Hence with the 48 hr study it was evident that the cell viability decreed significantly. The IC_{50} value was calculated to be 56 µg/mL for 24hrs and 43 µg/mL for 4 hr. Although the IC_{50} for 12 hr was not obtained from the study. To support this Assay the morphological structures of the cell at different concentration and time point was also captured and it supported the assay results, with decrease in cell viability the morphology of the cells changed and there was cell shrinkage which indicates cellular death.

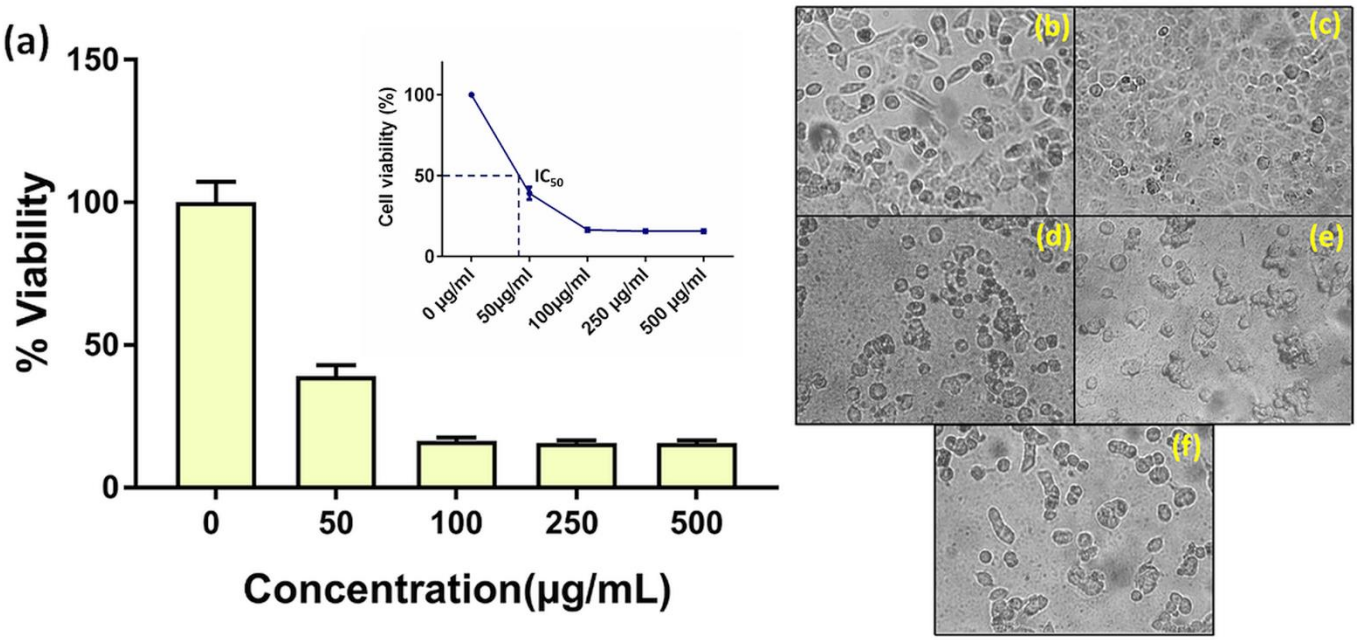


Figure 5.7: Cytotoxicity assay on HeLa Cell Line at a time point of 48 hrs.

The basic of MTT assay is that the mitochondrial dehydrogenase reduces the chemical MTT and produces dark purple colour formazan[13]. *In vitro* cytotoxicity test using HeLa cancer cell lines was accomplished to check the effect of the active compound present in hops extract on cellular functions and morphology. It was noted that the extract inhibited cellular growth in cancer cell line but it didn't have any significant cytotoxicity in normal PBMC cells. This selective toxicity in cancer cell line makes this extract a handful choice for anticancer treatment. Even the IC50 value for HeLa cell line was found to be very low and hence it can be considered very effective. The Hops extract are rich in polyphenols and flavonoids which might act as primary constituents for this selected toxicity in cancer cells. In addition to that the antioxidant agents present in the extract can be reason for this toxicity in the cancer cell line.

5.2 Result and Discussion for Silver Nanoparticle characterization and antibacterial and anti-inflammatory activity.

At the macro scale, silver always looks like silver. But solutions of silver nanoparticles can have many colors. The main reason behind this color change is the Surface Plasmon resonance. In the silver nanoparticles, electrons oscillate collectively .These oscillations affect how light interacts with the nanoparticles .The specific oscillations depend on the particles' size and shape, so particles of different sizes have different colors. Color Change Indicates Particle Size. Solution color gives an approximate idea of the particle size. The color we see is basically an integration of the absorption spectra Nanoparticle size can be monitored more accurately by taking absorption spectra.

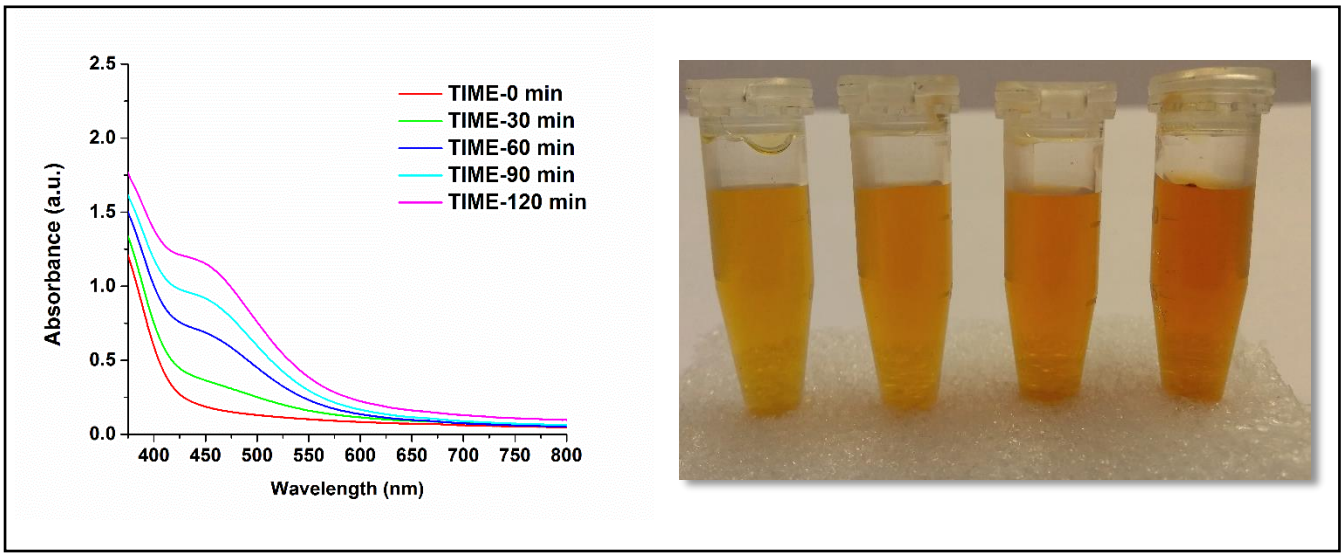


Figure 5.9: Change in color of Silver nanoparticle with respect to time

In the current study, silver nanoparticles were synthesized using Hops extract and evaluated for anti-bacterial and anti-inflammatory property. Silver nanoparticles using Hops extract were synthesized at normal temperature very rapidly within 1hr. The reduction of silver ions to silver nanoparticles by the Hops extract was evidenced by the change of colour of the solution to reddish colour, this change in colour was due to the excitation of the surface Plasmon vibrations**.**

5.2.1 Synthesis of Silver nanoparticle

Hops extract are rich in various phytochemicals majorly alpha acid and beta acids[14] . The proposed mechanism for synthesis of silver nanoparticles is attributed to these phytochemicals presnt in Hops. The silver ions get trapped on the surface of phytochemicals and are then reduced by the other phytochemicals leading to the materialization of silver nuclei. These formed silver nuclei accumulate and subsequently grow in size resulting in the formation of the silver nanoparticles. The silver nanoparticles are further capped by the phytochemicals to avoid their aggregation and also to make those chain like structure stable.

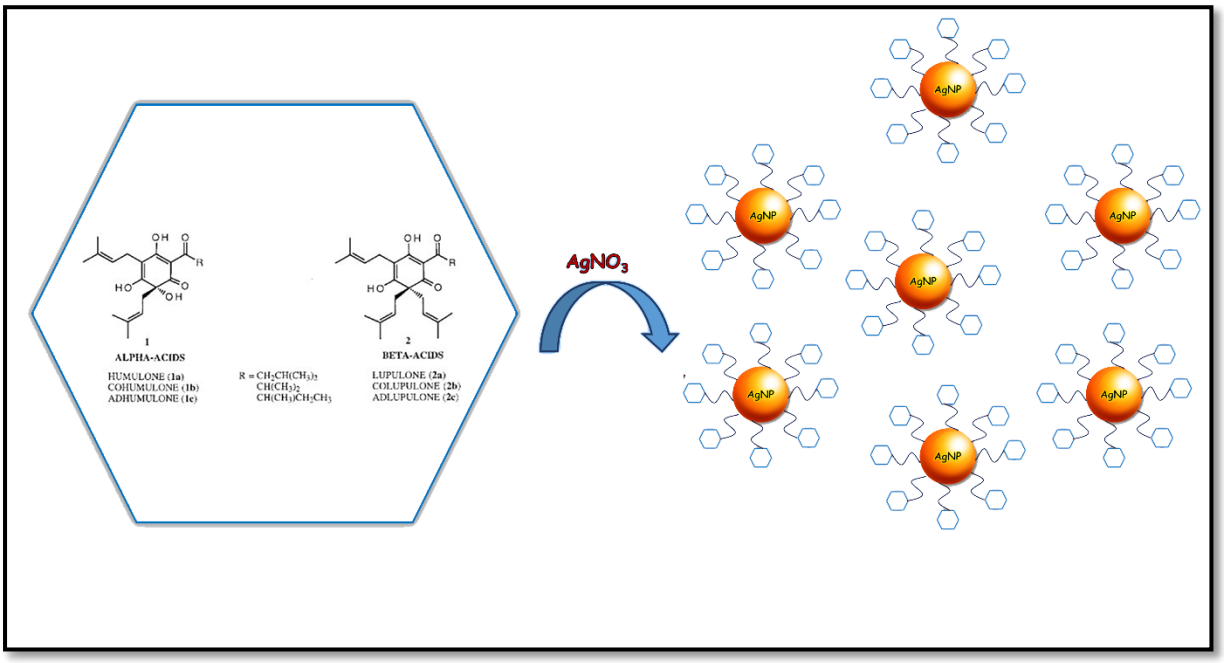


Figure 5.10: Scheme for biosynthesis of silver nanoparticles

5.2.2 Major Characterization of Silver nanoparticle

The UV - visible spectra indicated an absorption band at around 435 nm which was particular for the formed silver nanoparticles. The peak was witnessed after 2 hours of reaction time and the absorption band observed was narrow indicating the low polydispersity of the nanoparticles. The UV-Visible spectrum of the silver nanoparticles synthesized using Hops extract is depicted in Figure 5.11. From the UV-VIS study the 5mM sample was chosen for the future work as it showed the maximum peak among all four sample prepared.

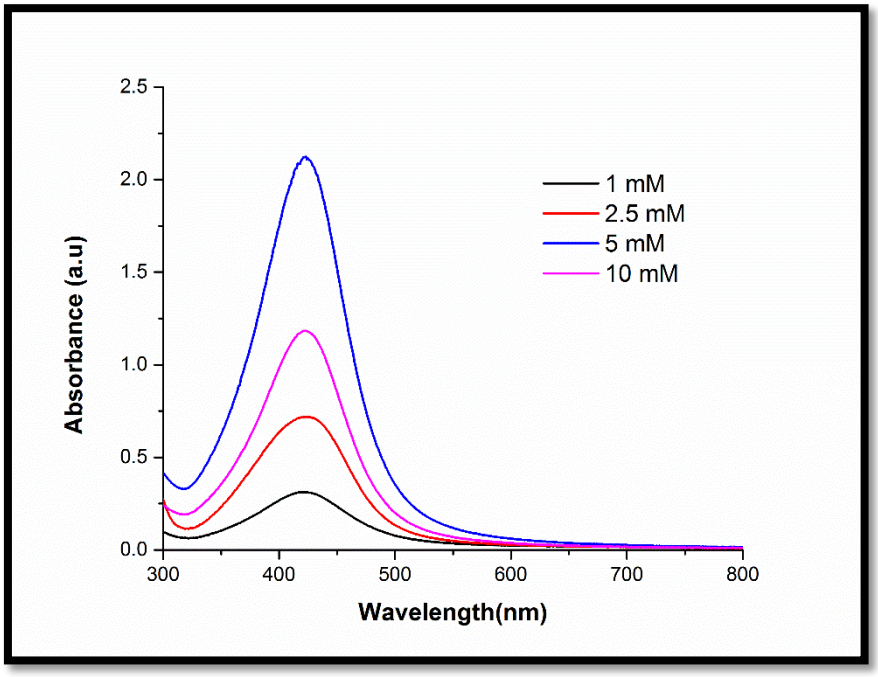


Figure 5.11: UV-VIS Characterization of Silver Nanoparticle

The DLS results indicated that the hydrodynamic size of the synthesized nanoparticles is in the range of 79.92 ± 1.23 nm. The PDI of the synthesized nanoparticles was found to be 0.182 indicating it be a monodisperse system hence less chance of agglomeration.

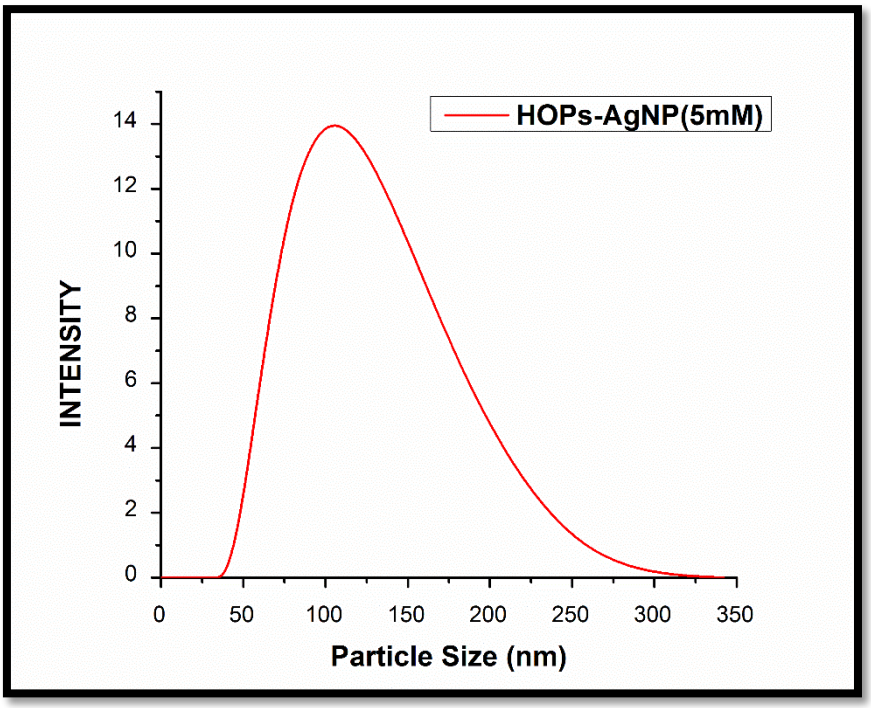


Figure 5.12: DLS Characterization of Silver Nanoparticle

The Zeta potential of the particles was found to be -19.3 ± .786 mV indicating a high negative value in the surface potential of the particles. This highly negative Zeta Potential indicates that the Nanoparticles are highly stable

To know the structural information and to confirm the presence of silver nanoparticles the biosynthesized silver nanoparticles was subjected to powder XRD. Figure5.13 shows the XRD pattern of the synthesized nanoparticles.

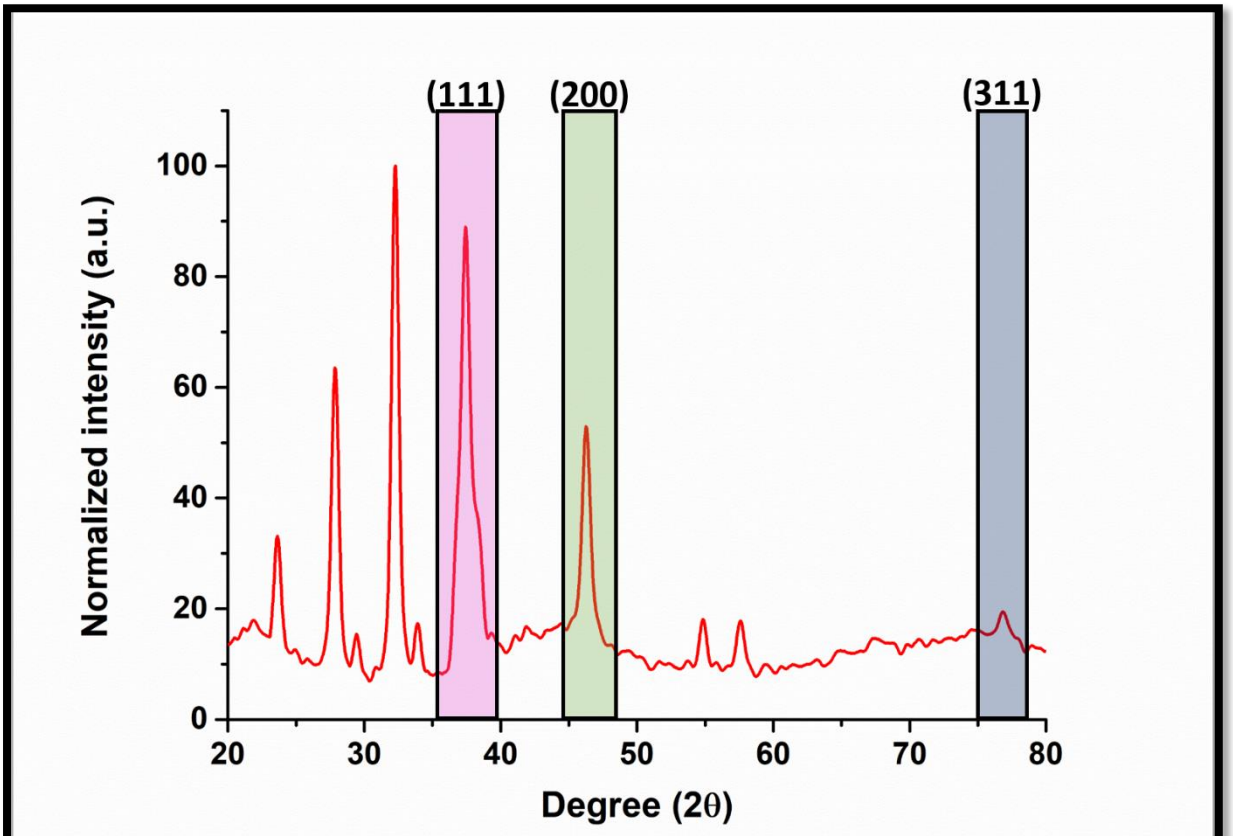
The pattern indicates 3 major peaks at (2θ) 37.44, 46.33 and 76.88 corresponding to (111).(200) and (311) planes respectively. The peaks when compared to JCPDS card no 04-0783, 1991 confirms the formation of silver nanoparticles. Beside this a sharp peak at 32.36 was noted which can be designated with the plane 101 as reported earlier[37]. The peak orientation corresponds to the fcc structure of the silver nanoparticles. The crystalline size of silver nanoparticles was calculated using he Debye–Scherer's equation[15]:

$$D = \frac{0.9\,\lambda}{\mathrm{b}\cos\theta}$$

By calculating the width of (111) Bragg's reflection, the average size of the nanoparticles was found to be 5.2 nm.

Few unassigned peak was found at (2θ) 27.82 54.83 and 57.72 but those peak have intensity weaker than silver nanoparticles. The cause for this peaks can be due to the presence of various phytochemical and

organic bio compounds over the surface of silver nanoparticles. Detailed crystalline size and lattice strain is given below.



Degree (2θ)	Plane Orientation	Crystalline Size (nm)	Lattice Strain
37.44	111	5.22	0.0216
46.33	200	8.05	0.0114
76.88	311	11.39	0.0051

Figure 5.13: XRD analysis of the biosynthesized silver nanoparticles

The FTIR analysis is depicted in the Figure 5.14 and it gave a brief idea about the functional group present in Hops extract+ which actually participated in capping of Silver Nanoparticles. Few peaks were prominent and the particles exhibited a prominent peak at 3135 cm^{-1} indicating the presence of C-H cm^{-1} (alkene) stretching. A sharp peak was observed at 1390 cm^{-1} which corresponds to O-H (phenol) bending. A very broad peak from 1863 cm^{-1} to 2267 cm^{-1} was present in the curve corresponding to N=C=O (isocyanate) stretching. A small peak at 1123 cm^{-1} corresponds to the C-O (secondary alcohol) stretching. One peak at 741 cm^{-1} indicated C=C (alkene) bending with disubstituted (cis).

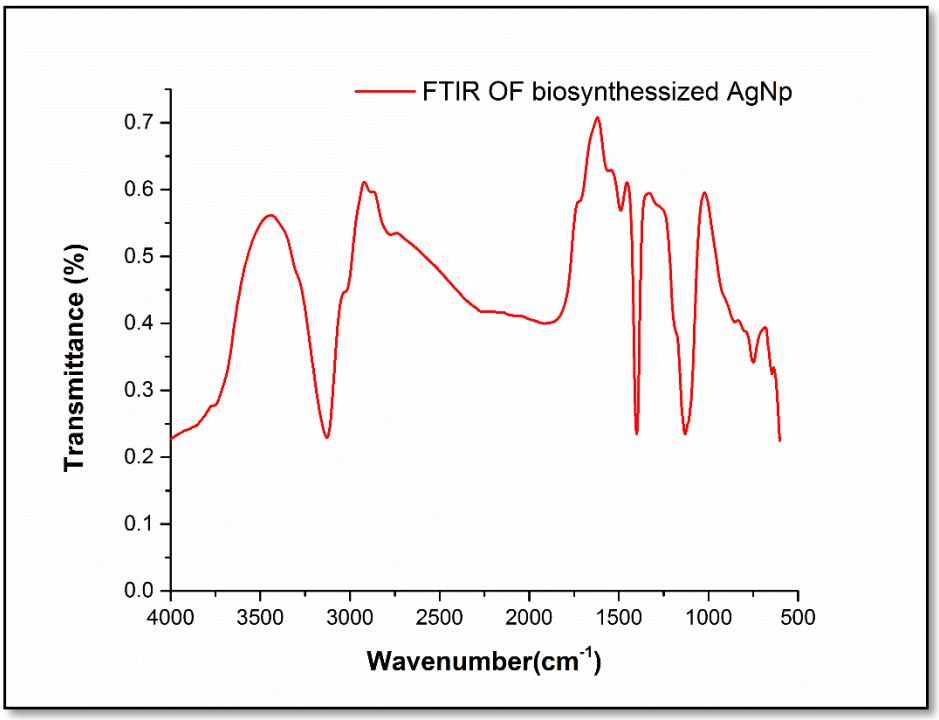


Figure 5.14: FTIR spectra of the synthesized HOPs stabilized silver nanoparticles

The actual shape, size and structure of the final particles were clarified with the help of TEM (Figure 5.15) .Diluted solution of Ag nanoparticles were casted on copper -grid and then the grids were dried for 48 hrs. before the TEM image was recorded . The TEM snapshots put forward that the sizes of the silver Nano particles were about 30-40 nm. Majority of the particles was spherical in shape. The size measured by TEM analysis was lower than that measured by DLS analysis. The atomic plane distance was found to be 1.4 Å .The EDAX study also pointed the presence of Silver ion and carbon in the particles.

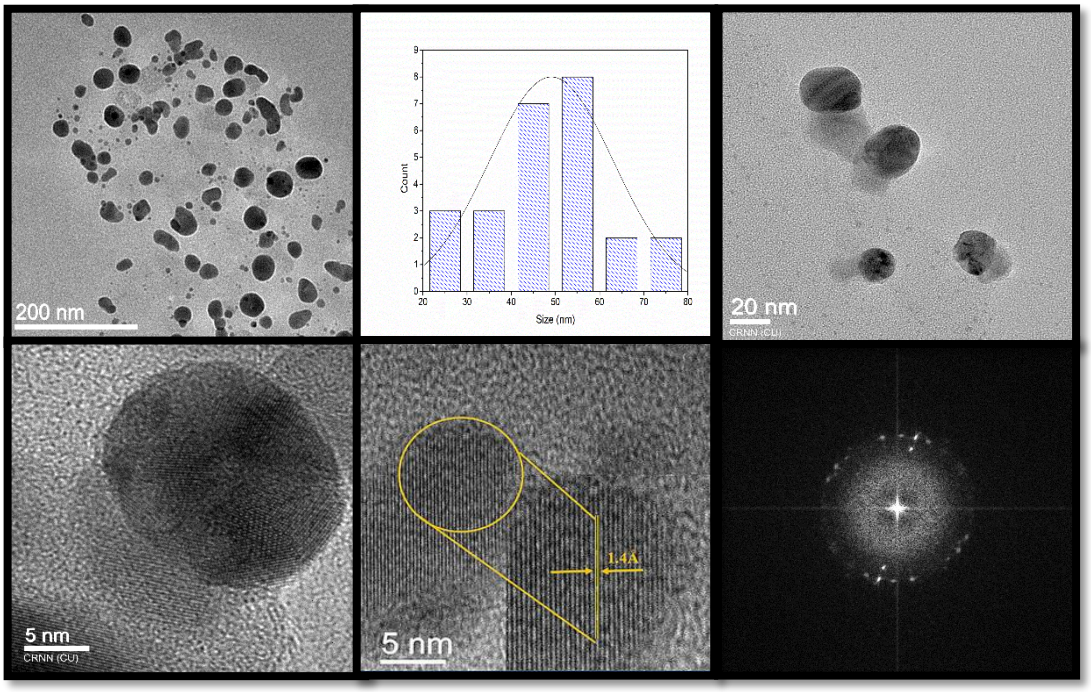


Figure 5.15 : TEM analysis of silver Nanoparticle

5.2.3 Antibacterial study for Biosynthesized Silver nanoparticle

The biosynthesized Silver Nanoparticles were examined about their antibacterial activity against two bacterial strains-E. coli (gram Negative)and S. aureus (gram Positive) we by determining the diameter of the inhibition zone. Figures 5.15 illustrate the zone of inhibition of the biosynthesized silver nanoparticles against the bacterial sample. The picture indicates zone of inhibition of Silver nanoparticles, Hops extract, Streptomycin and normal D.D water against the bacterial strains.

The Biosynthesized silver Nano particles exhibited an inhibition zone of 14.93 ± 0.57mm for S.aureus and standard drug i.e Streptomycin showed an inhibition zone of 9.03 ± 0.27 mm whereas Hops extract and normal D.D water showed no zone of inhibition. On the other hand the nanoparticles showed an inhibition zone of 18.13 ± 0.47 mm for E.coli and streptomycin exhibited an inhibition zone of 11.47 ± 0.23 mm , Hops extract and normal D.D water had no effect on the bacterial strains. Thus it is quite evident that the biosynthesized AgNPs showed excellent antibacterial property against S.aureus than E.coli when compared to standard drug. The effectiveness of the silver nano particles as an antibacterial agent can be explained by various ways , one possible way is that the positively charged silver ions might have attached to the cell surface of the bacterial membrane and penetrating the cell membrane and disrupting membrane permeability and cellular respiration , another possibility is that the silver nanoparticles are very small and can interact with the genetic materials and can hamper their normal function and hence causing cellular death and silver nanoparticles can also interact with the Sulphur and phosphorus containing compound production of Reactive Oxygen Species (ROS) similar to super oxide anions (O_2^-) and hydroxyl free radicals ($OH^\bullet$)[16].

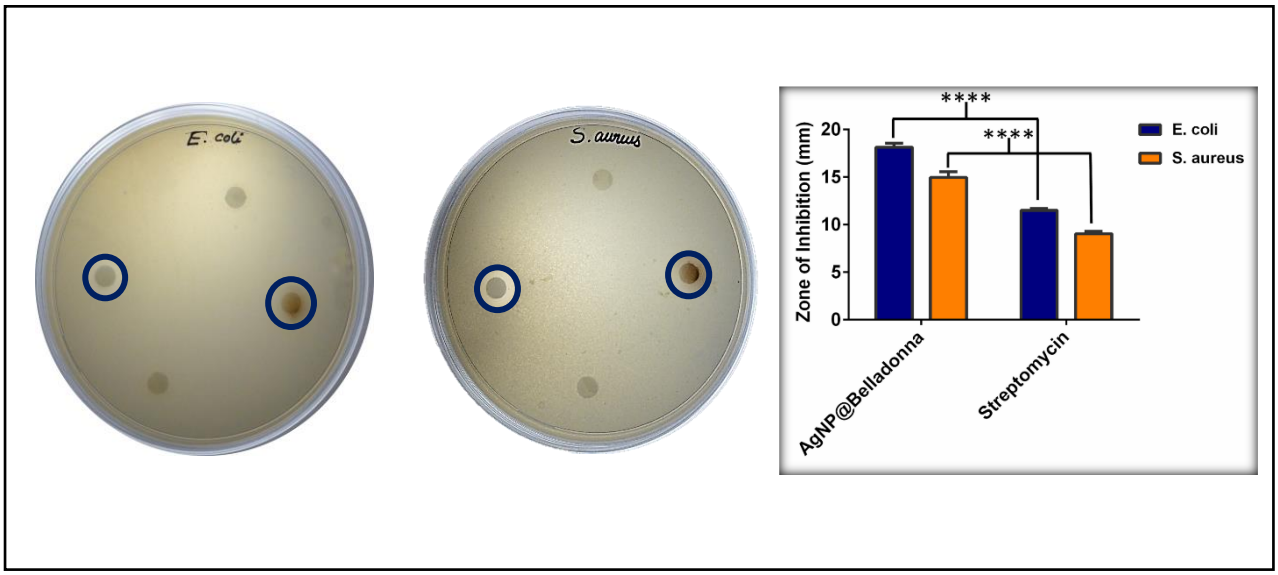


Figure 5.15 Images of plating for zone of inhibition experiments and bar graphs showing zone of inhibition diameter of biosynthesized AgNPs and control Streptomycin for E.coli and S. aureus

The MIC test is used to evaluate the lowermost concentration of a material or compound where there is not as such visible growth, or, in instances like bacteriostatic material, the minimum concentration where turbidity is more than the faint turbidity present in other samples[17].

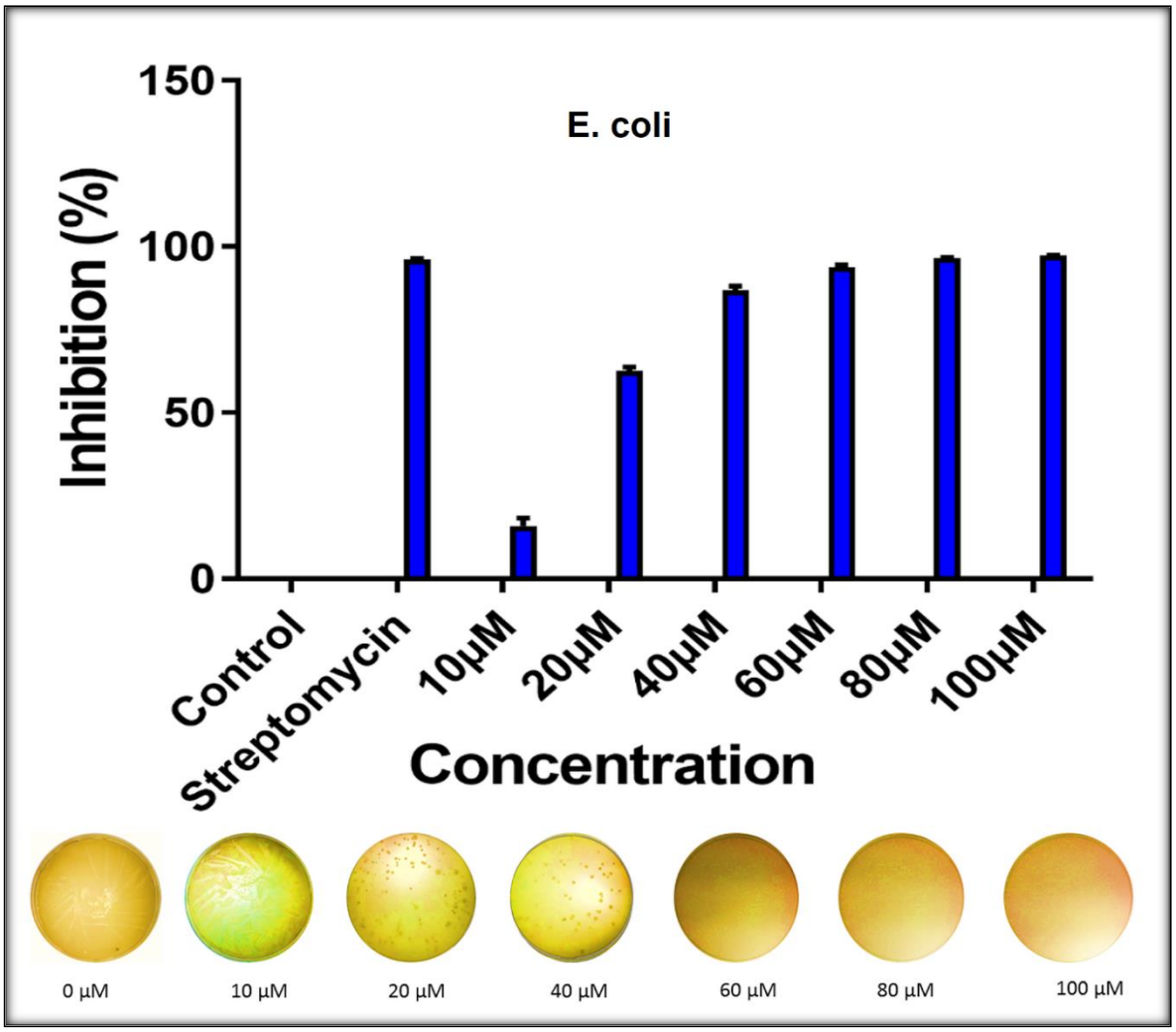


Figure 5.16 : Bacterial Inhibition of E.coli at different concentration of silver nanoparticles and Colony formation change at different concentration.

It is already reported that silver nanoparticles exhibit good antibacterial activity against various pathogens and it is also proved in many experiments, nevertheless the testified MIC values varies from experiment to experiment depending on the material used. Thus it is very tough to compare different results, as there is no as such standard protocol for evaluating antibacterial activity of nanoparticles and different researcher uses different protocol.

The biosynthesized nanoparticles using Hops extact exhibited good antibacterial property. The lower concentration showed an inhibition of 31.3 % in case of S.aureus and 15.96% in case of E.coli. There was an inhibition above 90 % for the concentration of 60 µM and above for both the stains. The culture was spread over agar plate to observe the bacterial colony formation. The plate showed no growth from and above 60 µM concentration of the nanoparticles solution for both the strains.

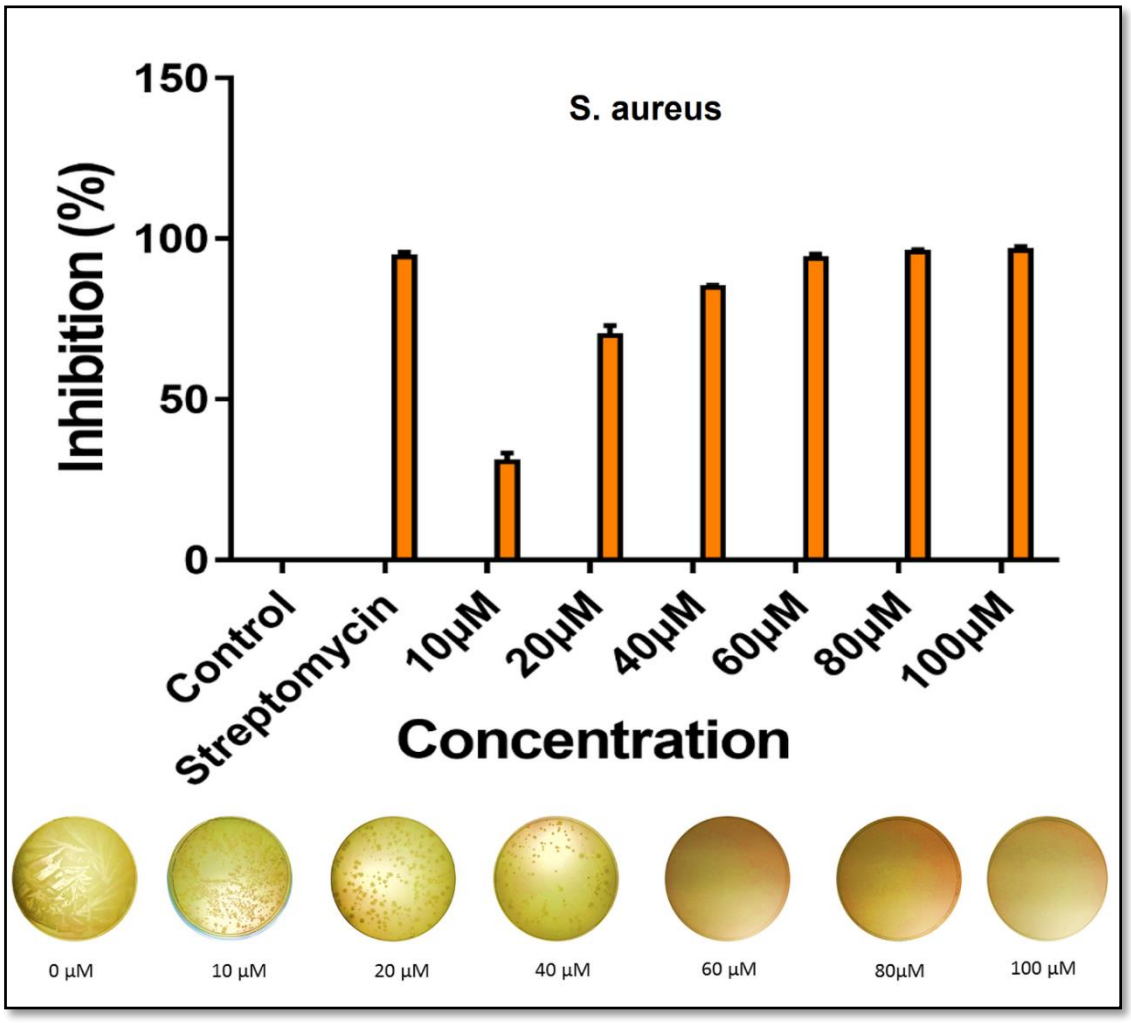


Figure 5.17: Bacterial Inhibition of S.aureus at different concentration of silver nanoparticles and Colony formation change at different concentration.

Earlier, Kim et al. stated that Gram positive *S. aureus* shows more resistance against silver nanoparticles than Gram negative *E. coli*[18] but our study showed that effect of silver nanoparticles on both E.coli and *S.aureus* are almost identical. The different percentage of inhibition are given below in table given below. The overnight treated culture was spread in Nutrient Broth plates to see the colony formation. The plates also depicted that there was no growth from and after 60 µM concentration of Silver Nanoparticles solution. The colonies gradually decreased as the concentration of nanoparticles increased.

E.coli		
Conc.	% Inhibition	S.D
10µM	15.9564	1.931669
20µM	62.54238	1.070651
40µM	86.90789	1.086595
60µM	93.75178	0.65047
80µM	96.54003	0.265585
100µM	97.25928	0.100994
Streptomycin	96.22636	0.155804

S.aurues		
Conc.	% Inhibition	S.D
10µM	31.36097	1.636528
20µM	70.63501	2.02652
40µM	85.50451	0.197707
60µM	94.69312	0.529005
80µM	96.54738	0.142163
100µM	97.17334	0.382138
Streptomycin	95.08287	0.680814

Table 5.3: Bacterial inhibition Percentage at different concentration of silver nanoparticle for E.coli and S.aurues

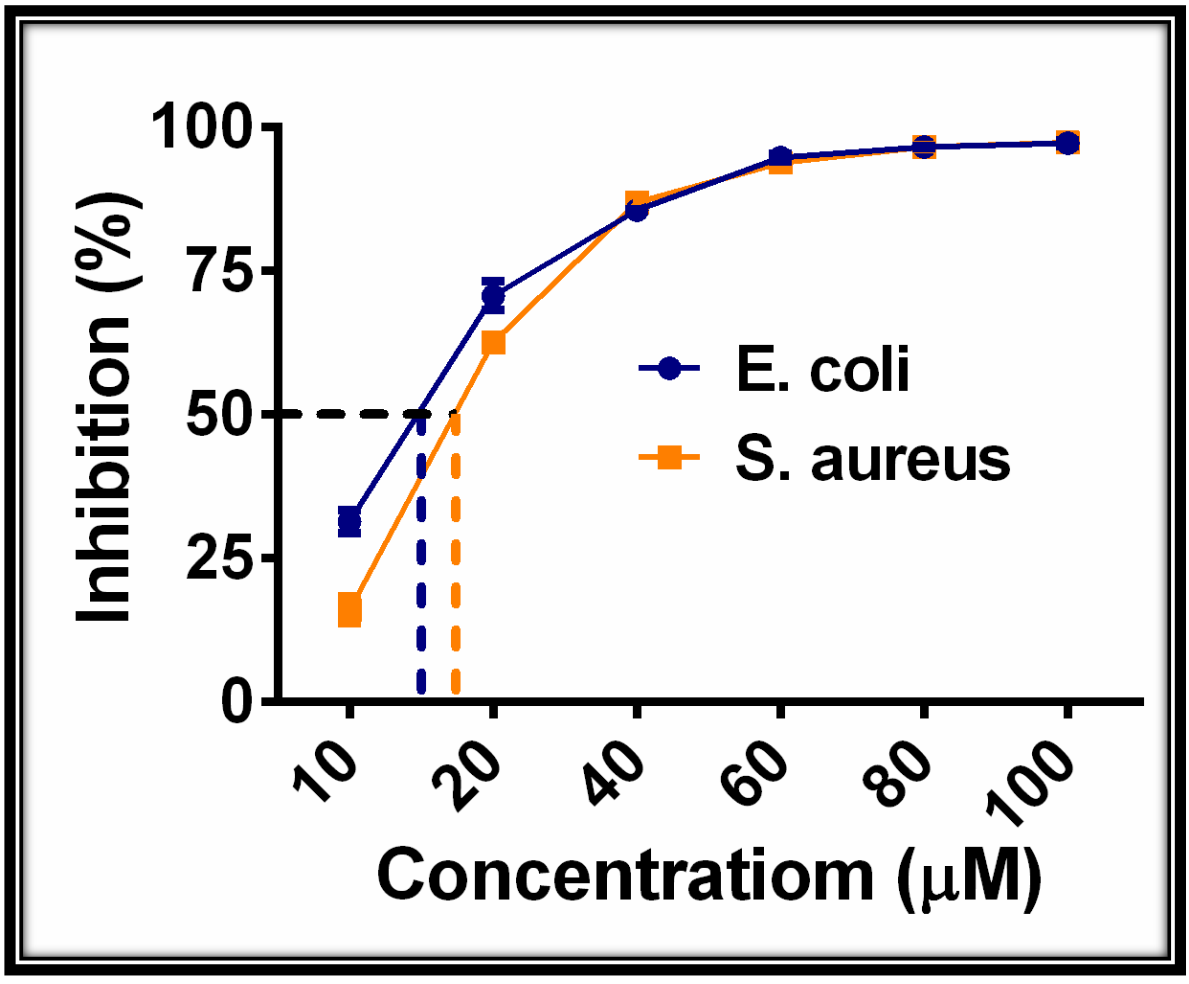


Figure 5.18 : . MIC_{50} curves for E.coli and S.aureus

The MIC_{50} for S.aureus and E.coli was calculated and for S.aureus it was 18 µM and for E.coli it was 15µM. From such low value of MIC_{50} it is quite evident that the biosynthesized silver nanoparticles by Hops extract are quite effective as antibacterial drug.

5.2.4 Anti-inflammatory effect of biosynthesized silver nanoparticle

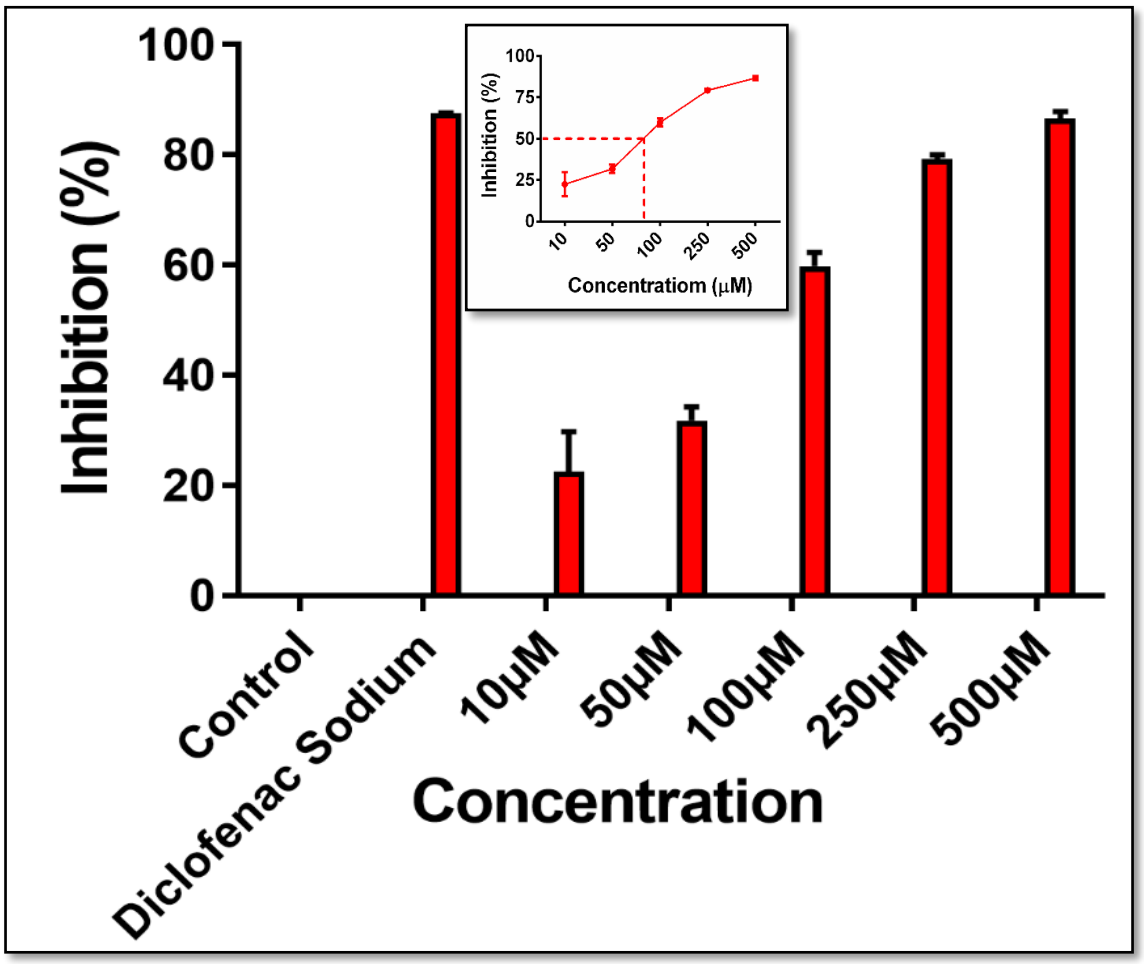


Figure 5.19 :. Protein denaturation inhibition and IC_{50} curve at different concentration of Silver nanoparticle

Anti-inflammatory effect of Hops extract capped silver nanoparticles showed significant inhibition in denaturation of BSA protein. The results are indicated in Figure 5.19. The inhibition level increased as we increased the concentration of silver Nanoparticles. It showed a significant anti-inflammatory effect when compared to standard drug diclofenac sodium (628.5 µM). The IC_{50} value for biosynthesized silver nanoparticles was found to be 84 µM with is much lower than the IC_{50} of standard drug Diclofenac sodium (145 µM) Thus it is quite evident that the Hops capped biosynthesized Silver nanoparticles exhibited potential inhibition of BSA protein denaturation even at lesser concentration in compared to commercially present anti-inflammatory drugs .This can be justified by the synergistic anti-inflammatory effect of Silver nanoparticles and the phytochemicals present inside Hops. Majorly alpha acids and beta acids are well known anti-inflammatory agent and their presence as capping agent over silver nanoparticles may have enhanced the property of the silver nanoparticles as an anti-inflammatory agent.

Protein Denaturation is a well-documented reason for Inflammation. So to investigate the effect of the silver nanoparticles as an anti-inflammatory agent and to find the mechanism of inhibiting protein denaturation the BSA was treated with the standard drug and silver nanoparticles so as to get a comparative information of its action. It is proved that denaturation of protein is one of the many causes for inflammation. Denaturation of protein causes production of autoantigens which in terms causes inflammation in few rheumatic diseases[19]. Earlier studies have pointed out how anti-inflammatory drug have been used to

inhibit denaturation of protein and how nonsteroidal anti-inflammatory drugs have inhibited protein denaturation at high temperature and are used as commercialized anti-inflammatory drugs[20,21]. Thus, agents having property of inhibiting protein denaturation can be used further for the development of anti-inflammatory drug. Moreover commercially available anti-inflammatory drugs cause's problems like gastric irritation but as the agent used in this study is derived from natural source it is expected to have a safe approach towards human body. In terms as we are using very low concentration of silver nanoparticles it is also expected to show very low toxicity.

5.3 Result and Discussion for Characterization and biomedical use of Silver nanocomposite Film

5.3.1 Characterization

The SEM analysis of the silver nanoparticles loaded Gelatin-PVA films are shown in Figure 5.20 The nanocomposite film has exhibited a dense and uniform plain microstructure. The nanocomposite film also shows the presence of defined nanoparticles in the film. The cross-sectional view of the film indicated a uniform structure with a width of 53.73 μm confirming the formation of thin film.

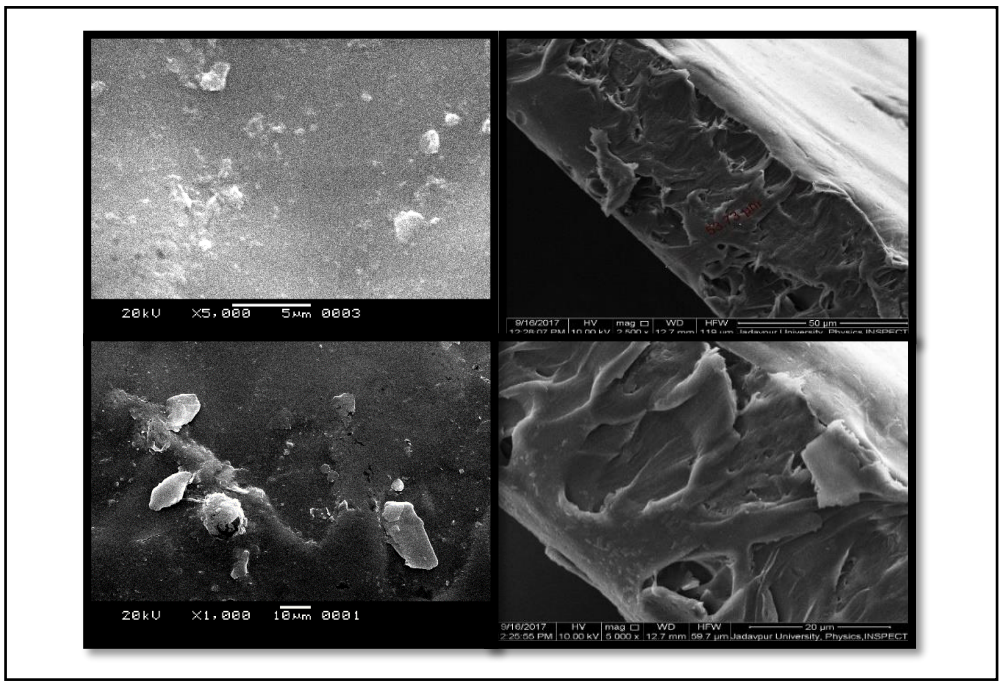


Figure 5.20: SEM analysis of nanocomposite film; a. surface view, b. cross-sectional view

The interfacial interaction between AgNPS and Gelatin –PVA nanocomposites was confirmed by FT-IR spectra (Figure 6). The infrared spectrum of film features band at 3702 cm^{-1} and 3717 cm^{-1} indicating presence of Amine (N-H stretching) which is for gelatin. Two major peaks at 3397 cm^{-1} and 3311 cm^{-1} indicates the presence of O-H stretching or this is majorly due presence of alcohol which is PVA in this case .Bands at 3084 cm−1 & 2927cm^{-1} due to the stretching of the (C-H) group. The peaks at 1641 cm^{-1}, 1534 cm^{-1}, 1449 cm^{-1}, 1335 cm^{-1}, 1229 cm^{-1}, and 1086 cm^{-1} could be attributed to the presence of aromatic C=C bonds stretching vibrations. In FTIR spectra, the bands at 674 cm^{-1} and 624 cm-1 confirm the presence of a skeletal vibration of C-C groups.

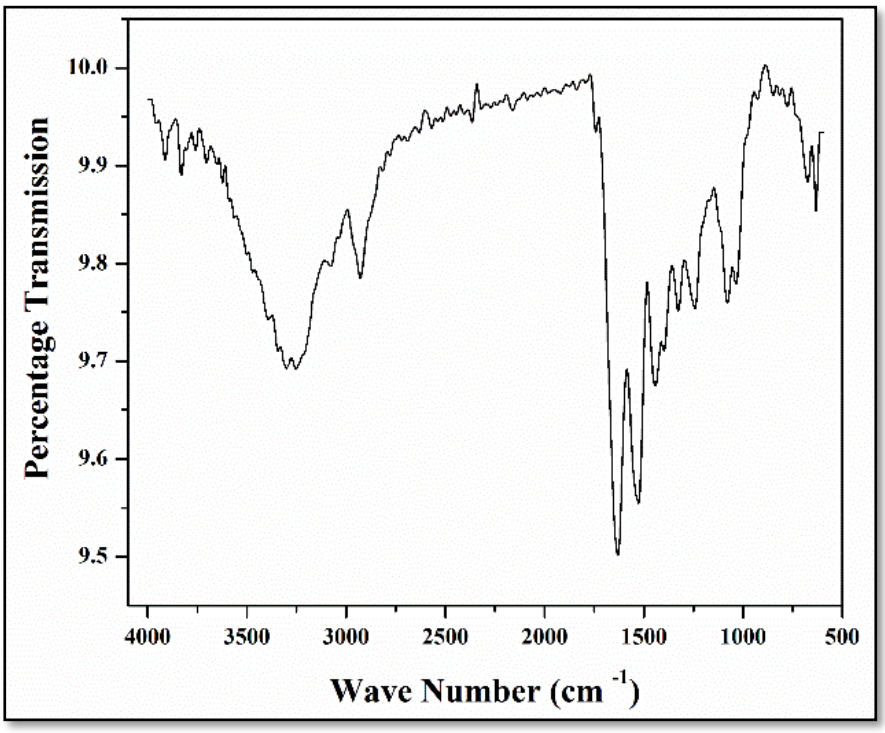


Figure 5.21: FTIR Spectrum of the Nanocomposite films

Figure 7 illustrates the thermogravimetric analysis of Nanocomposite films. The initial weight loss (below 100 ^{o}C) observed in the films is due to loss of moisture present in the films. ; The second weight loss starts at about 160 ^{o}C, indicating melting of nanocomposite chains and finally the film shows the greatest weight loss in the temperature range of 280–290 °C, which is believed due to the disintegration of intermolecular and partial breaking of the molecular structure. Overall the film showed a smooth degradation curve. Thermal degradation starts at around 275 °C. So it will be stable during the sterilization needed before medical application.

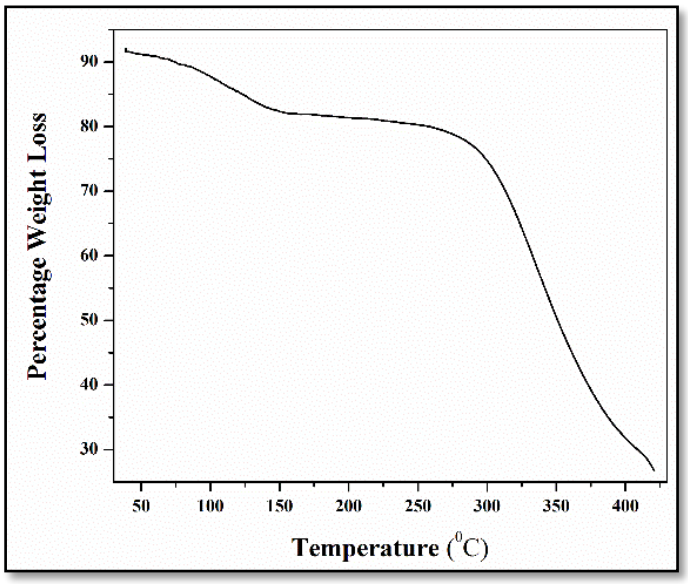


Figure 5.22: TGA of the Gelatin-PVA Nanocomposite films

The contact angle was measured using glycerin and was found to be 41.4 ° which indicates that the nanocomposite films have a good wetting property or else it can be interpreted that it is highly hydrophilic. As water and Glycerin have near values of surface energy so the contact angle with glycerin and water are likely to same i.e below 90° [22].

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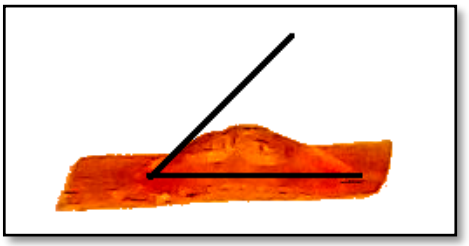

Figure 5.23: Contact angle with respect to Glycerin

The hardness of the polymer nanocomposite film was tested using Shore D meter and the harness of the film is 6 in the Shore D measurement scale. This indicates that the material is extra soft and can be used for biological purpose [23].

The mechanical properties of the AgNp nanocomposite film was calculated and it showed that the film has an Ultimate Force (N) of 78.6 and Ultimate Stress (MPa) 39.3. So, this membrane could be used for wound covering, as it can withstand some frictional stresses during day-to-day activities. The membrane is stitched around the wound surface so as to cover the wound. If there are any frictional stresses, the membrane absorbs the energy without breaking and thus protects the wound.

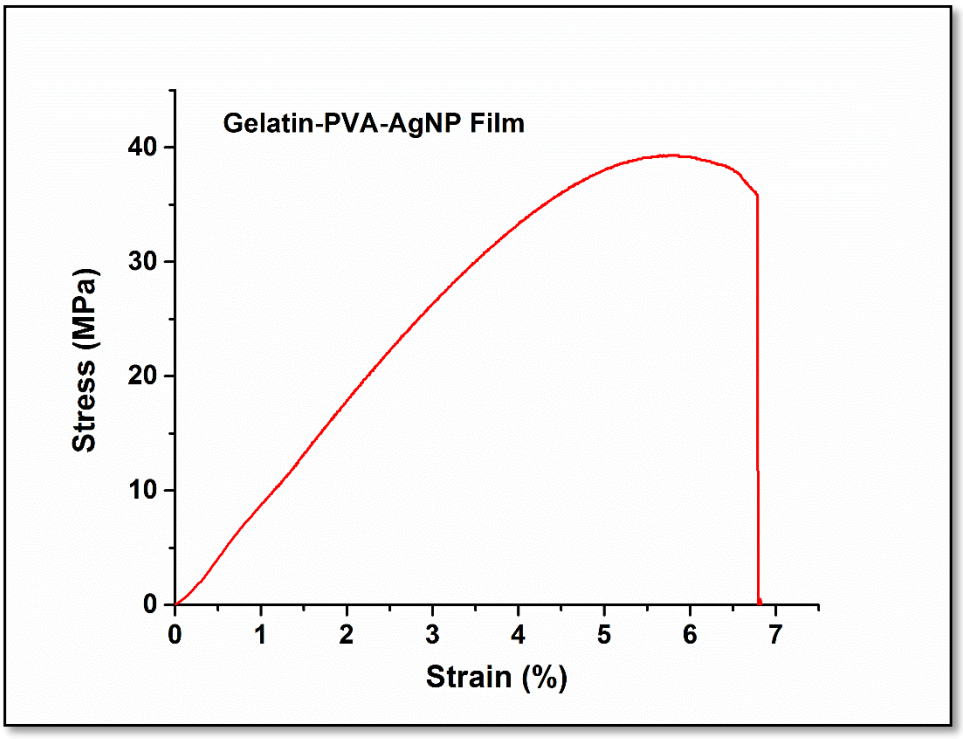

Figure 5.24 : Stress vs Strain curve for AgNp nanocomposite film

The swelling study reveals that the nanocomposite films gave maximum swelling at basic pH followed by SBF solution. The Swelling (%) reached up to 1500 % in terms of pH -9 solution. it can be categorized as superabsorbent. From the FTIR result for the membrane, it is clear that the whole carboxylic group of the gelatin has been esterified but the free primary amine groups are present. From this fact it can be hypothesized that these free amino groups play an important role in water uptake because of their hydrophilic nature.

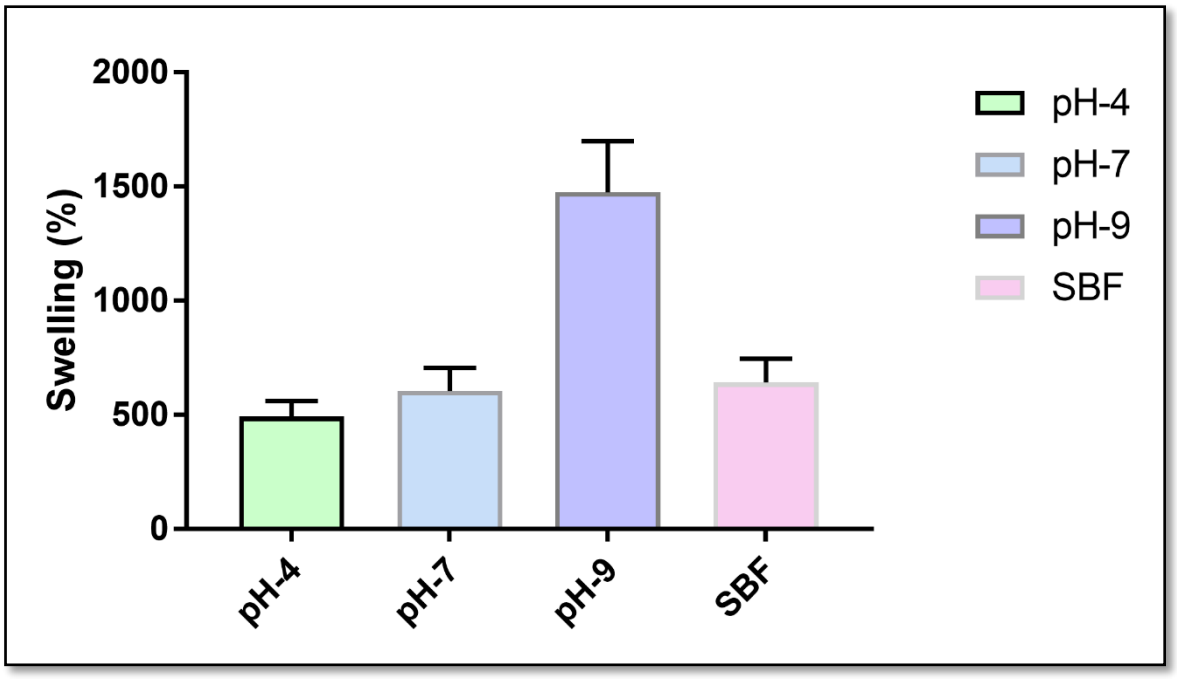

Figure 5.25 : Swelling Study of AgNp composite film

5.3.2 Hemocompatibility

Table 5.4: Hemocompatibility Test (Human Blood)

Sample	O.D at 545 nm	% Hemolysis	Remarks
+ve Control	0.6629		-
-ve Control	0.0038		-
Gelatin-PVA Silver Nanocomposite	0.0384	5.25%	Hemocompatible

It can be seen from Table 5.4 that the Nanocomposite films are highly compatible with human blood as the percentage hemolysis id around 5 % which is regarded as hem compatible range. It is reported earlier that Silver nanoparticles alone are highly toxic [24] but here in when it is used as a nanocomposite its toxicity is highly reduced as a total system. But this was only a preliminary test and vigorous biocompatibility tests need to be done with further experiment in animal model.

5.3.3 Antibacterial Activity of AgNp nanocomposite film.

The antibacterial activity is a demonstration of the release of silver nanoparticles from the polymer network. Silver nanoparticles exhibit relatively large surface area, thus increasing their contact with bacteria. Silver nanoparticles show powerful bactericidal activity by binding with microbial DNA, thereby preventing

bacterial replication .The use of Ag-containing Gelatin-PVA films as functional wound dressings is assessed by observing their antimicrobial activity (based on the disc diffusion method) against some common bacteria like *Escherichia coli & Staphylococcus aureus.*

Antimicrobial activity of pure, film and encapsulated silver nanocomposite films, were evaluated from their capacity to inhibit bacterial cultures along with one standard anti-bacterial medicine, Streptomycin (100 µg/mL) available in market was kept as control. The Minimum inhibition concentration of Streptomycin form E. Coli is 32 µg/mL, and that for S. aureus is 42 µg/mL [25]. Figure 9 shows the disc diffusion technique result for different bacterial culture. The experiment contained to sample to confirm the activity.

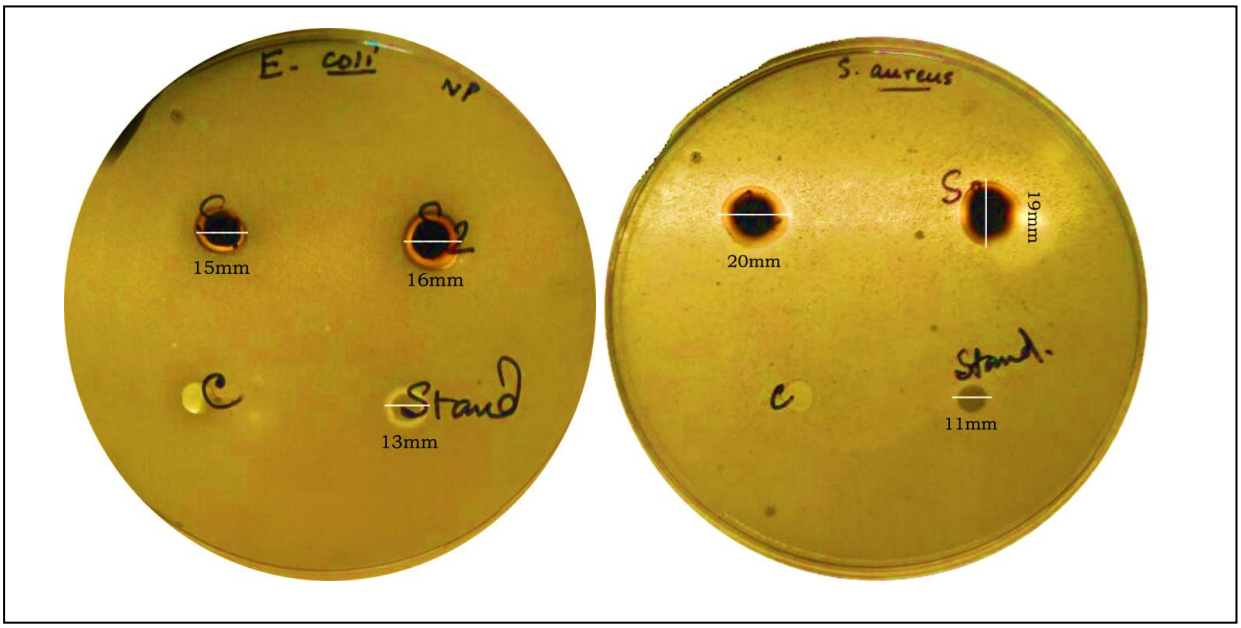

Figure 9-A Figure 9-B

FIGURE 5.26: Anti-Bacterial Assay using Silver Nanocomposite Gelatine/PVA films

The Ag nanoparticles synthesized and encapsulated in Gelatin /PVA films showed inhibition zone against all test organisms. The Zone of inhibition (ZOI) of AgNP for Escherichia coli (16mm) was greater than that of the standard antibiotic streptomycin (13mm). (Table 3) (Fig 9).

Table 5.5 : Zone of Inhibition using AgNp Composite film

Bacterial strains	**Zone of inhibition(AgNPs-Gelatine /PVA FILM)**	**Zone of inhibition (Standard Drug)**
Staphylococcus aureus	20mm	11mm
Escherichia coli	16mm	13mm

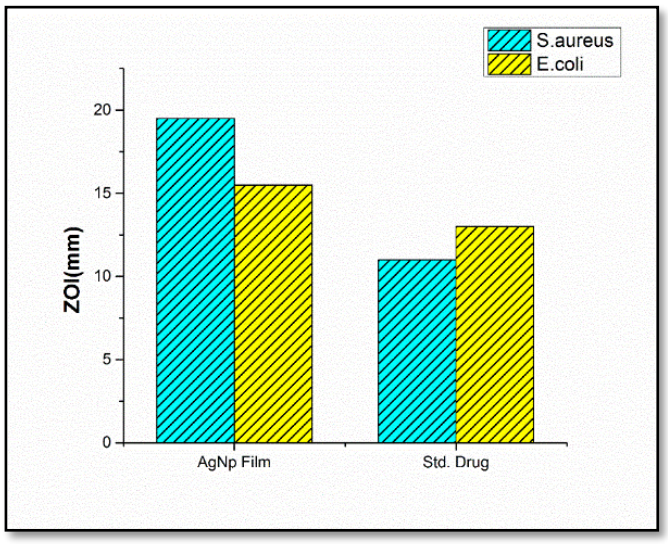

Figure 5.27: Antibacterial assay Results

5.3.4Toxicity of AgNp Film

The % Cell Viability of PBMC cells on the nanocomposite film was 82.14% (Figure 11) and the cytotoxicity grades of the nanocomposite films was 1 as indicated in Table 5.4. According to the cytotoxicity grading criteria, the nanocomposite film qualifies as a non-cytotoxic biomaterial.

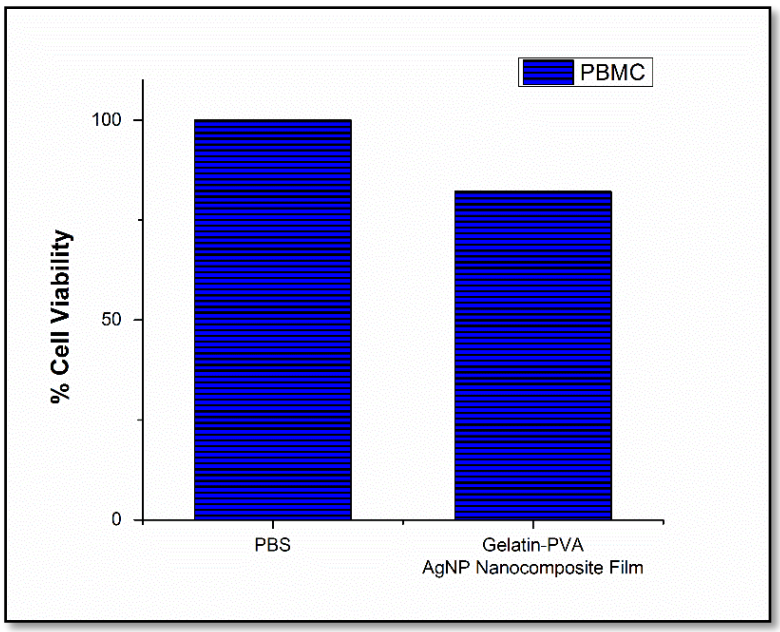

Figure 5.28: Percentage cell viability assay

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CHAPTER 6:

CONCLUSION AND FUTURE WORK

6.1 Conclusion

6.2 Future Aspects of Work

6.1 Conclusion

This study concludes that HALLERTAUER Hop extract is rich in polyphenols, flavonoids and tannin along with it is also evident that the extract is also enriched with various phytochemicals. This phytochemicals altogether contributed to the anti-oxidant and anti-inflammatory property of the extract which is very prominent from the results depicted above. Both anti-oxidant and anti-inflammatory property of the extract was screened keeping a standard as a reference to confirm the potency of the extract. The cytotoxicity effect of the extract was also tested on HeLa cell line and PBMC cells and the extract showed dose dependent and selective toxicity towards cancerous.

Silver is kwon for a long time as an agent with antimicrobial activity. However, with the appearance of antibiotics, silver's use decreased due to the high efficiency of antibiotics. With the onset of new bacterial strains resistant to several antibiotics, silver has regained its former usage when nanotechnology techniques started to be used. AgNPs are now considered promising candidates to be used in the combat of the several infections caused by different bacterial strains.

There are several methods to produce AgNPs. In this study, AgNPs were produced by green synthesis method by using HOPS extract. However, the AgNPs produced without stabilizing agents could aggregate and subsequently be toxic for human cells. But the phytochemicals in the Hops played a vital role in capping the silver nanoparticle and finally contributing to the biological activity of AgNp as well. The silver nanoparticle was characterized with DLS, UV-VIS, XRD, TEM which pointed the presence of biosynthesized silver nanoparticle. This silver nanoparticles showed good antibacterial effect and it also exhibited in-vitro anti-inflammatory effect.

This Silver nanoparticle was used finally to make a Silver nanocomposite film. . The Films were produced using traditional casting method and was finally cut into appropriate shape for its further characterization. The films which were finally obtained was tested against gram positive and gram negative bacteria for analyzing the antibacterial efficiency of the AgNP Films. The result of the antibacterial assay showed positive result in two of the bacteria tested i.e S. Auries and E.Coli. as compared to standard antibacterial drug Streptomycin.

The developed silver nanocomposite films have exhibited fairly good mechanical strength and superior hemocompatibility properties. Further, the current work demonstrates a promising method to combine silver Nano-composites with a natural compound (Lupulus). The synthesis of Gelatin PVA silver nanocomposite film showed substantial antibacterial activity on both Gram-positive and Gram-negative bacteria. Indicates a potential use of the nanocomposite in the pharmacological, biomedical and industrial fields, such as bandages, wounds dressing, anti-bacterial gloves and dental tools.

Gelatine-PVA hydrogel combined with the AgNPs produced by the reduction with HOPS, would be a good choice for coating an orthopedic implant to avoid the bacterial colonization, i. e., and the biofilm formation. Moreover, these particles may also be valuable candidates to be applied in skin regeneration, since AgNPs also possess an anti-inflammatory effect. Additionally, gelatin-PVA coated nanoparticles are currently used as drug delivery systems due their high efficiency in drug encapsulation. Therefore, it would be interesting to study the incorporation of growth factors in these

systems for bone and skin regeneration. In a near future, *in vivo* studies will allow to validate the results herein produced.

The present study presents a non-toxic as well as eco-friendly procedure for synthesizing AgNPs. This technique gives us a simple and efficient way for the synthesis of nanoparticles with good antibacterial properties. Antibiotic resistance by the pathogenic bacteria has been observed since last decade; hence, the researchers are focusing on the development of new antibacterial agents which can overcome such resistance. Hence, our current study proves to be an important step in this direction.

6.2 Future Aspects of Work

- Identification, isolation and purification of the antitumor bioactive compounds from Hops whole leaf extract.
- Dug Formulation from the potentially active component of Hops extract.
- Finding the correct pathway for inhibiting cancer growth.
- Evaluating the effect on cell cycle and apoptosis.
- In-vivo Anti-inflammatory effect of silver nanoparticle in mouse model
- Immunotherapy using AgNp for curing cancer.
- Gene delivery vector formulation.
- Bacteriological study to treat resistant bacteria
- In-vivo study of nanocomposite film for wound healing application
- Finally preparation of antibacterial gloves for defense