1

Introduction

Allicin (Diallyl thiosulfinate) is a main bioactive component derived from garlic (*Allium sativum*), which exerts a broad range of biological properties including anticancer, antioxidant, anti-inflammatory, and antimicrobial effects **(Choo *et al.*, 2020)**. Allicin is produced when garlic is crushed or chopped, through the enzymatic conversion of Alliin by Alliinase enzyme. Despite their broad therapeutic applications, the medicinal applications of Allicin is limited by their sensibility to heat, alkaline conditions, instability, poor bioavailability, rapid degradation in aqueous form and other physiological conditions. Therefore, this limits the efficacy of Allicin in clinical and pharmaceutical applications. There is lack of standardised protocol for the extraction of Allicin, stabilisation and formulation, which exhibits lack in research outcomes and further hinders their advancement from experimental use for clinical applications **(Borlinghaus *et al.*, 2014)**.

Considering the significance of Allicin and to address the existing research gap, the researcher take up this research to enhance the extraction yield, bioavailability and drug formulation for improving its pharmacological effects. Appropriate extraction solvents are essential for effective extraction of garlic. Ionic liquid (ILs) are increasingly preferred as green solvents to extract bioactive compounds from plants because they are water soluble, non-flammable, low nucleophilicity and vapour pressure. Hence, we propose this IL-extraction. To address the limitations associated with the instability and poor bioavailability of Allicin, advanced drug delivery systems such as floating tablets, and microsphere-based sustained-release formulations are being explored. Sustained-release drug carriers are essential components of drug delivery systems, designed to release the therapeutic agents gradually over time, thereby maintaining a consistent drug concentration into the bloodstream, reducing dosing frequency, and minimising side effects.

These systems are particularly advantageous in overcoming the challenges associated by conventional drug delivery system, which often lead to rapid degradation and short half-life of unstable compounds like Allicin. Floating tablets can prolong gastric residence time, enhancing the adsorption of drugs that are unstable or poorly adsorbed in the intestine, whereas microspheres offer protection from environmental degradation and allow controlled release. Furthermore, nanoencapsulation an innovative solution for improving drug stability and delivery.

Smaller quantity of nanoparticles acts as drug; hence we explored the use of nanoencapsulated formulations for pharmaceutical applications.

With the rapid advancements in nanoscience and nanotechnology, our understanding of materials at the nanoscale has significantly transformed. These scientific breakthroughs and innovative technologies have created massive attention over time. Nanoparticles, typically sized between 1 and 100 nm, play a vital role in nanotechnology. Nanoscience and nanotechnology are interdisciplinary fields which explore various scientific principles to create nanomaterials with specific functional properties **(Ozin *et al.,* 2009)**. Overall, nano science and nanotechnology has expanded the scope of nanoparticle applications across various fields, including nanomedicine, drug delivery, healthcare, electronics, biosensors, environmental science, food packaging, gene delivery, and agriculture **(Yattinahalli *et al.,* 2016)**.

These intriguing applications of nanoscience and nanotechnology in drug delivery is the impetus in choosing this research work. Based on the problem statement, the present research work is carried out in two main phases: (I) Drug innovations for the bioactive compound-based formulations in pharmaceutical applications, and (II) Sustainable synthesis of metallic nanoparticles aimed at enhancing the drug formulations and evaluating their in vitro biomedical applications.

1.1 Importance of Allicin and Organo sulphur bioactive compounds

Garlic (*Allium sativum L*) holds a long-standing history as both a culinary staple and a traditional remedy for various ailments and diseases. Garlic has thirty-three sulphur compounds which includes Alliin, Allicin, Ajoene, S-allyl-L-cysteine, Diallyl disulphide, S-allyl mercapto cysteine, Diallyl trisulfide, Vinyl dithiin, and several enzymes and seventeen amino acids, minerals, and volatile oils **(Omar and Wabel, 2010)**. Among its many bioactive constituents, Allicin is considered to stand out as a major bioactive compound, responsible for garlic's antibiotic properties. Allicin is produced when garlic is crushed or chopped, involving the conversion of Alliin by Alliinase enzyme. This compound was first identified by Cavallito and Bailey in 1944, marking the significant understanding of garlic's medicinal properties. The main compound Allicin, a thiosulfinate which comprises more than 70% thiosulphinate in fresh garlic extract. Allicin, a bright yellow, oily liquid characterised by its distinctive garlic odour; however, it is highly unstable. It readily decomposes when exposed to temperature, forming more stable compounds such as Ajoene's and Vinyl dithiins **(Choo *et al.,* 2020)**. Allicin shows a wide range of biological actions including neuroprotective, antimicrobial, antioxidant, antithrombotic,

antiplatelet, anticancer, antihypertensive, antilipidemic, anti-inflammatory, antidiabetic, and immunomodulatory effects. It also shows potential in liver protection, and wound healing **(Savairam *et al.*, 2023)**. However, the clinical utilisation of Allicin remains challenging due to difficulties in developing stable formulations for effective therapeutic delivery. Several factors affect the stability of Allicin, includes heat, storage duration, pH, and processing temperature.

Several extraction techniques can be used for isolation of Allicin, the efficiency of these extraction methods largely depends on the hydrophobic nature of the compound. The loss of Allicin activity has been observed in water and 100% ethanol compared to other solvents, primarily due to its chemical instability **(Harris *et al.*, 2001; Fujisawa *et al.*, 2008)**. The author studied the pharmacokinetics and stability of Allicin in simulated gastric fluid, intestinal fluid, blood, and various solvents, revealed more stability in methanol than in ethyl acetate. Moreover, 90% Allicin was retained in stimulated intestinal fluid and simulated gastric fluid, with pH values of 7.5 and 1.2, respectively after incubation at 37 ˚C for 5 h **(Batiha *et al.*, 2020)**.

The use of appropriate extraction solvents is required to ensure an effective extraction of garlic, which influence the yield, stability, and prevent rapid degradation of bioactive compound.

1.2 Ionic liquid Extraction and its importance

There are various approaches employed to isolate bioactive constituents from plant materials. Traditional extraction methods including maceration, percolation, infusion, distillation, and Soxhlet extraction are commonly used to produce organic extracts. However, researchers in natural product chemistry have increasingly attempting to substitute these conventional extractions with more advanced ionic liquid-based extraction, which include microwave assisted, ultrasound assisted, pressurized, high-speed homogenisation, and pulsed electric field **(Barcelo, 2017; Soquetta *et al.*, 2018; Chatel *et al.*, 2019)**. The conventional extraction methods required large quantities of solvent, extended extraction durations, and considerable energy demand, which resulted in reduced extraction efficiency and the degradation of thermolabile products **(Han *et al.*, 2016)**. To address the limitations associated with conventional organic solvent extraction, ionic liquids (ILs) are increasingly preferred as emerging alternatives for isolating bioactive constituents from plant materials. Conventional organic solvents such as methanol, chloroform and toluene are utilized widely in extraction and synthesis but causes serious environmental problems due to their volatility, flammability, and toxicity, considered to cause air and water pollution. In contrast, ILs are non-volatile, recyclable, and offer safer alternatives by

minimizes solvent loss and aligns with the green chemistry principles. ILs consist of salts in the liquid state, which are composed of an inorganic anion (such as Br^-, Cl^-, BF_4^-, PF_6^-) paired with an organic cation, including ammonium, imidazolium, pyridinium, or phosphonium. ILs exhibit characteristic properties including good water solubility, non-flammable, low nucleophilicity and no vapour pressure **(Jessop *et al.*, 2012)**. ILs also possess inherent characteristics that include low room temperature volatility, excellent thermal stability, and negligible vapour pressure which helps to avoid solvent loss to air. They also use small solvent volumes, offer high purity, can be recycled in separation and purification steps, improved yields, shorter extraction durations, exhibit good conductivity, and polarity, and requires less energy. These characteristics make ILs more environmentally friendly than traditional volatile organic solvents **(Xiao *et al.*, 2018)**. Although ILs serve as highly efficient extractive solvents, their cost remains higher than commonly used volatile organic solvents. In large-scale industrial process, ILs can be recycled and reused to some extent. The recycling and reusability of ILs are essential for both environmental and economic applications. Over the past few decades, scientists have introduced several methods for recovery and reuse of ILs, such as distillation, back extraction, crystallization, membrane separation, aqueous biphasic system, and adsorption process **(Zhou *e t al.*, 2018a)**.

Considering the importance of ILs as greener solvent systems for extracting bioactive constituents from plant materials, has prompted the utilisation of ILs as extraction solvent in this research work.

1.3 Pharmaceutical and Drug Delivery Systems

Pharmaceutical forms of drugs were designed to deliver the active ingredients into the biological system through various forms including oral dosage solutions, emulsions, suspensions, capsules, ointments, tablets, powders, injections, aerosols, and granules. Factors influencing the dosage form development includes drug properties such as solubility, stability, protein binding, and dissolution rate as well as the patients' disease state and route of administration. Oral dosage forms including pills, tablets, granules, capsules, and powders are frequently administrated due to feasibility, better patient compliance, effective therapy, and cost-effective manufacturing process. As described in the Indian pharmacopeia, pharmaceutical tablets are solid, flat, bilayered, unit dosage preparations produced by compressing the active ingredient together with a various excipient, and may be formulated with/without diluents **(Tekade, 2019)**. Drug delivery systems (DDS) have been effectively used in treating various diseases improving health conditions through systemic circulation and the pharmacological effect of drug. The controlled release of

drug formulation was first introduced in 1950s, which offer significant advantages over conventional drugs. Conventional DDS possesses certain limitations such as poor bioavailability, rapid drug metabolism, variations in plasma concentrations, required frequent dosing, and the inability to maintain prolonged drug delivery. In a sustained release system, the drugs are designed to be delivered at a controlled rate directly to the target site, ensuring maximum efficacy, improved bioavailability, and safety.

Controlled DDS has arrived from macroscale to nanoscale to attain targeted drug delivery with a single dose form, by reducing the multiple dosages requirements **(Ezike *et al.*, 2023)**. In controlled DDS, biomaterials are specifically designed to interact with biological environment for therapeutic use, which regulate pharmacokinetics of drugs through their physicochemical properties and drug release patterns. Various biomaterials, including polymers, lipid proteins, peptides, and polysaccharides, are used in drug delivery approaches in varying macro to nanoscale and different route of administrations. These materials need to be biocompatible and non-toxic **(Adepu and Ramakrishna, 2021)**. In drug formulation, the choice of polymers includes natural (biopolymer), synthetic and semi synthetic polymers depend on various factors like stability, biocompatibility, biodegradability, desired drug release profiles and specific drug formulation requirements. Synthetic polymers including poly ethylene glycol, polycaprolactone, poly acrylic acids, poly amides, poly vinyl alcohol, poly glycolic acid, poly vinyl pyrrolidone, and poly lactide-co-glycolide are utilised in drug formulations for their biocompatibility and gradual biodegradability **(Farshid *et al.*, 2022)**.

In oral DDS, sustained release dosage forms are developed to release the active compound at fixed time intervals, providing uniform drug release over an extended period with minimum side effects and preventing fluctuations in drug levels. Various drug delivery approaches are available such as extended release, controlled release, delayed release, site specific targeting, receptor targeting and sustained release. Each method has its advantages and disadvantages. It is essential to correlate the pharmacological activity and therapeutic effectiveness of drug with greater bioavailability, necessitating the validation of sustained release drug formulations **(Sampath *et al.*, 2012)**. Among these, floating tablets and microspheres have gained considerable attention for enhancing gastrointestinal retention time and improving sustained drug release with narrow absorption windows. Floating tablet remain buoyant in gastric fluids, which allow the drug to be released over extended duration in gastric region, and beneficial for long term chronic disorders. These oral dosage forms contain low-density excipients or gas-generating agents like sodium hydroxide or citrate which allows them to float

on the gastric fluids. On floating, the early passage to the intestine is avoided and provides the prolonged drug release in the stomach **(Davis *et al.*, 1986)**.

Microspheres has been emerged as promising approach in controlled or sustained drug delivery approaches as they are able to encapsulate active pharmaceutical ingredients within a polymer matrix, allowing the drug release profile in a controlled form. These are spherical particles measuring between 1 and 1000 µm, composed of either natural or synthetic polymers which provides protection of drug from the environmental degradation by allowing controlled drug release profiles. Microspheres offer several advantages, such as improved bioavailability, reducing dosage frequency, and enhanced patient compliance. Considering the advantages of sustained DDS, such as floating tablet and microspheres, enables us to formulate sustained release type formulations to enhance the therapeutic efficacy through prolonged drug release **(Ruan *et al.*, 2022)**.

In lieu of the aforementioned importance of drug delivery systems, this research work focuses on formulating sustained release tablets for pharmaceutical applications

1.4 Nanoparticles

Nanoparticles (NPs) are particulate substances whose characteristic dimensions falls below 100 nm, whereas submicron particles are with the size ranging from 0.1 µm to 1 µm. These nanomaterials are categorized into different structural categories: zero, one, two or three dimensional based on geometric shape **(Tiwari *et al.*, 2011)**. For example, 0D nanomaterials are confined spatial dimensions, whereas 1D nanomaterials, the electron movement occurs along a single direction (x-axis). Similarly, 2D and 3D nanomaterials allow electron movement along the x-y or x-y-z axis, and their dimensions are less than 100 nm. The importance of nanomaterials arises from their nanoscale and unique optical and physicochemical characteristics. NPs have classified based on carbon, metal, ceramic, semiconductor, magnetic, polymeric, and lipid NPs **(Khan *et al.*, 2017)**.

NPs can be uniform or composed of several layers. These layers often are (1) a surface layer, which can be modified with small molecules, polymers, metal ions or surfactants, (2) an intermediate shell layer, formed of material that differs from the inner core, (3) a core layer, refers an inner core forming central portion of NPs **(Shin *et al.*, 2016)**.

NPs synthesis works on two approaches: top-down and bottom-up. The top-down route works to produce smaller particles by breaking down larger materials through physical or mechanical techniques such as ball milling, mechanical milling, physical vapour deposition,

sputtering, laser ablation, electro-explosion, and chemical etching **(Iravani *et al.*, 2011)**. Similarly, the bottom-up method builds-up NPs from smaller components through certain techniques namely sol gel, spinning, green synthesis, template-support, chemical vapour deposition, plasma, flame spraying, atomic or molecular level condensation, laser pyrolysis, and biological synthesis (plant parts, algae, fungi, yeast, and bacteria). Recently, there have been growing interest in green and biogenic NPs synthesis method owing to its cost effectiveness, less toxicity, eco-friendliness, scalability, biocompatibility and easy to synthesise **(Khan *et al.*, 2017)**.

Based on the importance of NPs, the present study focuses on eco-friendly route for the metallic NPs synthesis, characterisation and their selected applications.

1.5 Metallic nanoparticles

Metal NPs have emerged as major focus within the nanomaterial research. Precious metals including gold, platinum, and silver are known for their therapeutic values and are commonly used as precursors in producing metal NPs **(Bhattacharya *et al.*, 2008)**. These NPs are produced by various synthesis route namely physical, chemical, and biological methods. The structure, shape, size and physicochemical characteristics of metallic NPs depend on the experimental techniques used during synthesis **(Chen *et al.*, 2001)**. NPs exhibit larger surface area than bulk materials, which enhances the functionality in various fields of biomedicine, cosmetics, biosensing, pharmaceuticals and drug delivery.

The noble metal gold has been widely used in medicine and Ayurvedic preparations in India and China for centuries **(Bhattacharya *et al.*, 2008)**. Gold nanoparticles (GNPs) are excellent candidates for diagnostic, target site specific drug delivery, and therapeutic applications. The size of GNPs increases as the Surface Plasmon Resonance (SPR) band shifts to longer wavelength. Functionalization and bioconjugation of GNPs helps to design a promising novel biomaterial for biomedical applications. GNPs also attributed to exhibit optoelectronic properties, redox activity, Surface Enhanced Raman Scattering, fluorescence quenching, low toxicity and biocompatibility **(Chen *et al.*, 2001; Dumur *et al.*, 2011; Sarkar *et al.*, 2011; Menon *et al.*, 2017)**.

Silver nanoparticles (SNPs) have extensive attention owing to their unique properties and their wide range broad applications in biomedical, food packaging, drug delivery, agriculture, antimicrobial textiles, biosensing, and water treatment **(Srikar *et al.*, 2016; Zhou and Tang *et al.*, 2018; Amini and Azadfallah *et al.*, 2018)**. The most notable characteristic is Localized Surface Plasmon Resonance (LSPR) which results from the excitation of free electrons

upon visible light. The excited electrons move to a higher energy level and returns to base level by releasing photons **(Thangaraju *et al.,* 2012)**. Successful formation of SNPs, resulting in a wavelength range between 380 and 470 nm, depends on particle shape and size **(Fiorati *et al.,* 2020)**. The phytochemicals present in plants could reduce Ag^{+} to Ag^{0}, leads to SNPs formation. The physical and chemical synthesis methods allow precise shape and size, they often require toxic chemicals. Therefore, biological and plant mediated synthesis approach offer safer, environment friendly, cost-effective, and scalable alternatives in pharmaceutical and biomedical fields **(Lee *et al.,* 2019 and Siddiqi *et al.,* 2018)**.

The longstanding use of gold and silver in traditional medicine inspired an exploration of their respective metal nanoparticles.

1.5.1 Plant-mediated Metallic Nanoparticle Synthesis

The metal NPs can be synthesised through physical or chemical methods, but these processes involve highly toxic or reactive reducing agents which are harmful to the environment and adversely affect human health. Green synthesis of NPs can be produced through different plant parts, microbes, fungi, and bacteria referred as "bio-nano factories" which are environmentally friendly, effective and affordable **(Dumur *et al.,* 2011; and Sarkar *et al.,*2011)**. The plant-based extraction process is encouraged for large-scale metallic NPs production to fulfil the increased global demand for NPs.

There are various reports, adopted the greener synthesis of metallic NPs in recent years. Phyto mediated green synthesis of SNPs using *Momordica cymbalaria* **(Sundar *et al.,* 2024)**, *Fraxinus angustifolia* **(Jallali *et al.,* 2024**), *Syzygium aromaticum* -Indian clove **(Aldabaan *et al.,* 2024)** and greener synthesis of GNPs using *Streptomyces albogriseolus* **(El-Naggar *et al.,* 2024)**, *Coleus scutellarioides* **(Al-Mafarjy *et al.,* 2024),** *Thespesia lampas* **(Nath *et al.,* 2024)** and *Passiflora ligularis* **(Al-Radadi *et al.,* 2023)** has been reported. ***Considering the advantages of green synthesis, the present study focusses on utilising plant derived materials for producing metallic NPs.***

We have utilised the following plants for the synthesis of GNPs and SNPs– *Amphilophium paniculatum* (AP) – leaf, *Tristellateia australasiae* – leaf, *Haematocarpus validus* – *fruit*, and *Phoenix dactylifera* – seed. The selected plants have been traditionally recognised for their medicinal, pharmacological and therapeutic effects, including anti-inflammatory, analgesic, antipyretic, antioxidant, antihyperglycemic and anthelmintic activities **(Nassar *et al.,* 2013; *Bazana et al.,* 2020; Mo, 1996; Momin *et al.,* 2018; Alkhoori *et al.,* 2022; Begum *et al.,* 2023).**

The bioactive compounds from the above selected plants were compiled and taken for *in silico* studies to further explore their potential biological interactions and applications.

1.6 *In silico* studies

The conventional approach for identifying and formulating new drugs is a long-term pathway that typically requires an investment of about 2.8 billion dollars to develop a single medication to market **(Wouters *et al.*, 2018*)***. Both *in vitro* and *in vivo* research are expensive and time consuming, and the developed new drug must be non-toxic and safe for use in humans. Therefore, an extensive screening of lead molecules is essential in the early stage of drug development. Computer-aided drug design tools, facilitate the speed, minimise drug inefficiency, requires lesser time in predicting the drug like lead compounds and minimising the cost involved in synthesis and discovery process. Recently, computer-aided drug design modelling has benefited researchers, by helping to predict the lead compounds that can inhibit the particular disease conditions. The virtual screening of large sets of compounds, which recognise a subset of particular compounds that binds to the target receptor, thus recognised as the potential lead compounds **(Rajalakshmi *et al.*, 2021)**.

There are various computer-aided drug design tools available, both commercially and as open source software. To design a drug-like compound, the proper screening of physicochemical properties of ligands, such as drug-likeliness, lipophilicity, Adsorption, Distribution, Metabolism, Excretion and Toxicity (ADMET). ADMET of selected lead compounds provides a route to drug discovery by filtering out the non-druggable compounds by Lipinski's rule of five. Subsequently, the toxicity of selected molecules is an essential parameter to screen the compound hazards such as eye irritation, skin sensitization, mutagenic, tumorigenic, reproductive effects, biodegradability, carcinogenicity and genotoxicity (**Sander *et al.*, 2015; and Guerra *et al.*, 2017).**

Furthermore, molecular docking programs helps to determine the drug lead compounds through active binding energy sites in the target receptor molecule through docking positions, conformations, and orientations. The stronger potential binder is indicated by the lead compounds with higher negative energy score. Various open source docking software are available. Among them Hex 6.3 is a tool for interactive molecular graphics with two types of docking models namely protein-protein and protein-ligand interaction. Energy calculations are accelerated through spherical Fourier correlations. Schrodinger software platform has benefitted biopharmaceutical, industries, academic institutions and also in government laboratories to attain

novel lead compounds for particular disease conditions **(Rajalakshmi *et al.*, 2021)**. ***So, we have utilised Hex, an internet freeware and commercially available Schrodinger software to carry out molecular docking.***

Diabetes mellitus has emerged as a significant economic challenge worldwide over the last thirty years. According to projections by the International Diabetes Federation (IDF), the number of individuals aged 20 to 79 living with diabetes could reach approximately 642 million by the year 2040 **(Ogurtsova *et al.*, 2017).** Prolonged use of commercially available synthetic drugs frequently resulting in severe adverse side effects and health issues **(Bi *et al.*, 2011)**. Target receptors for *Diabetes mellitus* includes glycogen phosphorylase, insulin receptor, aldose reductase and Dipeptidyl peptidase -4 (DPP-IV), among which DPP-IV has been recognised as key target involved in inactivation of incretin hormones. The development of DPP-IV inhibitors such as sitagliptin and vildagliptin, has provided an effective therapeutic option for managing Type 2 *Diabetes mellitus*, prolonged use of these drugs has been reported to cause gastro intestinal problems **(Vella *et al.*, 2007)**. Therefore, bioactive phyto component-based drug formulations are required for treating Type 2 diabetes **(Nisha, 2017 and Ahmed *et al.*, 2018)**.

The bioactive phytoconstituents from the selected plant derived extracts were retrieved from pub chem database and were evaluated for their physicochemical characteristics, ADME, toxicity profiles, and molecular docking interaction score with the DPP-IV target receptor.

1.7 *In vitro* Biomedical applications of metallic nanoparticles

Biomedical science is an interdisciplinary field that involving the application of biological and physiological principles, to study human health and disease mechanisms for enhancing healthcare. *In vitro* biomedical approach uses cell cultures, enzymes or microbial systems under controlled laboratory conditions by studying the biological interactions and therapeutic responses outside the living body **(Madhu *et al.*, 2022)**.

Green synthesised metallic NPs, GNPs and SNPs are used in drug delivery, anticancer drugs, antimicrobial agents, antidiabetic therapies, medical diagnostics, bioimaging, tumour targeting, multi-microbe resistant medicines, molecular biology and cellular biology.

The aforesaid application necessitates me to explore the biological activities of green synthesised GNPs and SNPs, particularly their antioxidant, antibacterial, anticancer, and antidiabetic properties.

1.7.1 Antioxidant activity

Unpaired electrons in the atomic orbitals of molecular species are referred as free radicals. Being unpaired, these free radicals are highly unstable or reactive as they either loss or gain electrons of other molecules. Free radicals damage biological cells, including DNA, carbohydrates, lipids, proteins, and nucleic acids, which lead to chronic diseases namely ageing, cancer, diabetes, arthritis, and neurodegenerative diseases (**Young *et al.,* 2001)**.

Free radicals, including various forms of reactive oxygen species (ROS), are produced within the body as part of cellular metabolism, such as during cell respirations and can also arise from external exposures including prolonged exposure to X-Rays, smoking, ozone (O_3), industrial chemicals, UV radiations, and other air pollutants **(Bagchi *et al.,* 1998)**. This necessitates the requirement of antioxidant rich substances, to break the undesirable chain reactions and making stable molecule by donating electrons, thus reducing the potential for cellular damaging. Antioxidants, available in natural sources such as bioactive compounds, nutritive-rich diets as well as nanoencapsulated antioxidant-rich supplements, can improve stability and efficacy **(Levine *et al.,* 1991)**.

Many bioactive compounds such as polyphenols and flavonoids, which are naturally found in plant sources, act as free radical scavengers **(Brown *et al.,* 1998)**. Recently, NPs have been considered for their superior antioxidant properties due to their higher effectiveness. Among several metal-based NPs (gold, silver, platinum), GNPs are of more interest due to their unique opto-electronic properties, high stability, biocompatibility and surface energy **(Ge *et al.,* 2022).** Several methods are available to assess the antioxidant capacity, such as 2,2 Diphenyl-picrylhydrazyl (DPPH) assay, ferric ion reducing antioxidant power, 2,2'-azinobis (3-ethyl benzothiazoline 6-sulfonic acid, oxygen radical absorbance capacity using trolox as a standard, and cupric reducing antioxidant capacity methods **(Chaves *et al.,* 2020).**

1.7.1.1 DPPH assay

One of the most widely used technique in assessing the antioxidant activity is the DPPH method, which was originally described by **Blois (1958)**. Single electron of nitrogen in DPPH is reduced to hydrazine by abstraction of hydrogen atoms from antioxidants. This method is extremely fast, and does not require expensive chemicals or sophisticated instruments **(Gulcin, 2012; Gulcin and Alwasel, 2023)**.

DPPH assay is used in this research work to determine the free radical neutralising ability of the extracts and biosynthesised GNPs and SNPs.

1.7.2 Antimicrobial activity

The emergence of Multidrug Resistant Bacteria's (MDRB) are considered to be a serious problem due to the continuous and inappropriate usage of antibiotics, improper selection of antimicrobial drugs and frequent switching between medications. This increased ineffectiveness of antibiotics has led to fatal outcomes and becomes riskier.

To overcome the associated MDRB, several strategies have been developed, including the modification of drug structures, drug combinations from plant extracts, as well as metallic and metal oxide NPs. Metallic NPs (Au, Ag, CuO, ZnO and Pt) have been reported for their antimicrobial capabilities due to particle size, geometric form, and surface functionalization, which ensures the potential candidates for MDRB **(Hancharova *et al.*, 2024)**.

Green synthesised metallic NPs, such as GNPs and SNPs, are widely recognised for their antimicrobial activity. The metal NPs can bind more well with the sulphur or phosphorous in the bacterial cell membrane, which hinder the cell wall synthesis, destroying bacterial proteins and leads to the loss of bacterial activity **(Alavi and Hamblin, 2023)**. GNPs and SNPs synthesised using bottlebrush extract exhibited strong inhibitory effects against multiple bacterial strains, including *E. coli*, *S. aureus*, *K. pneumonia*, and *P. aeruginosa* **(Khan *et al.*, 2024)**. Another promising formulation of contact lens preservation that was developed through conjugation of amikacin with GNPs was also found to be more effective against *P. aeruginosa* compared to both amikacin and GNPs **(Jawad *et al.*, 2024)**.

Pondering the need for the solutions to multi drug resistant bacteria's, the present study focuses on assessing the antimicrobial efficacy of synthesised GNPs and SNPs against bacterial strains.

1.7.3 Human lung cancer activity

Cancer is one of the most vulnerable disease, arising from the abnormal and unregulated cell proliferation. Recent data from the WHO, indicate that, in 2022, approximately 20 million individuals were newly diagnosed with cancer, and about 9.7 million deaths attributed to the disease worldwide **(WHO, 2024)**. Cancer has been predicted to increase by 77% by 2050, due to population growth, and life style choices such as tobacco use, unhealthy diets, obesity, physical inactivity, radiation exposure and alcohol consumption. According to global cancer observatory, the lung cancer remains predominately common among male and ranks second among female **(GLOBOCAN, 2022; WHO, 2023)**.

Treatment of lung cancer includes chemotherapy, radiotherapy, and targeted therapeutic approaches often resulting in adverse effects and development of drug resistance. Thus, there is a need for an alternative safe and new therapeutic drug formulations. Phyto-based metallic NPs have notable potential eradicating effects of cancer cells. GNPs and SNPs are more preferred because of their physicochemical and potential anticancer effects **(Mejía-Mendez *et al.*, 2023)**, their bioavailability, biocompatibility and can be easily functionalized with ligand molecules. Owing to the multifaceted properties, GNPs makes them better candidates for both disease detection and therapeutic applications **(Guinart *et al.*, 2020; Kumari *et al.*, 2023).**

Based on the preceding discussion, the present study focuses on assessing their efficacy against human lung cancer cell lines.

1.7.4 Antidiabetic activity

Diabetes mellitus considered to be chronic metabolic disorders that are treated by insulin resistance or deficiency due to high level of blood sugar. Over the past thirty years, Type 2 diabetes has been increased massively across all income level countries. Type 2 diabetes that is mostly known to affect adults arises as a result of resistant of body to insulin or lack of cellular response towards insulin. Juvenile (insulin dependent) diabetes, referred as Type 1 diabetes, whereby the pancreas produces less or no insulin **(Disanto *et al.*, 2015)**. The Indian Diabetes Federation (IDF), noted that the number of affected patients in worldwide will increase to 643 million adults by 2030 as opposed to 537 million (currently). For controlling Type 1 and Type 2 diabetes, regular check-ups and a healthy glycemic index are necessary. The range of glycemic index of the normal healthy blood glucose levels are 70 to 140 mg/dL or 4 to 8 mM **(American Diabetes Association, 2013)**.

Type 2 diabetes caused by combination of factors such a reduced physical activity, obesity, and unhealthy diet. If left untreated, it can lead to complications such as blindness, heart, nerves, kidney damage and increased susceptibility to microbial infections **(Sarwar *et al.*, 2010).** Gestational diabetes happens when the body is unable to produce sufficient insulin to sustain the rising glucose levels, resulting in high blood sugar levels. This condition fades away once after child birth; however, if not managed, it puts one at risk of evolving Type 2 diabetes in future **(Oliveira *et al.*, 2023)**.

Several orally medications are utilised for *Diabetes mellitus*, including metformin, glipizide, sitagliptin, sulfonyl urea, acarbose, are used to manage glycemic index. Medicinal plants offer promising benefits with minimal side effects due to the presence of bioactive

constituents. Most plant derived extracts containing phytoconstituents, which include flavonoids, saponins, terpenoids, alkaloids, glycosides, and carotenoids are considered as potential source for antidiabetic drugs. Reviews on the antidiabetic potential of medicinal plants, as well as the assessment of edible plants for α-amylase inhibitory activity, have been extensively explored **(Salehi *et al.*, 2019; Firdhouse and Lalitha., 2016)**. Similarly, the eco-friendly synthesised GNPs and SNPs from *Fagonia cretica* and *Terminalia bellirica* extracts possess better antidiabetic activity through α-amylase inhibition **(Khan *et al.*, 2023; Smina *et al.*, 2021)**. The encapsulation of metformin with the natural gum-based microspheres was developed using ionotropic gelation method. The cross linker - aluminium chloride was used to control and modulate its drug release profile where 90% sustained drug release was noted in the intestinal medium at 6 h (pH 6.8) **(Yahoum *et al.*, 2023)**.

The present research explores the prospective applications of selected plants, along with their synthesised GNPs, SNPs and nanoencapsulated microspheres, for in vitro antidiabetic activity through α-amylase inhibition

1.7.5 Toxicity Assessment of Nanoparticles

Growing demands for the development of nanomaterials for wide variety of medicinal applications is due to their surface-enhanced physicochemical properties. However, the increasing use of NPs for commercial purposes or direct/indirect human exposure has become a concern among the researchers/scientific community regarding NPs' toxicology. The primary goal of toxicological assessment is to identify the toxicity effects of NPs on both human health and environment. Rapid and simplified techniques for assessing, predicting and identifying the NPs toxicity needs to be developed. The use of animal-based models for testing the NPs' toxicity, is expensive and labour intensiveness. According to institutional animal care use committee guideline, *in vivo* study must ensure ethical clearance for the treatment of animal models **(Rajabi *et al.*, 2015)**.

Allium cepa (onion) root tip assay is recognised as a simple and valid plant-based test system to evaluate the genetic toxicity of various chemical substances. This is due to the continuous growth of its meristematic root which contain high proportion of actively dividing cells and clearly distinguishable monocentric chromosomes ($2n = 16$), required microscopic evaluation **(Fiskesjo, 1997)**. Moreover, it provides several observable cytogenetic markers such as chromosome damage, change in chromosome number, and disturbances in chromatid behaviour which makes its suitable for detection of genotoxicity. The use of *A. cepa* for studying

the chromosomal level aberrations has a long history for cytological investigations in the 1920s. Furthermore, international organizations including the United Nations Environment Programme and the International Programme on Chemical Safety, have recognized *A. cepa* bioassay as a standardised and acceptable method for assessing the pollutants and toxic chemical substances **(Grant, 1999; Mangalampalli *et al.*, 2017)**. ***Hence, A. cepa* root tip assay *has been used for toxicological evaluation of plants extracts and synthesised GNPs and SNPs.***

1.7.6 Textile Applications in Protective Clothing

Textiles have been an important part of human history as they act as essential protective barrier but also representation of cultural and ethnicity, aesthetic interest, and technological progress. History of textiles production reflects continuous innovation, and cross-cultural exchange that shaped the societies in various times **(Roach-Higgins and Eicher, 1992)**. Cotton fabrics are the most widely preferred due to their softness, comfort, excellent absorption of bodily fluid and biodegradability. Despite these benefits, cotton fabrics are more susceptible to microbe attack due to their high moisture retention properties; polymer linkage in fabrics is more prone to microbial enzymes after the removal of protective layers during fabrics processing. In addition, natural fabric act as energy sources for microbes in the form of carbohydrates/ proteins **(Gao and Cranston, 2008; Gupta, 2007)**. Microbial enrichment depends on the conditions of bacteria/fungi–fabric contact and the physicochemical properties of textile materials.

Staphylococcus aureus (*S.aureus*), micrococcus species, and odour causing *Corynebacterium* species are the most dominant skin-based bacteria. For e.g., pathogenic bacteria on hospital coats or uniforms worn by medical professionals contain *S. aureus* and *Enterococci* species **(Babb *et al.*, 1983; Wong *et al.*, 1991)**. Moreover, the hospital-based microorganism may stay alive on fabrics for several days to months **(Neely and Maley, 2000)**. Joseph Lister demonstrated the first report on the relationship between textiles and diseases in 1967, who investigated antiseptic-aided bandages for treating wound infections **(Lister, 1967).**

To address the rise of bacterial and fungal populations and to reduce the pathogenic infections, the development of targeted antimicrobial agents for textile applications has become essential. The increasing resistance of microorganisms to antibiotics, further contributes to the need of antimicrobial textile materials **(Balakumaran *et al.*, 2016)**. An effective way to reduce microbial attack is the frequent laundering of clothes, but this is impossible in a hospital

environment where microbial infection shifts from one person to another. The need for microbial-resistant clothes is particularly important for sanitary and sewage-related workers, where the infection rate is high.

Surface modification and treatment of fabrics include plasma treatment, ozone treatment, ultraviolet treatment, microencapsulation, sol-gel and electrospinning techniques, which are required to impart the functional properties of textile materials in water-repellent hydrophobic textiles, flame retardant, self-cleaning, and antimicrobial fabrics **(Nadi *et al.*, 2018)**.

The physical surface treatment methods help to improve the functional properties of fabrics. Eco-friendly treatment such as ozone/UV and oxygen plasma can alter fabric surface, by improving characteristics such as dye uptake and adhesion. The literature reports, pre-treated fabrics or plasma treatment of fabrics improves the sorption properties of silver coating in material **(Prysiazhnyi *et al.*, 2013; Rani *et al.*, 2018)**, material roughness **(Caschera *et al.*, 2013)**, enhances the flame-retardant properties **(Lam *et al.*, 2011)**.

Other technologies, such as Ozone gas (O_3) and Ultraviolet radiation (UV), achieve oxidizing efficiency and have the capacity to enhance the wettability of fabric materials. Therefore, several authors have utilised hydrophilic surface modification through O_3 treatment, UV treatment and combined O_3/UV treatment to modify the surface of polyester or blended polymer fabrics to consequently improve the sorption properties of dyes **(Gabardo *et al.*, 2021; Ibrahim *et al.*, 2010; Michael *et al.*, 2004)**.

Using chemicals and mordants in textiles exhibits health risks and disturbs the natural ecosystem. Overuse of chemicals and antibiotics leads to the development of multi-resistant microbes. Therefore, a sustainable green chemistry approach is required to dye the fabrics and simultaneously helps to inhibit microbes' growth in textile materials.

Nowadays, people are not much aware of skin damage and skin cancer that occurs due to the prolonged exposure to UV radiations like UVA (400 to 320 nm), UVB (320 to 280 nm) and UVC (280 to 200 nm), respectively. The increase in skin damage/cancer risk occurs a thousand times more when the radiation wavelength decreases at 280 nm. The clothing material is one such essential way to protect our body from various radiations exposure. The UV Protection factor (UPF) is categorised as per the Bureau of International Standard. If UPF (UV Protection Factor) ratings is less than 15 (no protection), 15 to 24 (good), 25 to 39 (very good) and > 40 (excellent) protection **(Sankaran *et al.*, 2021)**. UV protective textile materials are highly dependent on the kind of fabric; dye used, protective UVB agents or the use of nanoparticles.

Pondering the need for microbe resistant/UV-rays protective fabric, an attempt was made to utilise silver nanoparticles for coating on woven fabric.

1.8 Research gap

Identifying the research gap is most essential step in designing an effective research plan to address the research problem. Through a comprehensive review of existing literature, the research gap has been identified and addressed in the present study:

1. Lack of efficient extraction methods for isolating Allicin with high yield and stability
2. Poor stability of Allicin under heat, and other physiological conditions, leading to its rapid degradation.
3. Limited bioavailability of Allicin, hindering its clinical and pharmaceutical efficacy.
4. Absence of exploration of green solvents, such as ionic liquid, for the extraction of Allicin from garlic.
5. Minimal research on floating tablet and microsphere-based formulations designed to protect Allicin and prolong its therapeutic effect.
6. Limited exploration of sustained-release formulations incorporating natural bioactive compounds and metal nanoparticles to enhance and extend therapeutic efficacy.
7. Identification of suitable plant-based bioreductants, including *Amphilophium paniculatum*, *Tristellateia australasiae*, *Haematocarpus validus* and *Phoenix dactylifera* for the synthesis of GNPs and SNPs.
8. Lack of studies exploring the *in vitro* biomedical applications of synthesised GNPs and SNPs.
9. Development of microbial-resistant and UV-protective woven fabrics using synthesised SNPs and their nanocomposite, were explored.

These identified research gaps have inspired the present research to evaluate sustained drug-release formulation enhanced with nanoparticles, as well as to explore the selective biomedical applications of these NPs. A detailed study of these aspects is clearly outlined in the objectives of the present research.

1.9 Objectives of the study

1.9.1 Main objective

The advancement of nanotechnology has led to significant breakthroughs across diverse fields, particularly in biomedical and pharmaceutical research. The primary objective of the study is to develop innovative DDS *viz.* floating tablet and microspheres, for stable and sustained release of metabolites. Secondary objective includes enhancing the sustained release and selective *in vitro* biomedical applications.

1.9.2 Specific objectives

1.9.2.1 Ionic liquid-based Solvent Extraction of Organo Sulphur Compounds

- To extract garlic by using conventional organic solvent/ionic liquids.
- To employ chromatographic techniques for screening, isolation and quantitation of Allicin and organo sulphur compounds in garlic.

1.9.2.2 Sustained Release Floating Tablets and Microsphere- based Drug Delivery systems

- To standardise the formulation of sustained-release ciprofloxacin floating tablets
- To assess the pre and post compression evaluation of the developed tablet formulations.
- To formulate garlic loaded floating tablet for enhanced drug release applications
- To formulate garlic-loaded and drug-loaded microspheres to enhance sustained release
- To characterise the prepared formulations through analytical and microscopic techniques.
- To perform drug release (*in vitro*) of both tablets and microspheres
- To determine the drug release mechanism by applying mathematical models of drug release kinetics.

1.9.2.3 Sustainable Synthesis and Characterisation of Metallic Nanoparticles

- To prepare extracts of *Amphilophium paniculatum*, *Tristellateia australasiae*, *Haematocarpus validus* and *Phoenix dactylifera* and identified secondary metabolites through qualitative phytochemical screening.
- To synthesise GNPs and SNPs using the aqueous ethanolic extracts *via*. sustainable, green synthesis approach.
- To optimise the nanoparticle synthesis time by two different approaches: room temperature and solar irradiation.
- To evaluate the stability and surface charge of the synthesised NPs
- To characterise the synthesised metallic NPs using various analytical and microscopic techniques.

1.9.2.4 *In silico* Screening of Bioactive Compounds

- To screen physicochemical and ADME properties of bioactive compounds from selected plants

- To perform *in silico* toxicity predictions of selected compounds.
- To conduct molecular docking of selected ligands against DPP-IV receptor

1.9.2.5 *In vitro* Biomedical Applications of Biosynthesised Metallic Nanoparticles

- To assess the antioxidant activity of GNPs and SNPs
- To evaluate antibacterial activity of NPs against selected Gram-positive and Gram-negative bacterial strains.
- To investigate anticancer potential of the NPs using lung cancer (A^{549}) cell lines.
- To explore antidiabetic activity through *in vitro* enzyme inhibition studies
- To assess toxicity using *Allium cepa* root tip bioassay.
- To formulate and characterise nanoencapsulated microspheres
- To perform *in vitro* antibacterial and antidiabetic activity of garlic-nano encapsulated microspheres.
- To perform drug release (*in vitro*) studies of nano-encapsulated microspheres
- To determine the drug release mechanism by applying mathematical models of drug release kinetics

1.9.2.6 Industrial Applications of Metallic Nanoparticles

- To optimise cotton fabric surface treatment using O_3, UV and plasma under different exposure durations.
- To impregnate the synthesised NPs onto woven cotton fabric and assess its mechanical/ microbial/ UV protective property of coated woven fabrics

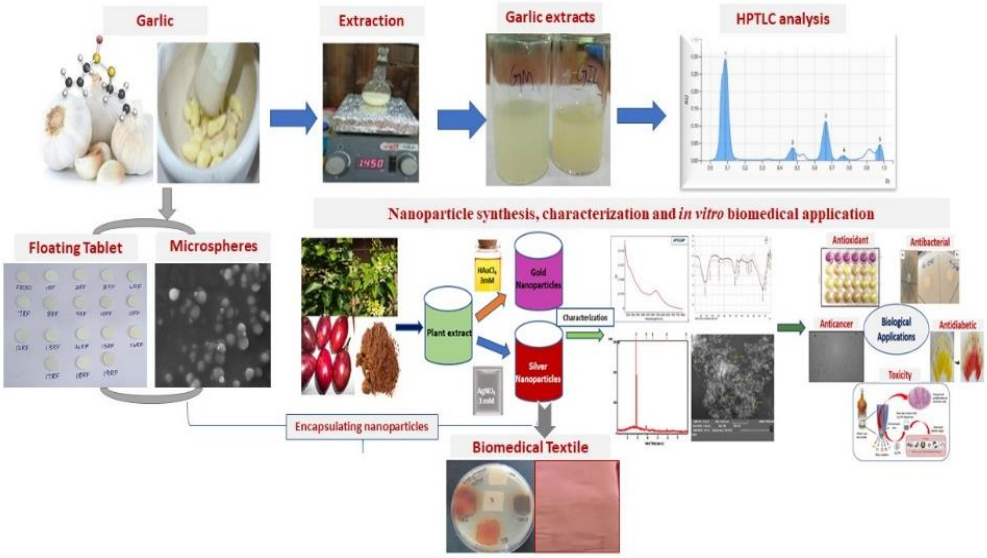

Figure 1. Systemic representation of the proposed work