3

Materials and Methods

3.1 General

The details of the chemicals, solvents, and instruments utilised in this study are provided in section 3.1.1 and 3.1.2. Necessary biosafety precautions were followed during the biological experiments.

3.1.1 Chemicals and Solvents

Solvents namely hexane, chloroform, ethyl acetate and methanol were brought from Loba Chemie Pvt. Ltd Mumbai, India. Hydrogen tetrachloroaurate trihydrate ($HAuCl_4.3H_2O$), polyethene glycol, silver nitrate ($AgNO_3$) and 2,2, diphenyl 1 picryl hydrazyl (DPPH) were acquired from Hi-Media. Ascorbic acid and potassium dichromate ($K_2Cr_2O_7$) were brought from Lancaster and Reachem chemicals. Alpha amylase (*Aspergillus oryzae* - plant amylase), bees wax, lactose, poly vinyl pyrrolidone, glacial acetic acid, acetocarmine, sodium bicarbonate, cellulose acetate, gelatin, 1-butyl 3-methyl imidazolium chloride and stearic acid which were purchased from Hi-Media laboratories Pvt. Ltd. (Mumbai, India). Sodium chloride (NaCl), starch, sodium potassium tartrate, dinitro salicylic acid, sodium hydroxide (NaOH), and sodium acetate trihydrate were obtained from Merck Pvt. Ltd Mumbai, India. All the solvents and chemicals utilised were analytical-grade reagents. Doubly distilled water was used throughout the experiment. Ciprofloxacin and metformin were purchased from a local pharmacy in Coimbatore.

3.1.2 Instruments and Equipment Used

Instruments used in the present research work are listed below.

- Digital weighing balance (Shimadzu ATY 224)
- Water bath (SAFIRE India Pvt. Ltd.)
- Magnetic Stirrer (REMI MS-500)
- Bio spec nano Spectrophotometer (Shimadzu 206-26300-48)
- pH meter (Deluxe (101))
- Fourier Transform Infrared Spectrophotometer (MIRacle 10, Shimadzu -00585; Thermoscientific Nicolet Summit X
- Spectrophotometer with Color lab (SS5100H (Colorlab + Color Matching Software))
- Thermal Gravimetric analyzer (EXSTAR/6300 analysis)
- Simultaneous Thermal Analyzer (STA) 000 PerkinElmer
- Field Emission Scanning Electron Microscope with EDX (TESCAN MIRA 3XMU)
- X-Ray Diffractometer (X-pert-Pro, PANALYTICAL)
- Plasma chamber (Ss304-Vacutech systems)
- Ozone purifier (Ozone Purifier & FVP 10-FG0206)
- Photo colorimeter (ELICO CL223)
- UV transmittance analyzer (UV-2000, Lab sphere)
- UV chamber (SUPERFIT, India)
- Heating mantle

- ❖ Texture analyzer (Shimadzu EZ-XS)
- ❖ Faraday Ozone (Ozone purifier & FVP 10-FG0206).
- ❖ Ball mill (RSB-1/060-718)
- ❖ Zeta potential analyzer (SZ-100V2-Horiba)
- ❖ ELISA multi plate reader (Thermo Multi-skan EX, USA)
- ❖ CAMAG HPTLC system with win CATS software
- ❖ Trinocular optical microscope (Bio blue lab)

3.1.3 Software

- ❖ Swiss ADME
- ❖ OSIRIS property explorer
- ❖ TOXTREE (v3.1.0)
- ❖ Hex (6.3)
- ❖ Maestro Schrodinger (v11.8)

3.2 Plant materials

The leaves of *Amphilophium paniculatum* (AP), *Tristellateia australasiae* (TA), *Allium sativum* cloves (G) and *Phoenix dactylifera* seeds (DS) were collected from Coimbatore district. The fruits of *Haematocarpus validus* (BF) were procured from Southern part of India. All plant specimens were examined and confirmed for authenticity by the Department of Botany, Avinashilingam Institute, and further verified by the Botanical Survey of India, Southern regional centre, Coimbatore, Tamil Nadu, India. Details of plant authentication with voucher specimen are provided in Appendix i, ii, iia and iii. The commercially available red sandal powder (RSP) was purchased from a nearby supermarket in Coimbatore.

The methodology of the present work has been carried out in the following phases:

Phase-I: Ionic liquid-based Solvent Extraction of Organo Sulphur Compounds
Phase-II: Sustained Release Floating Tablets and Microspheres for Drug Release Applications
Phase-III: Sustainable Synthesis and Characterisation of Metallic Nanoparticles
Phase-IV: *In silico* Screening of Bioactive Compounds Present in Selected Plants
Phase-V: *In vitro* Selected Biomedical Applications of Plant Extracts and Synthesised Metallic Nanoparticles
Phase-VI: Industrial Applications of Metallic Nanoparticles

Phase-I Ionic liquid-based Solvent Extraction of Organo Sulphur Compounds

- ❖ Extraction of Garlic
- ❖ Characterisation of Garlic Extract
  - ➢ Preparative Thin Layer Chromatographic (TLC) Analysis of Garlic Extract
  - ➢ Column Chromatographic Separation of Garlic Extract
  - ➢ FTIR analysis of Garlic Extract
  - ➢ Quantitation of Organosulphur Compound by HPTLC Analysis

Phase-II Sustained Release Floating Tablets and Microspheres for Drug Release Applications

- Floating Tablet for Sustained Drug Release Studies
  - Preparation and Standardisation of Floating Tablet
  - Pre-compression Study of Tablet Powders
  - Post-Compression Evaluation of Floating Tablets
  - Characterisation of Floating tablets
  - Preparation of Standard Calibration Curve of Ciprofloxacin
  - *In vitro* Dissolution Study
  - Stability of Floating tablet
  - Drug Release Kinetics
- Microspheres for Sustained Drug Release Studies
  - Preparation and Standardisation of Microspheres
  - Microsphere Preparation and Encapsulation Efficiency
  - Characterisation of Microspheres
  - Antibacterial Activity of Garlic loaded Microspheres
  - Preparation of Standard Calibration Curve of Metformin and Garlic Fresh Extract
  - *In vitro* Dissolution and Drug Release Kinetics of Microspheres

Phase -III: Sustainable Synthesis and Characterisation of Metallic Nanoparticles

- Extraction and Phytochemical Screening of Selected Plants
  - Extraction of Selected Plant Materials
  - Phytochemical Screening of Selected Plant Extracts
- Sustainable Synthesis of Metallic NPs
  - Preparation of Stock Solutions
  - Sustainable Synthesis of GNPs
    - ✓ Room Temperature
    - ✓ Solar Light Irradiation
  - Sustainable Synthesis of SNPs by Solar Light Irradiation
- Analytical Characterisation of Synthesised Metallic NPs
  - UV-Visible Spectroscopic Analysis of Synthesised Metallic NPs
  - FTIR Spectral Characterisation of Synthesised Metallic NPs
  - XRD Pattern of Synthesised Metallic NPs
  - Zeta Potential Measurements of Synthesised Metallic NPs
  - FESEM Imaging and EDS Analysis of Synthesised Metallic NPs

Phase-IV *In silico* Screening of Bioactive Compounds Present in Selected Plants

- Identification of Bioactive Compounds Present in Selected Plants
- Physicochemical properties and ADME Properties of Bioactive Compounds
- *In silico* Toxicity Prediction of Bioactive Compounds
- Molecular Docking Studies

Phase-V: *In vitro* Selected Biomedical Applications of Plant Extracts and Synthesised Metallic Nanoparticles

- Antioxidant Activity of Plant Extracts and Synthesised Metallic NPs
  - Preparation of Stock Solution

- DPPH Free Radical Scavenging Assay

❖ Antibacterial Activity of Extracts and Synthesised Metallic NPs
- Microorganisms used for Antibacterial Activity
- Antibacterial Screening of Extracts and Synthesised Metallic NPs

❖ Anticancer Activity of Synthesised Metallic NPs Against A^{549} Cells

❖ Antidiabetic α-amylase Inhibitory Potential of Extracts and Synthesised Metallic Nanoparticles

❖ Preparation of Starch and Phosphate Buffer Solutions
- Preparation of DNS Colouring Agent
- Preparation of Plant Amylase Solution
- Preparation of Standard and Sample Solution
- Test Procedure for α-amylase Inhibition Activity of Extracts and Synthesised Metallic NPs with *Aspergillus oryzae*
- Calculation of Percentage Inhibition

❖ *In vivo* Toxicity Evaluation of NPs - *Allium cepa* root tip assay

❖ Preparation of Nanoencapsulated Microspheres

❖ Characterisation of Nanoencapsulated Microspheres

❖ *In vitro* Antibacterial and Antidiabetic α-amylase Inhibitory Activity

❖ Thermal Stability Analysis of Bioactive compounds in Garlic/Garlic-nano Encapsulated Microspheres

❖ *In vitro* Dissolution and Drug Release Kinetics of Nanoencapsulated Microspheres

Phase-VI: Industrial Applications of Metallic Nanoparticles

❖ Fabrication of Microbial-Resistant and Ultraviolet Rays-Protective Fabrics for Textile Applications

❖ Desizing of Cotton Fabrics

❖ Preparation of Dye solutions

❖ Surface Pre-Treatment of Cotton Fabrics
- Effect of Exposure Time in O_3, UV and O_3/UV Treatment on Dyeing Performance of Cotton Fabrics
  - ✓ Combined dry O_3/UV Treatment
  - ✓ Combined dry O_3 (without water) /UV and wet O_3 (with water)/UV treatment
  - ✓ Combined dry O_3/UV/Plasma treatment
  - ✓ Plasma treatment

❖ Dyeing of Surface Pre-Treated Cotton Fabrics
  - ✓ O_3/ UV alone and Combined dry O_3/UV
  - ✓ Combined dry O_3/UV/ Plasma and Plasma alone

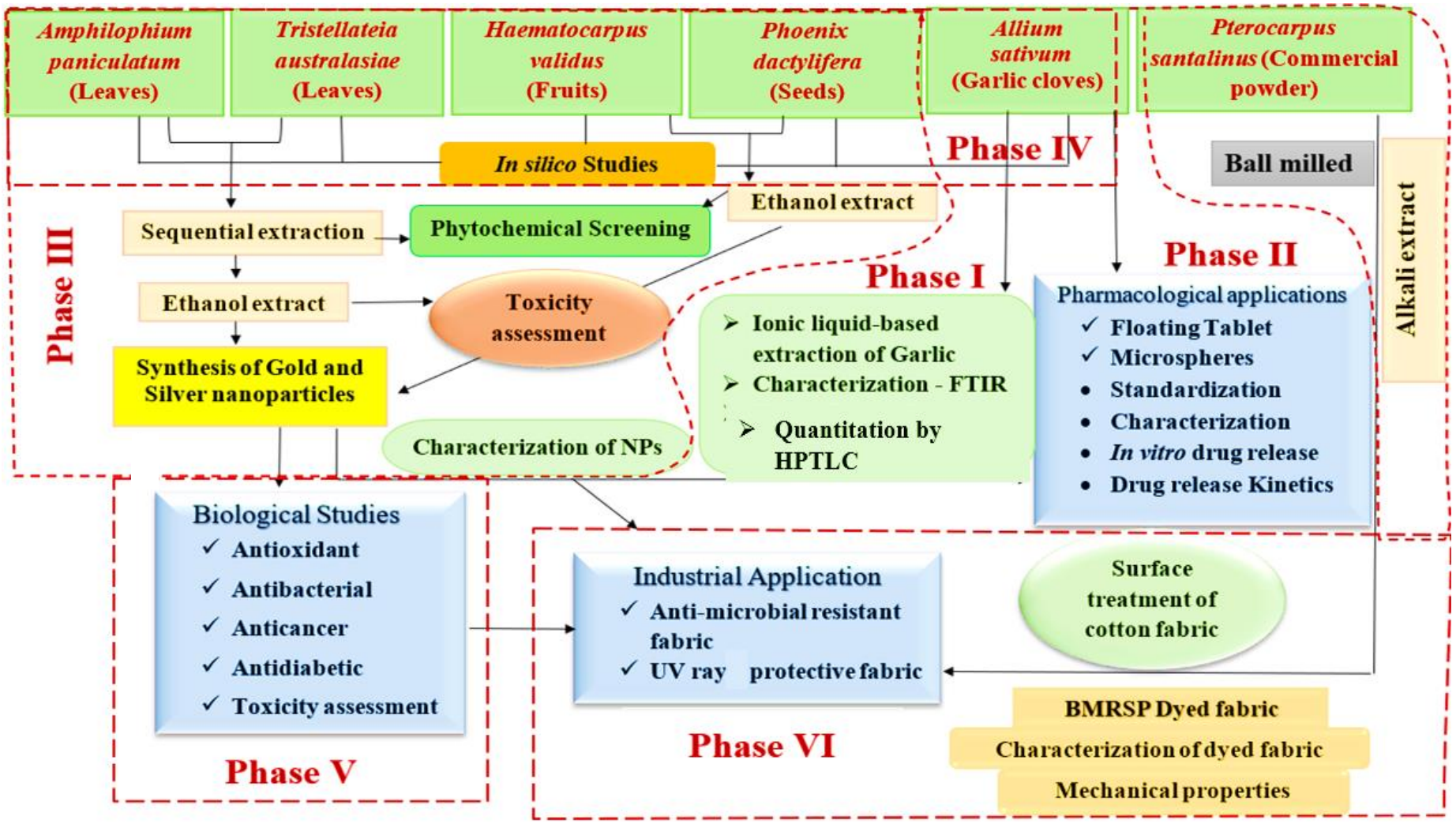
❖ Characterisation of Dyed Fabrics

❖ Evaluation Properties of Dyed Fabrics

❖ Antimicrobial Activity of Dyed Fabrics

❖ UV-Protective Dyed Fabrics

Flow chart represents the entire work carried out in the study



Phase-I Ionic liquid-based Solvent Extraction of Organo Sulphur compounds

This section focuses on the extraction, characterisation and quantitation of bioactive organosulphur compounds from garlic (*Allium sativum*), particularly Allicin, which is known for its potent antimicrobial and therapeutic properties. Conventional organic solvents often lead to degradation, lower yield or instability of Allicin due to their volatility and extraction conditions. Therefore, this phase aims to compare the conventional organic solvent extraction with ionic liquid-based extraction, a greener and more efficient to achieve enhanced extraction yield with chemical stability of sulphur based compound, which enabling their potential applications in further drug formulation and biomedical evaluations.

3.3 Extraction of Garlic

About 10 g freshly crushed garlic cloves were extracted with 20 mL methanol (98%) and mixture was stirred for 20 min at room temperature (RT). The extraction procedure was adapted from **Chavan *et al.* (2016)**, with minor modifications. Similarly, ionic liquid (IL) extraction was performed with 10 g crushed garlic in 20 mL methanolic-BMIMCl (0.7 M) and stirring at RT for 30 min, the procedure adapted from **Ahmad *et al.* (2017)**, with minor modification. Following extraction, the filtrates were collected and centrifuged at 1250 rpm for 10 min. The supernatant was transferred to clean containers and refrigerated at 4°C until further analysis. The methanol and IL based garlic extracts were labelled as GM (garlic-methanol extract) and GI (garlic-ionic liquid extract), respectively.

3.3.1 Characterisation of Garlic Extract

Considering the high cost of the standard compound Allicin (at Sigma-Aldrich) – 10 g costs around (Rs. 4,00,317) are required. We have utilised the relative standard reference method of Chavan *et al.* (2016) for the preparation and quantification of garlic extracts. Successful reports have highlighted the extraction of garlic using various solvents, such as ethanol, methanol and aqueous solvents; however, these solvents resulted in lower yield recovery **(Zhu *et al.*, 2016; Arthur** and **Ewald, 2006; Fujisawa *et al.*, 2008; Nguyen *et al.*, 2021)**. Therefore, utilisation of ionic liquid (1 butyl-3-methyl imidazolium chloride - BMIMCl) as an extractive solvent for garlic and its characterisation are discussed below.

3.3.2 Preparative Thin Layer Chromatographic (TLC) Analysis of Garlic Extract

As per the relative standard reference of **Chavan *et al.* (2016)**, both the 98% methanolic extract (GM) and IL based extract (GI) of garlic was prepared and subjected to TLC analysis. TLC was conducted on silica gel 60 (F_{254}) aluminium backed plates and the separation was achieved with a (2:1 v/v ratio) hexane-ethyl acetate eluent. Prior to sample application, the TLC chamber was saturated with the mobile phase for uniform development. The samples were spotted carefully on the baseline and the plates were developed up to three-quarters of their height. After TLC run, the plates were left to air-dry and then visualised under UV illumination at 254 nm and 365 nm, followed by visualisation in an iodine chamber. The retention factor (Rf) was calculated using following equation:

$$Rf = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent}}$$

3.3.3 Column Chromatographic Separation of Garlic Extract

Column chromatographic separation was carried out with 7.45 g methanolic extract of garlic cloves. A silica gel with methanol extract of garlic was prepared as a slurry, using silica gel (70 to 120 mesh) in a hexane solvent. The slurry was loaded to the pre-packed silica column. Further, elution was carried out *via* hexane–ethyl acetate mixtures of progressively increasing polarity, starting from 100% hexane and gradually increasing the ethyl aetate proportion ratios to 90:10, 50:50 and 20:80 (v/v). Fractions (100 mL each) were collected sequentially and monitored by TLC, with optimised hexane: ethyl acetate (2:1). The eluted fractions spotted on TLC plates were visualised under iodine vapour to assess the compound. Fractions showing similar TLC patterns (i.e., same RF values) were pooled together and concentrated for further characterisation.

3.3.4 FTIR analysis of Garlic Extract

The collected and combined fractions obtained through column chromatography were labelled as GS, while the methanolic garlic extract and IL-based garlic extract were labelled as GM and GI, respectively. All three samples were analysed using FTIR, to identify the characteristic functional groups associated with organosulphur compounds and other groups in the finger print region (1600 to 500 cm^{-1}).

3.3.5 Quantitation of Organosulphur Compound by HPTLC analysis

The freshly crushed garlic methanolic extract (GM), the compound obtained from column fractions labelled as GS and garlic ionic liquid-based extract as GIL were selected for HPTLC analysis. The mobile phase of hexane-ethyl acetate in a ratio of 2:1, was used to identify the bioactive compounds. Quantitative analysis of the bioactive compounds in the samples was performed using a Camag HPTLC system with a TLC scanner set to 254 nm. The samples were applied at 150 nL/s dosage speed, scanning speed (20 mm/s) and both deuterium and tungsten lamps were utilised.

Phase-II Sustained Release Floating Tablets and Microspheres for Drug Release Applications

In continuation of the previous phase, this section focuses on the utilisation of sulphur-rich, Allicin-containing garlic extracts in the development of sustained DDS, owing to their enhanced potential pharmacological activity. As part of this, we formulated sustained release floating tablets by optimising various polymeric compositions, with standardisation of over the counter medication such as ciprofloxacin, to compare the drug release profiles. Similarly, the bioactive microspheres incorporating fresh garlic juice containing Allicin were formulated to explore the effectiveness in targeting bacterial strains and managing Type 2 diabetes, through controlled drug delivery. These formulations, aimed to achieve prolong therapeutic action, reduce frequent dosing and improve bioavailability of the active compounds.

3.4 Floating Tablet

Floating tablet-based DDS are formulated to remain buoyant in the upper gastrointestinal tract, *i.e.,* stomach or duodenum, for optimal absorption of certain drugs and improved therapeutic effects over a prolonged period. The slow release of active ingredients minimises plasma peak concentrations and reduces adverse irritation and side effects, which often occurs with high dosage forms such as antibiotics or non-steroidal anti-inflammatory drugs. Due to the

importance of floating tablet-based DDS, we attempted to standardise compositions with an over the counter medication (ciprofloxacin), by varying excipients to provide sustained drug release, thereby reducing the need for frequent dosing.

3.4.1 Preparation and Standardisation of Floating Tablet

Floating tablets were formulated following the procedure reported by **Ganesh *et al.* (2021)**, with minor modifications. Ciprofloxacin floating tablets were prepared using various excipients, including bees wax (BW), lactose, poly vinyl pyrrolidone (PVP), sodium bicarbonate ($NaHCO_3$), cellulose acetate (CA), gelatin and stearic acid (SA). These excipients were blended *via* dry granulation, according to the weight proportions detailed in **Table 1 and Table 2**.

The powder components were thoroughly mixed and sieved, then compressed into tablets using the direct compression method with a hand-operated KBr pellet press, which has a flat, spherical punch with a diameter of 1.2 cm. Each tablet was compressed manually for 30 s, ejected and stored in an air-tight container.

Table 1 presents the preliminary trial formulations of ciprofloxacin floating tablets; which excipient concentrations were varied as follows: lactose (10 to 60 mg); PVP (5 to 20 mg); $NaHCO_3$ (10 to 70 mg); CA (52 to 82 mg); gelatin (0 and 10 mg). The amount of ciprofloxacin (70 mg); BW (3 mg) and SA (5 mg) were kept constant. Twenty different formulations were developed and assessed for their floating lag time (FLT) and total floating duration (TFT). The best formulation, based on floating characteristics, was selected for further modifications to optimise floating time, as shown in **Table 2.**

Table 1. Preliminary Trial Formulations of Ciprofloxacin Floating Tablets

Ciprofloxacin (CIP) at 70 mg, BW – 3 mg, and SA-5 mg were kept constant in all formulations. Additional excipients-lactose, PVP, sodium bicarbonate, CA and gelatin -were varied as shown below.

Sample code	Weight of Excipients (mg)					Total weight (mg)
	Lactose	PVP	$NaHCO_3$	CA	Gelatin	
F1C	30	5	50	82	10	255
F2C	40	5	50	48	10	255
F3C	50	5	50	62	10	255
F4C	30	5	60	70	10	253
F5C	40	5	60	60	10	253
F6C	50	5	60	55	10	258
F7C	30	5	70	67	0	250
F8C	40	5	70	57	0	250
F9C	60	5	70	37	0	250
F8C1	40	5	70	57	0	250
F8C2	50	5	60	57	0	250
F8C3	60	5	50	57	0	250
F8C4	40	10	50	67	0	245
F8C5	50	10	60	52	0	250
F8C6	60	10	70	32	0	250
F8C7	40	15	50	67	0	250
F8C8	50	15	60	47	0	250
F8C5a	50	10	10	52	0	200
F8C5b	10	10	60	52	0	210
F8C5c	50	20	60	52	0	260

Table 2. Formulated Composition of Ciprofloxacin Floating Tablets

Drug ciprofloxacin (Drug CIP) - 70 mg, Stearic acid (SA) – 5 mg, $NaHCO_3$ – 60 mg, and Cellulose acetate (CA) -52 mg were kept constant in all formulations. Additional excipients-BW, lactose, and PVP - were varied as shown below

Sample code	Weight of Excipients (mg)			Total weight (mg)	Sample code	Weight of Excipients (mg)			Total weight (mg)
	BW	Lactose	PVP			BW	Lactose	PVP	
F8C5c	3.0	50	20	255	11RF	6.5	10	30	250
1RF	3.0	30	30	255	12RF	6.5	30	20	250
3RF	10.0	50	20	255	13RF	10.0	30	30	250
5RF	10.0	10	20	253	14RF	6.5	10	10	245
6RF	3.0	10	20	253	15RF	3.0	50	20	250
7RF	10.0	30	10	258	16RF	35.0	50	50	250
8RF	6.5	50	30	250	17RF	0	50	50	250
9RF	3.0	30	10	250	18RF	35.0	50	35	250
10RF	6.5	50	10	250					

Similarly, floating tablets containing freshly crushed garlic juice as an active ingredient were prepared using the optimised excipient combinations identified in previous ciprofloxacin trials **(Table 2)**. The garlic-based tablet formulations used from the best performing excipients-including BW, lactose, PVP, sodium bicarbonate, CA and SA, are detailed in **Table 3**.

Table 3. Formulations of Garlic based Floating Tablet using Optimised Excipients

Sample code	Garlic juice (mg)	Weight of Excipients (mg)						Total weight (mg)
		BW	Lactose	PVP	$NaHCO_3$	CA	SA	
18RFG1	85.3	35	50	35	60	52	5	224.9
18RFG2	100.1	70	100	70	120	104	10	460.0
18RFG3	120.2	70	20	70	20	104	10	404.2
18RFG4	120.7	70	10	10	5	150	50	415.2

3.4.2 Pre-compression Study of Tablet Powders

The Pre-compression evaluation of the formulated floating tablet powders was studied according to procedure described by **Aulton, (2002)**. The blended powder, containing the active drug and polymer-excipients, were assessed for the physicochemical properties to determine their suitability for direct compression. The following parameter evaluated are colour, drug solubility, bulk density, tapped density, compressibility, Carr's Index, Hausner's ratio and angle of repose. These parameters help in evaluating the flowability, compressibility, and uniformity of the powder blends, during tablet formulation and in manufacturing process.

3.4.2.1 Solubility and pH of Tablets

The solubility of the formulated tablets was examined in various solvents, including water, ethanol, dil. HCl (0.1 N), aqueous-ethanol mixtures, and phosphate buffer solutions. Each

formulation was individually dispersed in 5 mL of respective solvent. The mixture was vortexed for 30 min in tightly sealed containers to reach saturation equilibrium. After 24 h, the saturated solutions were filtered using Whatman filter paper (pore size of 44 µm). The resulting filtrates are appropriately diluted with their respective solvents and the absorbance was measured at 276 nm with a UV BioSpec-Nano spectrophotometer. In addition, the pH of all sample solution was determined, with the digital pH meter pre-calibrated using standard buffer solutions to ensure measurement accuracy.

3.4.2.2 Flow Properties

The flowability and compressibility of the powdered formulations were determined using standard bulk and tapped density values. The known weight of powder was placed in a graduated cylinder, and its initial volume was referred as the bulk volume. The cylinder was subsequently tapped for 2 min to obtain the tapped volume. Using these values, Carr's index, and Hausner's ratio were calculated using the standard formula, as described by **Aulton, (2002).**

- Bulk density = weight of powder/volume of packing powder
- Tapped density = weight of powder/volume of tapped powder
- Hausner's ratio = Tapped density/Bulk density
- Carr's compressibility index = 100*(Vo -Vf)/Vo

where Vo – Initial volume of powder and Vf – Final volume of powder

3.4.2.3 Angle of Repose

The angle of repose for each formulation was measured using funnel method, as described by **Aulton, (2002)**. A pre-weighed amount of powder was allowed to flow freely through a funnel onto a flat surface, forming a cone without external vibrations or manual disturbance. The height (h), and mean base diameter (D) of the pile were measured, with the latter determining by averaging four perpendicular diameters (D1+D2+D3+D4)/4. The angle of repose (θ) was then calculated using the standard equation.

$$\theta = \tan^{-1} (h/r)$$

where h – the height of the powder cone
r- radius of cone base (D/2)

The flowability of the powders was determined based on angle of repose, where lower angles indicate better flow characteristics.

3.4.3 Post-Compression Evaluation of Floating Tablets

3.4.3.1 Physical evaluation of Tablets

All formulated floating tablets were observed to be white in colour and spherical in shape. The total of eighteen tablets from each batch were evaluated for their physical properties. The thickness and diameter of the tablets were measured using a Vernier caliper. Tablet hardness (crushing strength) were determined using a texture analyzer, which measures the breaking force in Newton (N). To assess weight uniformity, random selection of tablets from each batch were individually weighed and the mean values with its standard deviation was calculated.

3.4.3.2 Friability

Friability testing was examined following the procedure of **Ashish *et al.* (2022)**. Accurately weighed tablets were placed into the centrifuge-type friability tester and it was rotated for 5 min. After which the tablets were removed and any loose particles were gently brushed away before recording the final weight. Mechanical integrity was assessed by calculating the percentage weight loss (≤1% are acceptable) of tablets. Percentage friability was calculated as:

$$\text{Friability (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \text{ X } 100$$

3.4.3.3 Buoyancy Study

The *in vitro* buoyancy of all eighteen ciprofloxacin-based and garlic juice-loaded floating tablets was examined by following the procedure of **Roy *et al.* (2009) and Jiménez-Castellanos *et al.* (1994)**. Each tablet was placed in 0.1 N HCl, and the two parameters were noted. Floating Lag Time (FLT): The time required for the tablet to rise to the surface of the dissolution medium. Total Floating Time (TFT): Duration of the tablet remaining buoyant on the surface of the dissolution medium without sinking.

3.5 Characterisation of Floating tablet

The formulation 18RF was subjected to FTIR, XRD, and TGA-DSC analysis. FTIR spectral analysis detected the characteristic functional groups and interactions, while XRD pattern assessed the crystalline or amorphous nature of the formulation. TGA-DSC analysis was carried out from 0 to 900 ˚C at a heating flow rate of 10 ˚C/min under a nitrogen atmosphere (20 mL/min). Drug release samples were analysed with a UV BioSpec nano spectrophotometer using 0.1 N HCl as the blank solvent.

3.6 Preparation of Standard Calibration Curve of Ciprofloxacin

The standard CIP HCl (0.1 mg/mL) stock solution was prepared using 0.1 N dil. HCl (pH 1.2) and further diluted to obtain concentration ranging from 10-90 µg/mL. The solutions were scanned from 220 to 800 nm using a UV BioSpec nano to obtain the absorbance spectra. The maximum absorbance (λ_{max}) of ciprofloxacin was observed at 276 nm, and the absorbance of each concentration was recorded at a specific wavelength. The calibration curve of absorbance versus concentration (µg/mL), which helps to calculate the drug content in the formulations.

3.6.1 *In vitro* Dissolution Study

In vitro dissolution of the CIP-floating tablet was carried out using the method described by **Reddy *et al.* (2018)**. The developed tablets containing various excipient compositions and CIP as the model drug were tested in simulated gastric fluid (0.1 N dil. HCl). The dissolution study was performed with 0.1 N HCl (900 mL) as the medium, maintained at 37˚±0.5˚ C and stirred at 100 rpm. The required volume of the solution was withdrawn and replaced with the same volume of fresh dil. HCl solution at predetermined time intervals. The collected drug solutions were diluted tenfold, and the following samples were measured for absorbance using UV BioSpec nano at a wavelength of 276 nm.

3.6.2 Stability Studies of Floating Tablet

The stability of the floating tablet formulation (18RF) was evaluated under accelerated storage conditions at 25˚ to 32˚C. The tablet was stored in a tightly sealed sample bottle and observed over six-month period. At 1, 3, and 6 months, the tablet was withdrawn for evaluation. These tablets were tested for their physical appearance, pH measurement, and *in vitro* drug release. These studies were conducted to determine the physical and chemical stability of the formulations over time (**Irfan *et al.*, 2016)**.

3.6.3 Drug Release Kinetics

The drug release mechanisms of the formulations were analysed using various mathematical kinetic models, such as zero order, first order, Higuchi, and Korsmeyer-Peppas (**Egbaria and Friedman, 1990)**. The model with the highest correlation coefficient (r^2) value provided the best fit and was therefore chosen to describe the release profile. The release exponent n, obtained from the Korsmeyer-Peppas model, was utilised to interpret the diffusion mechanism as follows: $n < 0.5$ signifies Fickian diffusion (Case I transport); $0.45 < n < 0.89$ represents anomalous (non-Fickian) diffusion; $n = 0.89$ indicates zero-order release (Case II transport); and $n > 0.89$ indicates super case II transport. These classification helps to determine whether drug release is controlled primarily by diffusion, swelling, erosion, or a combination of these mechanisms **(Dash *et al.*, 2010; Gouda, 2017)**.

3.7 Microspheres

Microspheres can also ensure the bioavailability of conventional drugs and minimise side effects. Microspheres are small microparticles, made from biodegradable polymers, which encapsulate active substances such as NPs or organosulphur containing fresh garlic juice, providing a sustained release profile. Based on the biological stability of active compound like Allicin and other sulphur compounds, which may be sensitive or degrade in the stomach's acidic environment. Owing to the complete dispersion of active ingredient in the standardised floating formulations, we have attempted to encapsulate garlic fresh juice in microspheres to provide better stability and extended release profile.

3.7.1 Preparation and Standardisation of Microspheres

The microspheres were formulated using the emulsion solvent evaporation method by **Banstola *et al.*, 2019** with a modified procedure. Briefly, the matrix formulation of microspheres was prepared using various biocompatible synthetic polymers, including poly caprolactone (PCL), poly ethylene glycol (PEG), and hydroxy propyl methyl cellulose (HPMC). The emulsifiers used in this study were poly vinyl pyrrolidone (PVP) and poly vinyl alcohol (PVA). Standard metformin was dissolved in distilled H_2O (1 mg/mL) and used as the drug solution. Four different blank microspheres (MS1, MS1a, MS2 and MS2a) were prepared by varying the presence of emulsifier and PEG, with details provided in the **Table 4**.

Table 4. Preparation and Standardisation of Blank Microspheres

Sample code	Organic Phase in DCM/ethanol		Aqueous phase (1% Emulsifier)	
	PCL + HPMC (mg)	PEG (mg)	PVP (mL)	PVA (mL)
MS1	50 + 50	50	5	0
MS1a		50	0	5
MS2		0	5	0
MS2a		0	0	5

The organic phase consisted of PCL (50 mg) dissolved in 3 mL dichloromethane (DCM) and stirred for 10 min. PEG and HPMC (in a 1:1 ratio) were dissolved in a mixture of DCM-ethanol (2:1 ratio) to obtain a clear solution. The combination of these solutions, containing PCL, HPMC and PEG, were referred to as the organic phase. The aqueous phase containing 1% solution of either PVP/PVA as emulsifier in H_2O, was kept under stirring conditions. The prepared organic phase was added slowly (drop wise) to the aqueous phase and stirred at 1500 rpm for 4 h at RT. The organic solvent was then evaporated, and the formed microspheres were washed with distilled H_2O and centrifuged at 9500 rpm. The residue containing microspheres were air dried and utilised for further analysis.

3.7.2 Microsphere Preparation and Encapsulation Efficiency

3.7.2.1 Preparation of drug/garlic encapsulated Microspheres

For the preparation of fresh garlic juice encapsulated microspheres, we utilised freshly crushed garlic juice for encapsulating with microspheres for sustained drug release. Briefly, the preparation of garlic encapsulated microspheres involves the addition of garlic fresh juice (1 mL) to an aqueous phase containing 1% PVP (5 mL) which was stirred well. The organic phase, containing PCL, HPMC and PEG was dissolved in DCM and ethanol (2:1) were added dropwise to the aqueous phase under stirring condition. The solution was stirred for 4 h at RT. After homogenisation, the emulsion containing organic solvent was evaporated, and the dried microspheres were collected. The garlic loaded microspheres were labelled as MS1G. Drug-encapsulated microspheres were prepared using the same procedure, with the addition of metformin (5 mL) to the aqueous phase. The metformin-loaded microspheres were labelled as MMS1.

3.7.2.2 Encapsulation Efficiency of drug/ garlic Loaded Microspheres

Encapsulation efficiency (EE%) of the prepared microspheres was evaluated following **Costa *et al.*, (2025)**, with minor modifications. Encapsulation was assessed by quantifying unentrapped drug in the dispersion medium. Briefly, 20 mg dried microspheres were dispersed in 5 mL phosphate buffer (PBS, pH 6.8), vortexed for 5 min, and centrifuged at 10,000 rpm for 20 min at RT. The collected supernatant was analysed for free (unentrapped) drug using a UV Bio Spec-nano spectrophotometer. Absorbance was measured at 231 nm for metformin and 251 nm for bioactive garlic extract. The free drug concentration was determined using the calibration curve (mentioned in section 3.7.5) in PBS. Encapsulation efficiency (EE%) was determined using following formula.

$$\text{Encapsulation efficiency (EE\%)} = \frac{\text{Wi} - \text{Wf}}{\text{Wi}} \text{ X } 100$$

Wi – initial amount of drug/extract added during microsphere preparation
Wf – amount of free (unentrapped) drug/extract in the supernatant

3.7.3 Characterisation of Microspheres

The formulated blank microspheres, and garlic encapsulated microspheres were characterised for drug compatibility using ATR-FTIR spectroscopy, with a wavenumber from 4000 to 400 cm^{-1}. The amorphous/ crystalline nature of the formulated microspheres was analysed using XRD. UV bio nano spectrophotometer was used to record the drug-release solutions, with PBS (0.05 M) as the blank. FESEM morphology and elemental analysis of formulated microspheres were performed.

3.7.4 Antibacterial Activity of Garlic loaded Microspheres

In vitro antibacterial activity of blank microspheres, garlic encapsulated microspheres (MS1G) and heated crushed garlic extract (GH) was evaluated against four bacterial strains: *S. aureus, B. subtilis, K. pneumoniae*, and *E. coli*. Ciprofloxacin was utilised as a standard reference antibiotic. The antibacterial activity was evaluated as outlined in section 3.9.2.2, *via.* agar well diffusion method. After inoculating Mueller-Hinton agar plates with respective bacterial cultures, the wells were loaded with the test samples. The culture plates were maintained at 37˚C for 24 h under standard conditions. After incubation, the zone of inhibition (ZOI) around the wells was measured in millimetre using a Hi-Media standard scale to assess and compare the antibacterial activity of microsphere formulations.

3.7.5 Preparation of Standard Calibration Curve of Metformin and Garlic Fresh Extract

A standard metformin HCl solution (0.1 mg/mL) was made using 0.05 M PBS at pH 6.8. To obtain the stock solution, 10 mg metformin was weighed and dissolved in 100 mL PBS. Serial dilutions were prepared from the stock to produce solutions ranging from 1 to 15 µg/mL. The samples were scanned over a wavelength of 220 to 800 nm, and the absorbance was recorded at λ_{max} 231 nm using a UV BioSpec nano spectrophotometer.

For the garlic fresh juice analysis, a stock solution with a concentration of 0.25 mg/mL in 0.05 M PBS. From the stock, a series of dilutions (1-30 µg/mL) was prepared. The absorbance of these diluted samples was measured at 251 nm. The calibration curve obtained from garlic extract was adopted as the reference due to the high cost of the standard Allicin.

3.7.5.1 *In vitro* Dissolution and Drug Release Kinetics of Microspheres

The drug release study of the microsphere formulations enclosed in a dialysis bag was carried out by following the method of **Yu *et al.* (2019)**. The study included the microsphere formulations MMS1 and MS1G, was conducted in a simulated physiological medium (0.05 M PBS), to mimic body fluids such as blood, and the extracellular fluid surrounding the cells. Drug dissolution was performed at 100 rpm in 900 mL PBS medium. At regular intervals, aliquots were withdrawn and replaced with equal volumes of fresh PBS. The samples were diluted tenfold prior to analysis, and the absorbance was measured at 231 nm for metformin and 251 nm

for the garlic solution. The drug release kinetics of the microsphere formulations were assessed using kinetic models, following the procedure outlined in section 3.6.3

Phase -III Sustainable Synthesis and Characterisation of Metallic Nanoparticles

This section focuses on the sustainable synthesis of metallic NPs using four different plant materials, providing an eco-friendly approach to conventional chemical synthesis. Sustainable, plant-based nanoparticle synthesis is often considered environmentally benign, as they eliminate toxic chemicals and reduces energy requirements. The plant-based sustainable synthesis of NPs is cost-effective, energy efficient, non-toxic, and biodegradable. The first step involves the extraction and phytochemical screening of the plant materials, followed by the formation and characterisation of metallic NPs.

3.8 Extraction and Phytochemical Screening of Selected Plants

3.8.1 Extraction of Selected Plant Materials

The details of the plant materials used in this research are provided in **Table 5**. The plant leaves were collected, washed, shade-dried, and pulverised. Sequential extraction was performed for *Amphilophium paniculatum* (AP) and *Tristellateia australasiae* (TA) using various solvents (hexane, chloroform, ethyl acetate, methanol, and ethanol), by reflux method. Similarly, *Haematocarpus validus* (BF) fruits was extracted using ethanol, and *Phoenix dactylifera* (date palm seeds -DS) were extracted using 75% ethanol by reflux method. The powdered leaves of AP (65 g), TA (35 g), BF (20 g), and DS (22 g) were refluxed with non-polar and polar solvents (250 mL) for 6 h. After extraction, the solutions were filtered and distilled to obtain the extracts. The extracts were then dried and refrigerated until further use. The obtained solvent-based plant extracts were assigned the following sample codes, as provided below.

Table 5. List of selected plants utilised in the study

Selected Plants (Botanical name)	Common name	Family	Sample code	Parts used
Amphilophium paniculatum	Duckbill/Cuello Creeper	Bignoniaceae	AP	Leaves
Tristellateia australasiae	Australian gold vine, Shower of gold climber	Malpighiaceae	TA	Leaves
Haematocarpus validus	Blood Fruit	Menispermaceae	BF	Fruits
Phoenix dactylifera	Date palm	Arecaceae	DS	Seeds

Sample code	Description of extracts	Sample code	Description of extracts
APH	AP hexane	**TAC**	TA chloroform
APC	AP chloroform	**TAEA**	TA ethyl acetate
APEA	AP ethyl acetate	**TAM**	TA methanol
APM	AP methanol	**TAE**	TA ethanol
APE	AP ethanol	**BFE**	BF ethanol
TAH	TA hexane	**DSE**	DS ethanol

3.8.1.1 Phytochemical Screening of Selected Plant Materials

Phytochemical screening was carried out for the above assigned extracts. Each sample was tested with various reagents, and any resulting colour change was used to determine whether the secondary metabolites were detected (+) or not detected (-), following standard procedures **(Harborne, 2013; Shaikh *et al.*, 2020).**

3.8.2 Sustainable Synthesis of Metallic Nanoparticles

The sustainable synthesis of metallic NPs, such as gold and silver NPs, were carried out using four extracts such as APE, TAE, BFE and DSE, as assigned in section 3.8.1. For gold nanoparticle (GNPs) synthesis, two different methods were employed: room temperature and solar light irradiation. For silver nanoparticle (SNPs) synthesis, only the solar light irradiation method was adopted.

3.8.2.1 Preparation of Stock Solutions

Stock solutions containing 3 mM auric chloride and silver nitrate were prepared in distilled H_2O. The auric chloride stock solution was refrigerated at 3°C, while the silver nitrate solution was stored in a brown (amber) bottle and kept in a dark condition to avoid light-induced decomposition.

3.8.2.2 Sustainable Synthesis of Gold Nanoparticles at Room Temperature

Gold nanoparticles (GNPs) were synthesised by adding a constant volume of auric chloride (3 mM) to varying volumes of plant extracts (APE, TAE, BFE, and DSE) dispersed in aqueous medium (1 mg/mL). Extract solution of varying volumes were added to the same volume of auric chloride in a ratio of 1:1, 2:1, 3:1, 4:1, and 5:1, and the mixtures were allowed to reduce the metal (Au^{3+} to Au^0) at room temperature (27°C to 30°C). The change in colour and the time of GNPs formation were monitored visually and further confirmed by spectroscopic techniques. The sample codes assigned to the synthesised GNPs from four different extracts, using room temperature method are given below.

Sample Code	Method	Description
APEGRT	Room Temperature (RT)	AP ethanol extract aided GNPs by RT
TAEGRT		TA ethanol extract aided GNPs by RT
BFEGRT		BF ethanol extract aided GNPs by RT
DSEGRT		DS ethanol extract aided GNPs by RT

3.8.2.3 Sustainable Synthesis of Gold Nanoparticles by Solar Light Irradiation

Gold nanoparticles (GNPs) were synthesised by adding a constant volume of auric chloride (3 mM) to varying volumes of plant extracts (APE, TAE, BFE, and DSE) dispersed in aqueous medium of 1 mg/mL concentration. Different volumes of extracts and an equal volume of auric chloride in ratio of 1:1, 2:1, 3:1, 4:1, and 5:1 were used to reduce the metal (Au^{3+} to Au^0) under sunlight (30°C to 35°C). This process is referred to as the solar light irradiation method. The change in colour and the time of GNPs formation were monitored visually and further confirmed by spectroscopic techniques. The sample codes assigned to the synthesised GNPs from four different plant extracts, using the solar light irradiation method are given below.

Sample Code	Method	Description
APEG	Solar light irradiation (SL)	AP ethanol extract aided GNPs by SL
TAEG		TA ethanol extract aided GNPs by SL
BFEG		BF ethanol extract aided GNPs by SL
DSEG		DS ethanol extract aided GNPs by SL

3.8.2.4 Sustainable Synthesis of Silver Nanoparticles by Solar Light Irradiation

Silver nanoparticles (SNPs) were synthesised by mixing different volumes of plant extracts (APE, TAE, BFE, and DSE) dispersed in an aqueous medium (1 mg/mL) with silver nitrate at the ratios of 1:1, 2:1, 3:1, 4:1, and 5:1. The mixture was allowed to reduce the metal Ag^{+} to Ag^{0} under sunlight at 30°C to 35°C. The change in colour from yellow to brown was identified visually, and the time of formation was recorded. The sample codes assigned to the synthesised SNPs from four different plant extracts, using the solar light irradiation method are given below.

Sample Code	Method	Description
APES	Solar light irradiation (SL)	AP ethanol extract aided SNPs by SL
TAES		TA ethanol extract aided SNPs by SL
BFES		BF ethanol extract aided SNPs by SL
DSES		DS ethanol extract aided SNPs by SL

3.8.3 Analytical Characterisation of Synthesised Metallic Nanoparticles

The synthesised GNPs and SNPs were characterised using several analytical techniques to determine their formation, size, shape, morphology, and elemental composition. The analytical techniques used are described below.

3.8.3.1 UV Visible Spectral Characterisation of Synthesised Metallic Nanoparticles

UV-visible spectra of synthesised GNPs and SNPs were recorded using a UV BioSpec nano in photometric mode with a path length of 0.7 mm. Samples were scanned at the wavelength ranging from 220 to 800 nm. The synthesised GNPs and SNPs in liquid form were recorded with blank H_2O as the solvent. The conversion of Au^{3+} to Au^{0} and Ag^{+} to Ag^{0} generates characteristic SPR bands of GNPs and SNPs.

3.8.3.2 FTIR Spectral Characterisation of Synthesised Metallic Nanoparticles

The synthesised NPs were characterised using ATR-FTIR to reveal the chemical functional groups present. The NPs was placed on a Zn-Se crystal and recorded in the mid-IR region, with wavenumber from 4000 cm^{-1} to 400 cm^{-1} in % transmittance mode. A background scan was recorded against atmospheric interference. The FTIR spectra of the GNPs and SNPs were obtained using the samples in liquid form.

3.8.3.3 XRD Pattern of Synthesised Metallic Nanoparticles

XRD analysis was performed to examine the structural arrangement of atoms, ions, or molecules within the crystal lattice. The crystalline or amorphous nature of the NPs was determined from the diffraction pattern. For the analysis, the GNPs and SNPs were drop-cast onto sterile glass plates (2 x 2 cm) and allowed to air-dry. The crystallite size of the synthesised

NPs was determined using the Debye-Scherrer equation [$D = K\lambda / (\beta \cos \theta)$], where K represents Scherrer constant (0.94), λ is the X-Ray wavelength (1.54 Å), and β is the full width at half maximum (FWHM). The instrument was operated with a monochromatic source at 45 kV and 30 mA, and scans were recorded over a 2θ range of 10^{o} to 80^{o} at a rate of 2^{o}/min.

3.8.3.4 Zeta Potential Measurements of Synthesised Metallic Nanoparticles

The stability and surface electric charge of GNPs and SNPs were evaluated by measuring the most stable particles when electrostatic repulsion occurs between them. Samples were analysed in triplicate, in an aqueous medium and the average zeta potential was determined.

3.8.3.5 FESEM Imaging and EDS Analysis of Synthesised Metallic Nanoparticles

The morphological characteristics and average sizes of the synthesised GNPs and SNPs were determined using FESEM analysis. The NPs were centrifuged and dispersed for 10 min. The clear supernatant was coated on double-sided carbon tape, which was fixed on aluminium metal stubs. To make the samples conductive, they were gold-sputtered for 60 s with an electron voltage of 5 kV to 30 kV and a current leak of 10 mA. The sputtered samples were then loaded onto the FESEM chamber. The elemental mapping of synthesised GNPs and SNPs was analysed using an EDS instrument with Apex software. The overall distribution of elements present in the samples was determined through EDS mapping analysis.

Phase-IV *In silico* Screening of Bioactive Compounds Present in Selected Plants

3.8.4 *In silico* Screening of Bioactive Compounds Present in Selected Plants

In this phase, *in silico* screening was carried out to assess the potential bioactive compounds from selected plants, including *Amphilophium paniculatum* (AP), *Tristellateia australasiae* (TA), *Haematocarpus validus* (BF), *Phoenix dactylifera* (DS) and organosulphur compounds in *Allium sativum*, as inhibitors of *Diabetes mellitus*.

3.8.4.1 Identification of Bioactive Compounds Present in Selected Plants

Bioactive compounds from the selected plants such as *Amphilophium paniculatum* (AP), *Tristellateia australasiae* (TA), *Haematocarpus validus* (BF), *Phoenix dactylifera* (DS), and organosulphur based compounds in garlic were compiled from literature reports. The two-dimensional chemical structures of twenty-two ligands from AP, eight ligands from TA, twenty-three ligands from BF, sixteen ligands from DS and thirty-four organosulphur based compounds from garlic were obtained from the PubChem database and are depicted in **Figure 2a, 2b, 2c, 2d and 2e**

.

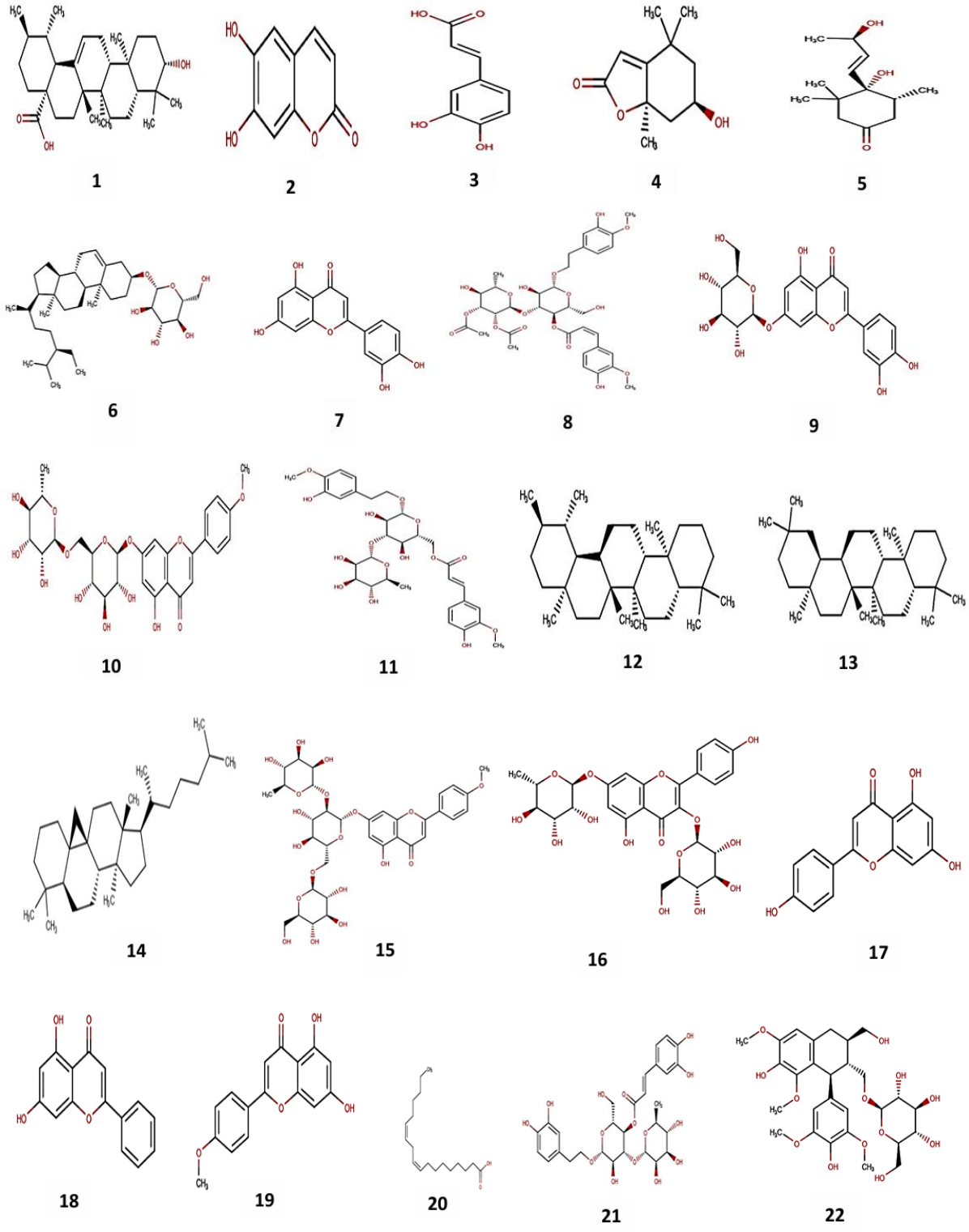


Figure 2a. **2D representations of bioactive compounds isolated from *A. paniculatum*** 1. Ursolic acid, 2. Aesculetin, 3. Caffeic acid, 4. Epiloliolide, 5. 4,5-dihydroblumenol A, 6. Daucosterol, 7. Luteolin, 8. Diacetylmartynoside, 9. Cynaroside, 10. Linarin, 11. Isomartynoside, 12. Ursane, 13. Oleanane, 14. Cycloartane, 15. Glucopyranoside, 16. kaempferol 3-O-beta-D-glucopyranosyl-7-O-alpha-L-rhamnopyranoside, 17. Apigenin, 18. Chrysin, 19. Acacetin, 20. Linoleic acid, 21. Acteoside, 22. (+)-lyoniresinol-3-alpha-O-beta-D-glucopyranoside.

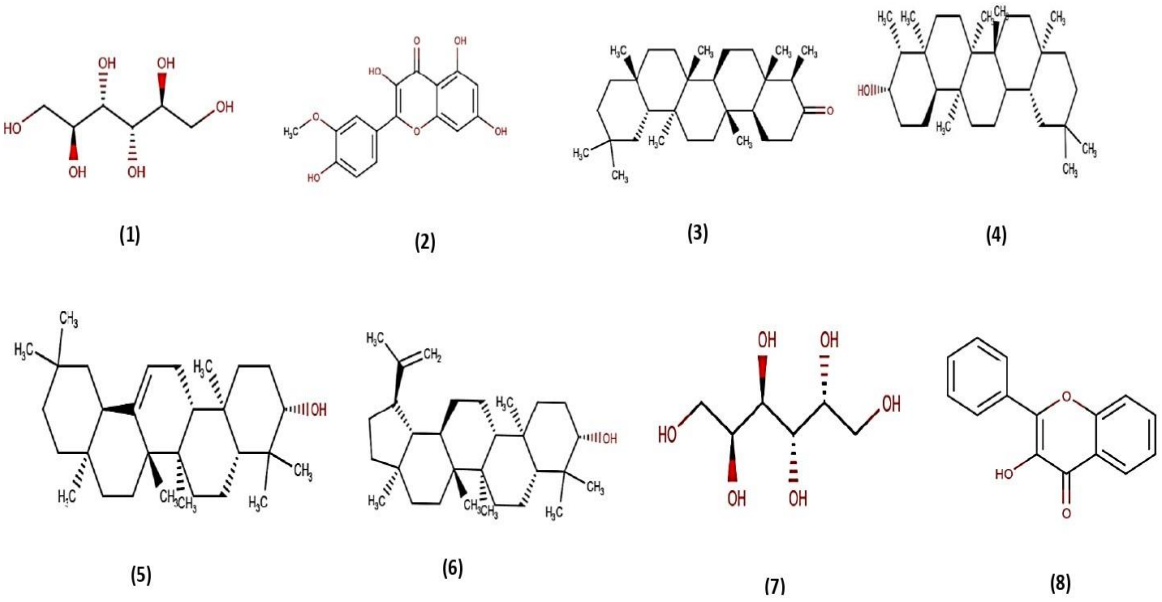
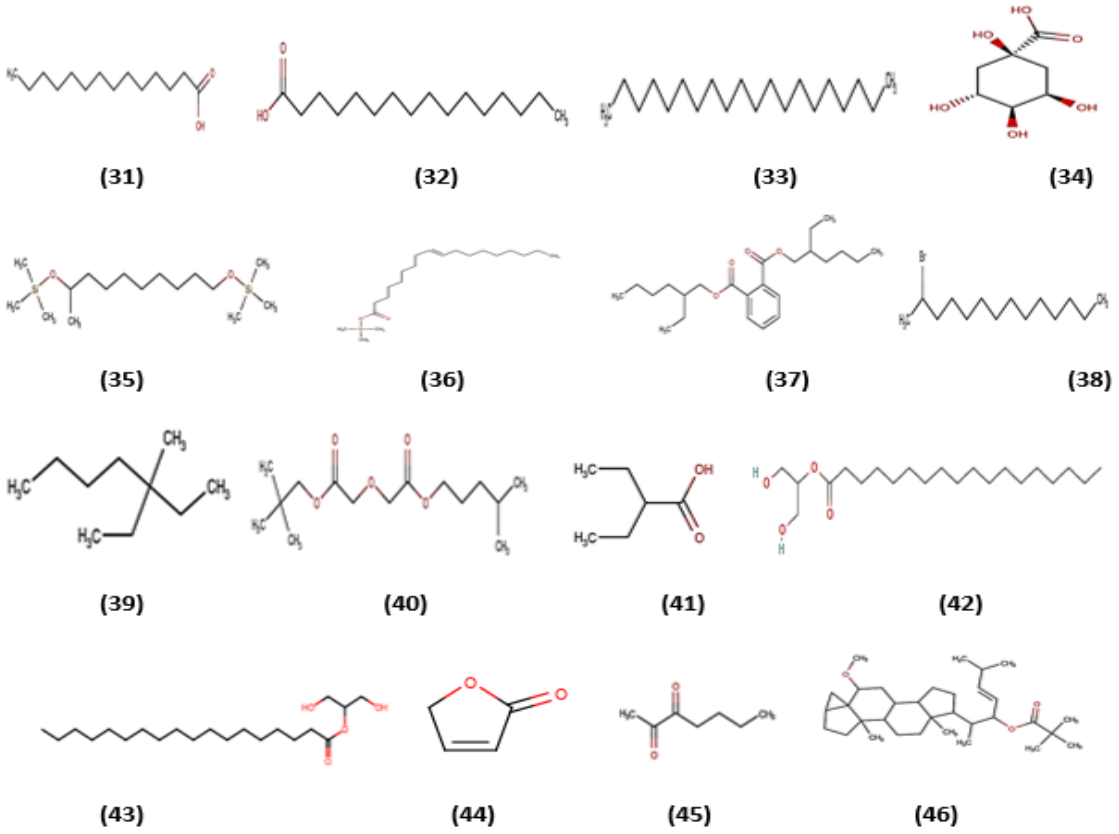


Figure 2b. 2D representations of bioactive compounds isolated from *T. australasiae* (23) Hexitol (24) Isorhamnetin (25) Friedelin (26) Epifriedelinol (27) Beta-Amyrin (28) Lupeol (29) Galactitol (30) Flavonol



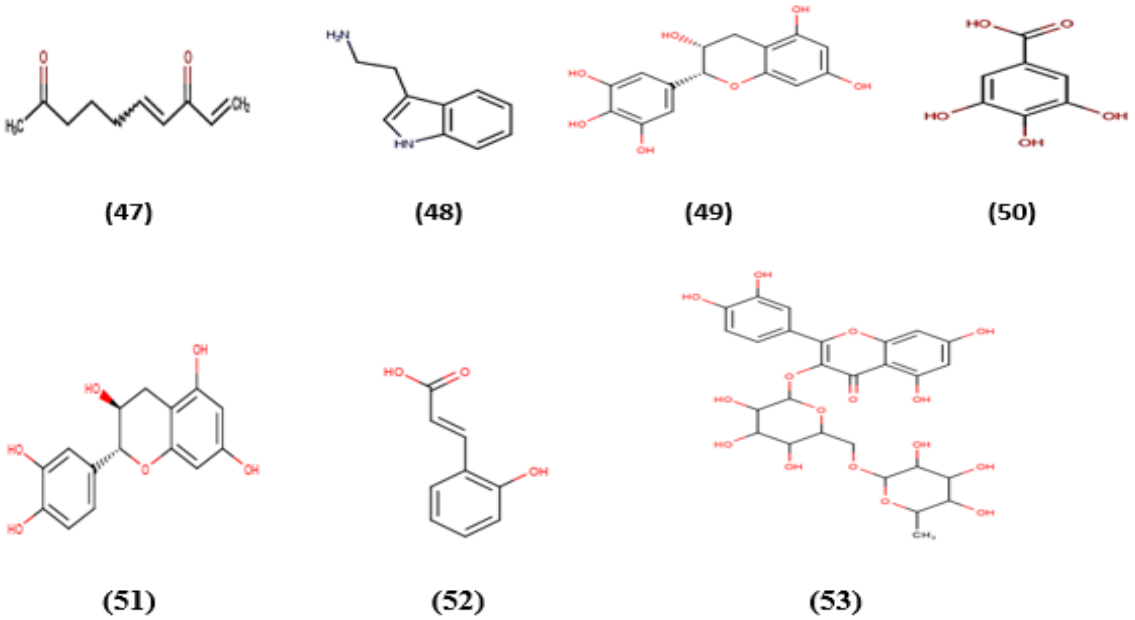
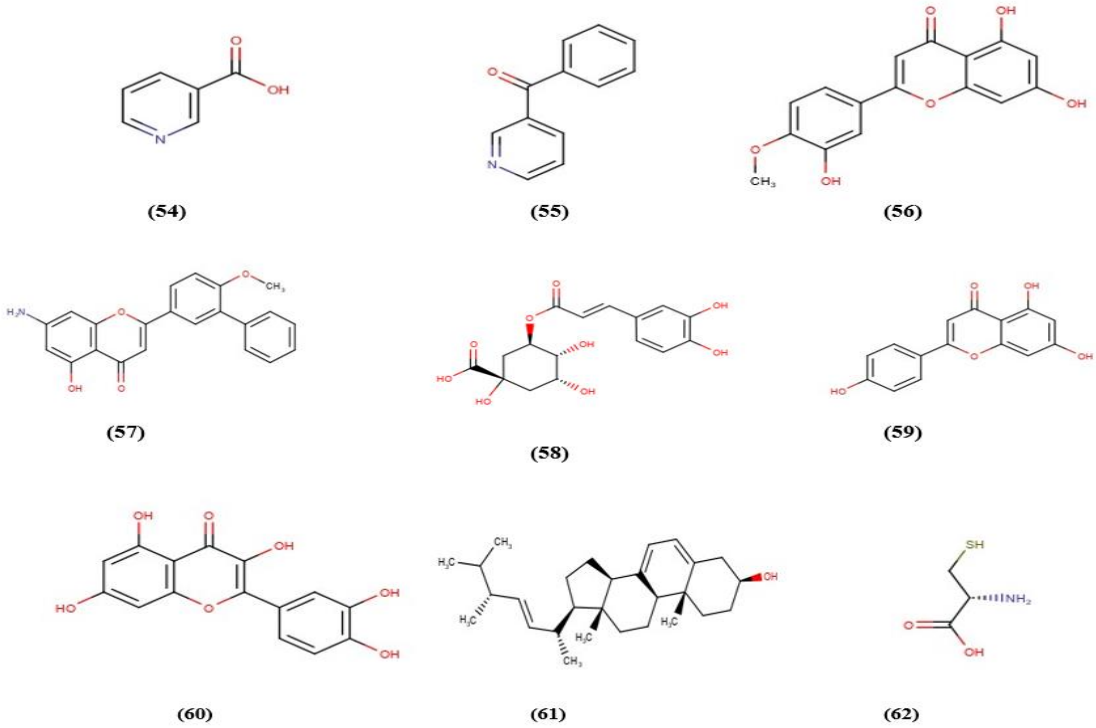


Figure 2c 2D representations of bioactive compounds isolated from *Haematocarpus validus* (31) Myristic acid (32) Palmitic acid (33) Eicosane (34) Quinic acid (35) Decane,1,9-bis[(trimethylsilyl)oxy] (36) Trimethylsilyl (9E)-9-Octadecenoate (37) bis (2-ethylhexyl) phthalate (38) 2-Bromotetradecane (39) 3-Ethyl-3-methyl heptane (40) Isohexyl neopentyl ester (41) 2-Ethyl butyric acid (42) 2-Monostearin (43) 2-Palmitoyl glycerol (44) Furanone (45) Acetyl valeryl (46) 3,5-Dehydro-6-methoxy pivalate (47) Deca-6,9-diene-2,8-dione (48) 1H-indole-3-ethanamine (49) Epigallocatechin (50) Gallic acid (51) Catechin (52) 2-coumaric acid (53) Rutin



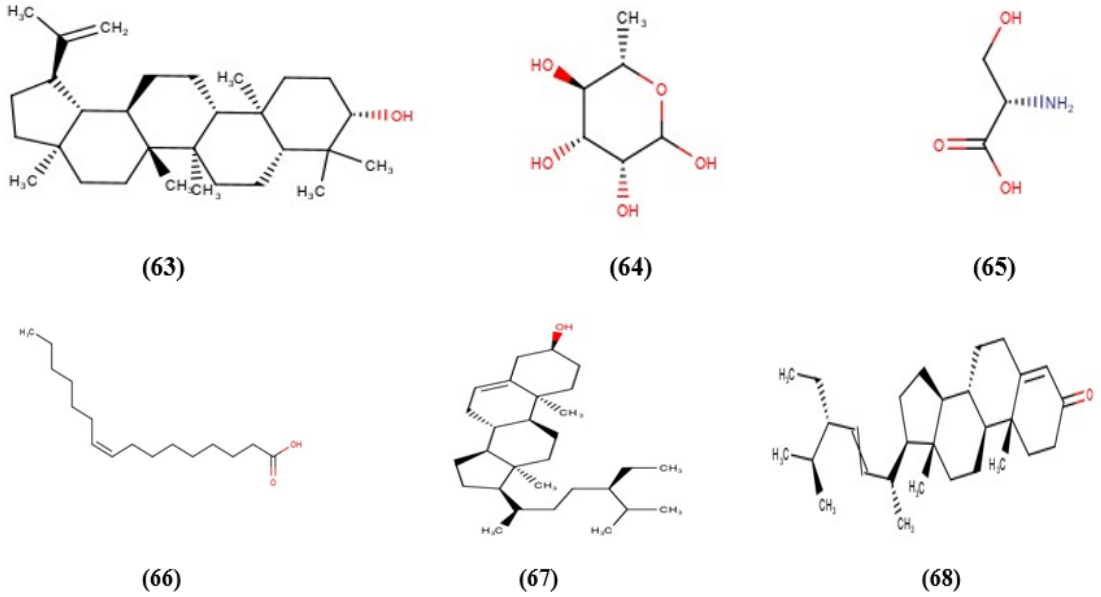
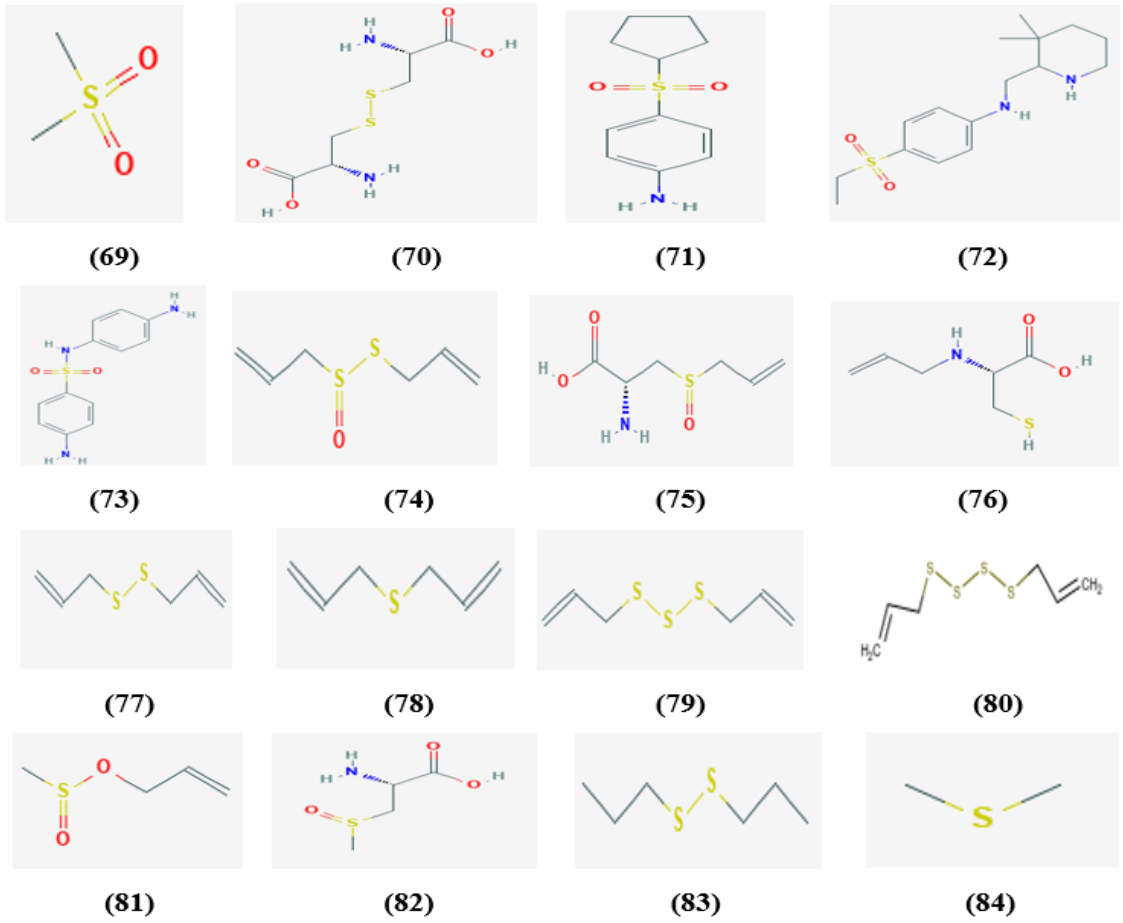


Figure 2d. 2D representations of bioactive compounds isolated from *P. dactylifera* (54) Nicotinic acid (55) 3-benzoyl pyridine (56) Diosmetin (57) 3-O-caffeoylshikimic acid (58) Chlorogenic acid (59) Apigenin (60) Quercetin (61) Ergosterol (62) L-cysteine (63) Lupeol (64) L-rhamnose (65) L-serine (66) Palmitoleic acid (67) Beta Sitosterol (68) Stigmasta-4,22-dien-3-one



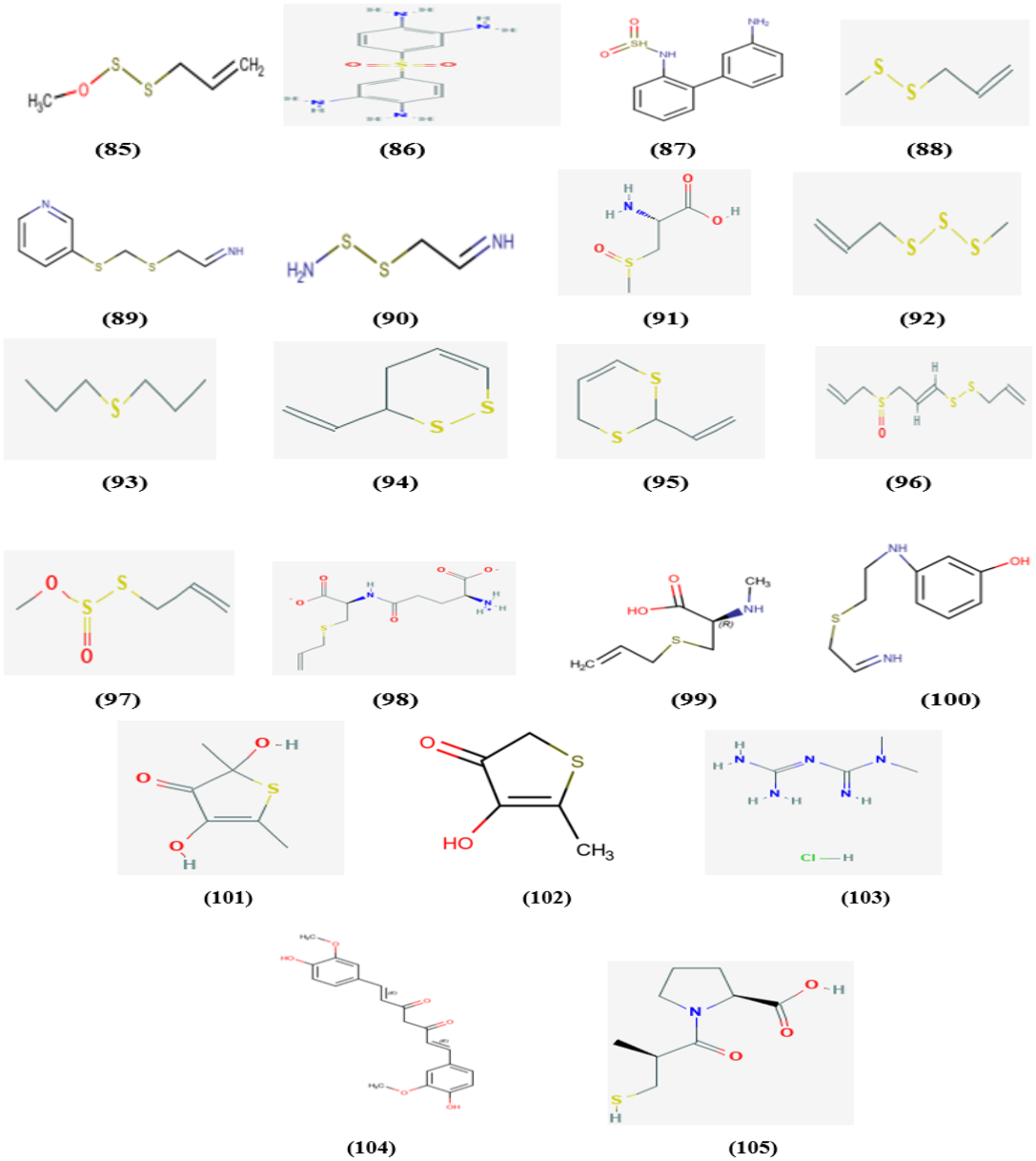


Figure 2e. 2D chemical structures of Organo sulphur compounds (69) Methyl sulphonyl methane (70) L-cystine (71) 4-(Cyclopentyl Sulphonyl) aniline (72) N-[(3,3-dimethyl piperidin-2-yl)methyl]-4-ethyl sulphonyl aniline (73) 4-amino-N-(4-aminophenyl) benzene sulphonamide (74) Allicin (75) Alliin (76) Allyl cysteine (77) Allyl disulphide (78) Diallyl sulphide (79) Diallyl trisulphide (80) Diallyl tetra sulphide (81) Allyl methyl sulphinate (82) S-propyl cysteine sulphoxide (83) Dipropyl disulphide (84) Dimethyl sulphide (85) S-Methyl 2-Propene-1-Sulphinothioate (86) 2-amino-4-(3,4-diaminophenyl) sulphonylbenzene-1,2-diamine (87) 2-(3-aminophenyl) sulphonyl aniline (88) Allyl methyl disulphide (89) Modified Structure of 20 IUPAC: 2- {[(pyridin- 3- yl sulphanyl) methyl] sulphanyl} ethan-1-imine (90) Modified Structure of 20 IUPAC: [(2- iminoethyl) disulphanyl] amine (91) S-methyl cysteine sulphoxide (92) Methyl allyl trisulphide (93) Dipropyl sulphide (94) 3-vinyl-4H-1,2-dithiin (95) 2-Vinyl-4H-1,3-dithiin (96) E-Ajoene (97) Methyl Allyl thiosulphinate (98) Gamma-L-Glutamyl-[(S)-Allyl]-L-Cysteine (99) Modified structure of 30 IUPAC:(2R) - 2- (methylamino) - 3- (prop- 2- en- 1- yl sulphanyl) propanoic acid (100) 3- ({2- [(2- iminoethyl) sulphanyl] ethyl} amino) phenol (101) Thiacremonone (102) 4- hydroxy- 5- methyl- 2H- thiophen- 3- one, (103) Metformin hydrochloride, (104) Curcumin, (105) Captopril

3.8.4.2 Physicochemical Properties and ADME Properties of Bioactive Compounds

The selected bioactive compounds were evaluated for their physicochemical properties, polar surface area (PSA), molecular weight, molecular surface area (MSA), lipophilicity (log P), polarizability, Hydrophilic-Lipophilic balance (HLB), and the number of hydrogen bond donors (HBD), and acceptors (HBA), along with refractivity. In addition, ADME parameters - absorption, distribution, metabolism, and excretion were assessed. Absorption characteristics were determined through the predicted gastrointestinal uptake, solubility, P-glycoprotein substrate, skin permeability (log Kp), and bioavailability scores. Distribution properties were evaluated on the basis of blood-brain barrier (BBB) penetration. Metabolism was analysed *via.* cytochrome P450 (CYP) enzymes, and the excretion potential was evaluated using total clearance values.

3.8.4.3 *In silico* Toxicity Prediction of Bioactive Compounds

The toxicity prediction is essential component in development of new drugs. The effective therapeutic drug must be safe, soluble within the gastrointestinal tract, and ability to cross physiological barriers like BBB. In this study, toxicity profiles were examined along with the solubility, drug score, and other safety parameters, including skin irritation, reproductive toxicity, tumorigenicity, mutagenicity, and eye irritation. The toxicity results were obtained using a colour-coded system, where red indicates high toxicity, yellow represents moderate toxicity, and green represents no toxic risk.

3.8.4.4 Molecular Docking Studies

The two-dimensional structures of the selected ligands were prepared and saved in both .pdb and .sdf formats for the following molecular docking studies. Three-dimensional structures of the target protein, dipeptidyl peptidase (DPP-IV), with PDB ID: 2RIP, was obtained from RCSB Protein Data Bank. The 2RIP structure, reported to have a resolution of 2.1 and 2.5 Å, was obtained in .pdb format, and the protein preparation was completed using a protein preparation wizard, which involved the removal of water molecules and heteroatoms. The active binding site was identified on chain A, with residue 34Q, as the inhibitor site. After structural refinement, the energy minimisation was carried out using the OPLS force field. The receptor grid was prepared to determine the ligand interactions with the amino acid residues. The output files were saved in Excel and .png formats. Molecular docking was performed to know the interactions between the selected ligands and the DPP-IV receptor (2RIP). The molecular docking studies helps in the identification of potential lead compounds based on their docking scores.

Phase-V *In vitro* Selected Biomedical Applications of Plant Extracts and Biosynthesised Metallic Nanoparticles

This section explores the potential biological applications of the biosynthesised metallic NPs, specifically gold nanoparticles (GNPs) and silver nanoparticles (SNPs). The evaluation includes their *in vitro* antioxidant, antibacterial, anticancer, and antidiabetic activities along with their toxicity profiles. In addition, the most effective NPs were employed for the nano-encapsulation of microspheres and assessed for their antibacterial and antidiabetic efficacy.

3.9 Antioxidant Activity of Plant Extracts and Synthesised Metallic NPs

3.9.1 Preparation of Standard and Samples

The ascorbic acid stock solution (1 mg/mL in H_2O) was initially prepared. Serial dilutions were carried out to obtain five different concentrations (3, 6, 9, 12, and 15 µg/mL) each made up to a final volume (10 mL) using distilled H_2O. DPPH (0.3 mM) solution in methanol was prepared for analysis. Similarly, extracts were prepared at 1 mg/mL concentration. Hexane and chloroform extracts were dissolved in ethanol, while other extracts were soluble in distilled H_2O. Additionally, various volumes of biosynthesised GNPs and SNPs, prepared by solar light irradiation method, including APEG**,** TAEG, BFEG, DSEG, APES**,** TAES**,** BFES**,** and DSES NPs were also evaluated for antioxidant activity.

3.9.1.1 DPPH Free Radical Scavenging Assay

The DPPH radical scavenging assay of extracts and synthesised metallic NPs from AP, TA, BF and DS were conducted with slight modification as described by **Nikhat *et al.*, (2009)**. To perform the assay, 2 mL methanol and 1 mL DPPH (0.3 mM) was added to 2 mL sample/standard solution at varying concentrations. The samples were then left undisturbed and kept under dark conditions for 30 min. Following this, the absorbance values for the standard and samples were recorded at 520 nm using a photo colorimeter. A control was prepared without adding the sample. The ability of the extracts and metallic NPs, to donate hydrogen bond donors and scavenge DPPH free radicals was determined with the equation given below:

$$\text{Inhibition (\%)} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \text{ X } 100$$

3.9.2 Antibacterial Activity of Biosynthesised Metallic Nanoparticles Against Microorganisms

3.9.2.1 Microorganisms used for Antibacterial Activity

The antibacterial activity of the plant extracts, biosynthesised metallic NPs, and the standard (ciprofloxacin) was tested against bacterial strains: two gram-positive strains - *Staphylococcus aureus* (*S. aureus*) and *Bacillus cereus* (*B. cereus*) and three gram-negative strains including *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) *via* agar well diffusion method **(Athanassiadis *et al.*, 2009)**.

3.9.2.2 Antibacterial Activity of Extracts and Synthesised Metallic Nanoparticles

Each bacterial strain was separately transferred into 5 mL sterile nutrient broth and allowed to grow at 37°C for 24 h. About 200 µL each bacterial culture was transferred into 30 mL sterile nutrient broth and incubated for 2 to 4 h to standardise the bacterial culture to 10^8 CFU/mL (colony forming units). Once the inoculum suspension was standardised, a sterile cotton swab was dipped into the suspension, rotated several times, and firmly pressed against the tube wall to remove excess inoculum. Each bacterium was then swabbed and streaked separately on the entire surface of a sterile dried Muller Hinton agar plate. After 3 to 5 min, the wells (6 mm) were made in the agar using a sterile cork borer. The samples, including APE, TAE, BFE extracts (1 mg/mL) and metallic NPs (GNPs and SNPs) such as APEG, APES, TAEG, TAES,

BFEG and BFES, were loaded (50 µL) into the wells. For APES NPs (100 µL) were performed by disc-diffusion method, using the clinically isolated inoculums (*Bacillus subtilis, K. pneumoniae, P. aeruginosa, S. aureus*). The culture plates were incubated for 24 h at 37°C. After incubation, the maximum zone of inhibition (ZOI) was measured in millimetre using a Hi-Media standard scale. Ciprofloxacin was used as a standard (std) to compare the obtained results.

3.9.3 Anticancer Activity of Synthesised Metallic Nanoparticles Against A^{549} Cells

The anticancer activity of extracts (APE and TAE) and biosynthesised metallic NPs (APES, TAEG, TAES, BFEG, and BFES) against human lung cancer cells (A^{549}) were assessed using MTT dye reduction method, following the procedure reported by **Igarashi and Miyazawa (2001)**. Details on cell lines, culture maintenance, and procedures are provided in **Appendix iv**. The standard drug etoposide (50 µg) and control cells without treatment were evaluated in triplicate. The samples (APE, TAE, APES, TAEG, TAES, BFEG, and BFES) were tested using various concentrations (25, 50, 75, 100, 125, 150, 175, and 200 µg) in triplicate. The percentage viability of the samples and the standard was determined with the equation given below:

$$\text{Viability (\%)} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \text{ X } 100$$

3.9.4 Antidiabetic α-amylase Inhibitory Potential of Extracts and Synthesised Metallic Nanoparticles

The α -amylase inhibition for the plant extracts and biosynthesised metallic GNPs and SNPs were conducted according to the modified method reported by **Wickramaratne *et al.* (2016)**. The antidiabetic activity of plant extracts, GNPs, and SNPs were assessed at various concentrations against α-amylase using the chromogenic DNS method through an *in vitro* inhibition assay. The details of the solution preparation, and test procedure are provided in **Appendix v**. The α-amylase inhibition (%) was determined with the equation given below:

$$\alpha \text{ amylase Inhibition (\%)} = \frac{\text{Blank absorbance} - \text{Sample absorbance}}{\text{Blank absorbance}} \text{ X } 100$$

3.9.5 *In vivo* Toxicity Evaluation of Nanoparticles

Toxicity studies are essential for evaluating the potential toxicity of plant extracts, isolated bioactive compounds, and synthesised metallic NPs. Minimal to no toxicity is crucial for the successful development of pharmaceutical and cosmetic products **(Sharma and Kharel, 2019).** The *in vivo* toxicity of the extracts and biosynthesised GNPs and SNPs were evaluated using *Allium cepa* root tip bioassay.

3.9.5.1 *Allium cepa* root tip Bioassay of Synthesised Metallic Nanoparticles

Allium cepa root tip bioassay was performed for the plant extracts and their synthesised GNPs and SNPs following the standard procedure by **Rajeshwari *et al..*, (2016)**, with minor modifications. Healthy onion bulbs weighing 15-30 g each, were selected. The outer scales of the bulbs were removed, and bulbs were placed in glass test tubes containing distilled water and maintained at room temperature (27°C). The water in the test tube was replaced every 24 h with fresh distilled water. The roots of length reached to 2 to 4 cm, they were excised from the bulbs and treated with the test samples at various concentration (25, 75, and 150 µg/mL) for 4 h.

Potassium dichromate (PD) at a concentration 1 mg/mL was used as the positive control, and distilled H_2O severed as negative control (blank). Tested samples included the plant extracts (APE, TAE, BFE, and DSE), and their corresponding synthesised GNPs and SNPs, all prepared at 1 mg/mL concentration.

After 4 h treatment, the exposed root tips were removed, and 1-2 mm section were cut from the tips using a sterile blade. These meristem regions were further fixed in a solution of glacial acetic acid-ethanol (1:3) for 1 h at 3˚C. Further, the fixed meristems were hydrolysed in 2 N HCl for 5 min, rinsed twice with distilled water, and stained with acetocarmine. The stained root tips were gently heated and squashed under a cover slip for microscopic observations. The mitotic phases were observed under trinocular (Bio blue lab) optical microscope at 1000x magnification. To determine the mitotic index and chromosomal aberrations, approximately 100 to 250 cells were counted for each sample, including control. The percentage mitotic index (MI) was determined with the equation given below.

Mitotic index (MI) % = [Number of dividing cells / Total number of observed cells] *100

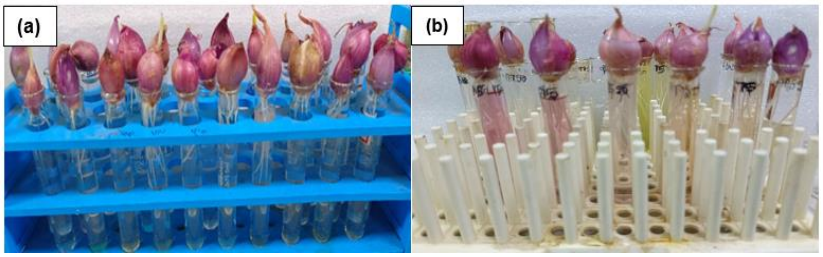


Figure 3. *Allium cepa* root tips (a) Roots grown in distilled H_2O; (b) Root tips treated with synthesised nanoparticles

3.9.6 Preparation of Nanoencapsulated Microspheres

Nanoencapsulated microspheres were prepared following a modified emulsion solvent evaporation method described by **Banstola *et al.*, (2019)**. Three types of active nanocomponents include APE-aided silver nanoparticles (APESNP), DSE -aided gold nanoparticles (DSGNPs) and squeezed fresh garlic juice were selected due to their potent biomedical applications. These nanocomponents were incorporated into a biodegradable polymer matrix to achieve sustained drug release. For the preparation of MMS1AS and MMS1DG formulations, the synthesised NPs (APES or DSEGNPs) and metformin (1 mL) was dispersed in 1% (w/v) PVP solution (5 mL) and stirred to obtain an aqueous phase. Similarly, for the garlic-nano loaded formulations, the aqueous phase was prepared using 1:1 ratio of NPs (APES and DSEG) and freshly squeezed garlic juice extract in 5 mL PVP solution (1%), which was magnetically stirred for homogeneous mixing.

The organic phase, composed of PCL, HPMC and PEG in equal proportions, were dissolved in a mixture of dichloromethane (DCM) and ethanol (2:1 v/v) which was added dropwise to the aqueous phase under continuous stirring at room temperature for 4 h to form a stable emulsion. After homogenisation, the organic solvent was allowed to evaporate, resulting in the formation of solidified microspheres. The obtained microspheres were washed with distilled H_2O to remove the unencapsulated residues and air-dried at RT. The prepared microspheres were labelled according to the compositions as MMS1DG, MMS1AS, MS1G2 and MS1G2S **(Table 6)**.

Table 6. Preparation of Nanodrug and Garlic-nano Encapsulated Microspheres

Sample code	Organic Phase in DCM/ethanol	Aqueous phase			Method
		Emulsifier (1%)	Drug	Active ingredients	
MMS1DG	PCL +HPMC + PEG (50+50+50 mg)	PVP – 5 mL	5 mL (1 mg/mL)	DSEGNP	Emulsion Solvent Evaporation
MMS1AS				APESNP	
MS1G2			-	DSEGNP + Garlic juice	
MS1G2S			-	Garlic juice + DSEGNP + APES	

3.9.6.1 Characterisation of Nanoencapsulated Microspheres

The formulated drug-loaded nanoencapsulated microspheres were characterised for drug compatibility using ATR-FTIR spectroscopy, with a wavenumber range from 4000 to 400 cm^{-1}. The amorphous/ crystalline nature of the formulated microspheres was analysed using XRD. UV BioSpec nano spectrophotometer was used to record the drug release solutions, with PBS (0.05 M) as the blank. FESEM morphology and elemental analysis of formulated microspheres were also performed.

3.9.6.2 *In vitro* Antibacterial and Antidiabetic α-amylase Inhibitory Activity

In vitro antibacterial activity of blank microspheres (MS1), and garlic encapsulated microspheres (MS1G2, and MS1G2S) was evaluated against four bacterial strains: *S. aureus*, *B, cereus, K. pneumoniae*, and *E. coli.* Ciprofloxacin was utilised as standard reference antibiotic. The antibacterial activity was performed according to the procedure described in section 3.9.2.2, using agar well diffusion method. After inoculating Mueller-Hinton agar plates with respective bacterial cultures, the wells were loaded with the test samples. The culture plates were kept under standard conditions at 37 ˚C for 24 h. Following incubation, the ZOI around the well were measured in millimetre using a Hi-Media standard scale, to assess and compare the antibacterial efficacy of microsphere formulations.

The α -amylase inhibition of garlic encapsulated and garlic-nano encapsulated microspheres (MS1G, MS1G2, MS1G2S,) and standard metformin was determined using a modified method given by **Wickramaratne *et al.* (2016)**. The antidiabetic activity was evaluated through *in vitro* chromogenic assay based on DNS method. Details of the preparation of solutions, and step-by-step assay procedure are provided in **Appendix v**.

3.9.6.3 Thermal Stability Analysis of Bioactive compounds in Garlic/Garlic-nano Encapsulated Microspheres

To evaluate the thermal stability of the bioactive compounds in garlic/garlic-nano encapsulated microspheres (MS1G and MS1G2S), the sample was divided into three portions and subjected to different temperature treatments. Each portion was mixed with 5 mL distilled H_2O and heated under controlled conditions: RT (27-29˚C); moderate temperature (40-50 ˚C); and high temperature (90 -100˚C). The heating duration was maintained at 5 min, and conditions

were monitored using a thermometer. After heat treatment, all the samples were analysed using FTIR, to assess the retention of sulfoxide and allyl related functional groups in the sample.

3.9.6.4 *In vitro* Dissolution and Drug Release Kinetics of Nano encapsulated Microspheres

The drug release study of the nanoencapsulated microsphere formulations enclosed in a dialysis bag was carried out by following the method of **Yu *et al.*, (2019)**. The study included the formulated microspheres such as MMS1DG, and MMS1AS was conducted in a simulated physiological medium (0.05 M PBS), to mimic body fluids such as blood, and the extracellular fluid surrounding the cells. Drug dissolution was performed at 100 rpm in 900 mL PBS medium. At regular intervals, aliquots were withdrawn and replaced with equal volumes of fresh PBS. The samples were diluted tenfold prior to analysis, and the absorbance was measured at 231 nm for metformin and 251 nm for garlic. The drug release kinetics of the nanoencapsulated microsphere formulations were assessed using kinetic models, following the procedure outlined in section 3.6.3.

Phase-VI Industrial Applications of Metallic Nanoparticles

3.9.7 Fabrication of Microbial-Resistant and Ultraviolet Rays-Protective Fabrics for Textile Applications

This section focuses on industrial-based textile applications for enhancing the performance of cotton fabric against microbe resistant pathogens and at fabricating UV-resistant fabrics using synthesised NPs.

3.9.7.1 Desizing of Cotton Fabrics

The bleached plain weave cotton fabric with the following characteristics: GSM-135 g/m^2, thickness (0.319 mm), and yarn count of 86 yarns/cm in warp direction and 84 yarns/cm in weft direction. The cotton fabric was scoured with 2% NaOH (material liquor ratio 1:40) at 90°C for 30 min to remove both soluble and insoluble impurities from fabric materials. After scouring, the fabric was thoroughly washed and shade dried. The dried cotton fabric, was cut into 2*2 cm^2 pieces and subjected to dyeing with and without surface treatments, followed by dyeing process. The fabrics were pre-weighed before surface treatment and after dyeing. The experiments were performed in triplicate (n=3), and mean values were noted.

3.9.7.2 Preparation of Dye solutions

3.9.7.2.1 Preparation of Ball Milled Red Sandal Powder

Commercially available red sandal powder -RSP (140 g) was placed in a ball mill chamber with 12 large stainless-steel balls (average weight - 16.3096 g/ball; diameter - 10 to 20 mm) and subjected to ball milling for 16 h. The entire process included 15 min break after every 2 h to prevent heat build-up. The resulting sample was labelled as BMRSP and used for further studies.

3.9.7.2.2 Preparation of BMRSP Alkali Dye Solution

About 1% NaOH solution was used to extract dye from alkali extraction of BMRSP using boiling method (80˚C/20 min). Sixty gram of ball-milled red sandal powder was mixed with hexane and the solvent was evaporated. BMRSP was added to one litre of NaOH solution

and heated at 90˚C for 30 min with occasional stirring. The solution was filtered, and the filtrate was used as a natural dye for surface treated cotton fabrics. The solution was labelled as BMRSP alkali dye solution.

Similarly, red sandal powder (RSP, 60 g) was mixed with an alkali solution (material-to-liquid ratio 1:20) and heated at 90˚C for 30 min. After dyeing, the fabrics was dried at RT. The following solution was labelled as RSP dye solution.

3.9.7.2.3 Preparation of TA and BMRSP Alkali dye

TAEA (*Tristellateia australasiae* ethyl acetate) extract (150 mg) was mixed with 15 mL NaOH (1%) solution and vortexed for 20 min to prepare the TA alkali dye. Similarly, BMRSP (150 mg) was mixed with 15 mL NaOH solution (1%) and vortexed for 20 min to prepare BMRSP alkali dye.

3.9.7.2.4 Preparation of Composite Dye

Three different composite dye solutions were prepared using TA alkali dye, BMRSP alkali dye, ionic liquid (IL) and synthesised SNPs. The first composite dye solution was prepared by mixing equal volumes of TA alkali dye and BMRSP alkali dye in a 1:1 ratio and was labelled as TBD. The second composite dye solution was prepared by adding 5 mL 1-butyl 3 methyl imidazolium chloride-BMIMCl (0.1%) to the TBD solution, followed by stirring and vortexing for 20 min, this was labelled as TBID. Finally, the third composite dye solution was prepared by adding SNPs to the TBID solution and vortexed for 20 min to obtain the final composite mixture, labelled as TBISD.

3.9.8 Surface Pre-Treatment of Cotton Fabrics

Surface treatment helps in increasing the fabric functionality and improve the adhesion properties of dye to the materials. For the surface treatments of cotton fabrics, we utilised ozone (O_3), UV rays (UV), plasma and combined O_3/UV and O_3/UV/Plasma treatments

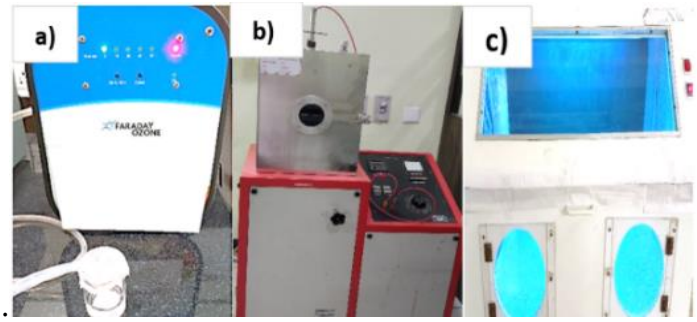


Figure 4. Images of the equipment used for surface pre-treatment of fabrics a) Faraday ozone generator; b) Plasma chamber; c) UV chamber

3.9.8.1 Effect of Exposure Time in O_3, UV and O_3/UV Treatment on Dyeing Performance of Cotton Fabrics

Cotton fabric (2*2 cm^2) samples were individually exposed to O_3 at varying time intervals (30, 60, 90, and 120 min) using a Faraday Ozone generator. Similarly, another set of cotton fabric were exposed to UV (254 nm, 180 W) treatment in a UV chamber for 60, 120, 180 and 240 min. Additionally, a separate set of cotton fabrics were subjected to O_3 irradiation (30,

60, 90 and 120 min), and then exposed to UV irradiation for 120 min, referred to as combined dry O_3/UV treatment. The sample code assigned for the surface treated fabrics are given below.

Sample code	Treatment time (min)	Sample code	Treatment time (min)	Sample code	Treatment time (min)	
O_3		**UV**		**Combined O_3/UV**		
S1CO	30	**S1CUV**	60	**S1COUV**	30	120
S2CO	60	**S2CUV**	120	**S2COUV**	60	
S3CO	90	**S3CUV**	180	**S3COUV**	90	
S4CO	120	**S4CUV**	240	**S4COUV**	120	

3.9.8.2 Combined dry O_3/UV Treatment on Dyeing Performance of Cotton Fabrics

Based on the effectiveness of O_3 alone, UV alone, and the dry combined O_3/UV treatment as given above, the combination of dry O_3 treatment (90 min) and UV irradiation (120 min) showed the highest colour strength, after dyeing the fabric. Therefore, the remaining cotton fabrics were treated with combined dry O_3/UV treatment at specified time intervals.

3.9.8.3 Combined dry O_3 (without water) /UV and wet O_3 (with water)/UV treatment

Cotton fabrics were surface treated under ozone (O_3) with or without water for 90 min. The assigned sample codes were given below. For the CWOUVS3 sample, the fabric was immersed in water (20 mL), treated with O_3 for 90 min, dried, and then treated with UV irradiation for 120 min. For the COUVS3 sample, the fabric was treated with dry O_3 (without water), under closed condition for 90 min followed by UV treatment (120 min). Both sets of pre-treated fabrics were left for dyeing.

Sample code	with water/ without water	Combined O_3/UV Treatment time (min)	
		O_3	**UV**
CWOUVS3	with water	90	120
COUVS3	without water	90	120

3.9.9 Dyeing of Surface Pre-Treated (O_3/ UV alone and Combined dry O_3/UV) Cotton Fabrics

The conventional heating method was used to dye the pre-treated cotton fabrics (1/2 meter) with BMRSP alkali extract. The material-to-liquor ratio was 1:20 and the dyeing bath temperature was set at 80˚C to 90˚C for 45 min. The dyed samples (COUVS3 and CWOUVS3) were dried at RT and then used for further analysis.

3.9.9.1 Combined dry O_3/UV/Plasma treatment and Plasma-alone treatment

Based on the effects observed from wet /dry O_3 treatment for 90 min (with or without water) followed by UV treatment for 120 min, it was found that dry O_3 exposure (without water) followed by UV treatment resulted in high colour strength. Consequently, for the remaining surface treatment of fabrics, dry O_3 without water is applied for 90 min, followed by UV

treatment for 120 min. For the combined dry O_3/UV/Plasma treatment, the cotton fabrics were first exposed to O_3 for 90 min, then to UV irradiation for 120 min, and finally to plasma treatment for 45 min.

A separate set of fabric samples was treated with plasma for 45 min using an atmospheric plasma system. Atmospheric O_2 was introduced into the chamber, a vacuum was created (2 to 12 bar), and electrical discharge occurred through the plasma electrode. Plasma glow discharge was achieved after 15 to 20 min with applied plasma current of 1.18 A^o.

3.9.9.2 Dyeing of Surface Pre-Treated (Combined dry O_3/UV/Plasma and Plasma alone) Cotton Fabrics

The surface treated cotton fabric samples (2*2 cm^2) were immersed in dye solutions (80°C to 90°C) for 30 to 45 min. During dyeing process, the cotton fabric was immersed in the dye solution and it was stirred every 5 min to avoid coagulation and to attain an efficient dye coating. After dyeing, the dyed fabrics were squeezed, and dried at RT. The dried coated fabric was weighed, and the difference in weight before and after coating was calculated. Similarly, the conventional cotton fabric (C5) was dyed without any surface pre-treatments. The standard fabric (std) was coated with ciprofloxacin (100 ng/mL). The sample blank cotton fabric (B) was used as control. The fabric surface treated by combined dry O_3/UV/plasma dyed fabric and plasma treated dyed fabric samples were assigned the sample code as given below.

Sample code	Treatment Time (min)	Coated samples
TOUPD	90/120/45 (Combined O_3/UV/plasma)	TAEA alkali extract
TPTD	45 (plasma)	TAEA alkali extract
TPTBD		TAEA + BMRSP alkali extract
TPTBID		TAEA + BMRSP alkali extract+ IL
TPTBISD		TAEA + BMRSP alkali extract+ IL + SNP
C5	No surface treatment	TAEA + BMRSP alkali extract+ IL + SNP
Std	45 (plasma)	Commercial ciprofloxacin

3.9.9.3 Characterisation of Dyed Fabrics

The surface-treated dyed fabrics [CWOUVS3, COUVS3, TPTBID, TPTBISD, and blank cotton fabric (B)] were characterised using FTIR, XRD, spectrophotometer with color lab, FESEM, and EDS analysis.

3.9.9.4 Evaluation Properties of Dyed Cotton Fabrics

The tensile strength (ASTM-5034-09 (2017)), stiffness (ASTM 1388-14 (2015)) and thickness (ASTM-D1777-96 (2015)) of the samples were tested according to standard procedures. The surface plasma-treated and dyed fabrics (TPTBID and TPTBISD) were evaluated for colour on screen, colour fastness to rubbing (wet/dry conditions), colour fastness to light, and leaching properties. Colour analysis was performed using a UV color lab with a spectrophotometer. Colour fastness was assessed by rubbing the samples in wet and dry

conditions and exposing them to sunlight for 10 days. Colour strength values were used to determine any change in fabric colour.

3.9.9.5 Antimicrobial Activity of Dyed Fabrics

The antimicrobial activity of eco-friendly dye-impregnated cotton fabrics was evaluated against gram-positive bacteria (*S. aureus*) and fungal species (*C. albicans*), using AATCC standard Kirby-Bauer method. The gram +ve bacteria were precultured in a nutrient broth medium, adjusted with sterile saline, and incubated at 35°C until the turbidity suspension reached 1 to 2 x 10^8 CFU/mL.

The fungal inoculum (*C. albicans)* were precultured on potato dextrose agar at 25°C for 4 to 5 days. The potato dextrose agar plates were streaked with a swab dipped in the standardised inoculum and left to dry for 3 min. The samples were placed on the steaked agar plates and incubated at 30°C for 4 to 7 days. Ciprofloxacin-coated fabrics (100 ng/mL) served as the standard for both bacterial and fungal test. After incubation, the ZOI was measured in millimetre.

3.9.9.6 UV-Protective Dyed Fabrics

The fabric samples (B, C5, CWOUVS3 and COUVS3) were subjected to UPF analysis. UPF values were determined with a UV-2000 transmittance analyzer which measures the diffuse transmittance in UV wavelength (250 to 450 nm). After passing through the analyser to the fabric at five different directions, determines the mean UPF values assigned to the samples providing UVA (wavelength of 315 to 400 nm) and UVB (280 to 315 nm) transmittance values. Fabrics were classified based on the standard UPF values as follows: good protection (15-24), very good protection (25-39), and excellent ($\geq$40) according to **Gambichler *et al.*, (2002)**.