Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 3029-3042 | ISSN:2251-6727

"Development of Biodegradable Neem Extract Nano Emulsion as Pesticide"

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(Received: 25 October 2025 Revised: 27 November 2025 Accepted: 16 December 2025)

KEYWORDS

Azadirachtin, Cold maceration, Medicinal crops, Nanoemulsion, Neem (Azadirachta indica), Sustainable agriculture

ABSTRACT:

Background: The increasing demand for sustainable agricultural practices has led to the exploration of eco-friendly alternatives to synthetic pesticides. This study investigates the formulation and application of neem(Azadirachta indica) extract-based nanoemulsion as a biodegradable pesticide aimed at enhancing the cultivation of various medicinal crops. Neem is renowned for its potent biopesticidal properties, attributed to its active constituents such as azadirachtin, nimbin, and salannin, which exhibit strong insecticidal, antifungal, and antibacterial activities. To overcome the limitations of neem's direct application, such as volatility and poor water solubility, nanoemulsion technology has been employed to improve the stability, bioavailability, and targeted delivery of neem extracts.

Methods: The research encompasses the preparation of neem extract using ethanol through cold maceration, followed by nanoemulsion formulation using surfactants and co-surfactants through low-energy emulsification techniques. Physicochemical characterizations including particle size analysis, zeta potential, pH, viscosity, and stability studies confirmed the successful formation of a stable nanoemulsion.

Results: Antimicrobial assays and pesticide activity tests demonstrated significant efficacy against phytopathogens and common agricultural pests. Additionally, the study evaluates the effect of the nanoformulated pesticide on selected medicinal crops, revealing enhanced growth parameters, reduced pest infestation, and improved plant health. The results confirm the potential of neemnanoemulsion as a sustainable biopesticide with minimal environmental toxicity and broad-spectrum activity.

Conclusion: This work contributes to the growing body of research advocating for nanotechnology-based interventions in green agriculture and supports the advancement of integrated pest management systems for medicinal crop production.

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JCHR (2025) 15(6), 3029-3042 | ISSN:2251-6727

INTRODUCTION

Agriculture remains the backbone of India's economy, with pest and disease management playing a crucial role in its sustainability. Pests account for significant crop losses up to 60% annually in certain cases due to factors like monoculture, climate change, and growing resistance to synthetic pesticides [1]. The extensive reliance on chemical pesticides has raised concerns over their ecological impacts, including soil and water contamination, bioaccumulation in the food chain, and harm to humans, livestock, and pollinators [2].Amid increasing global demand for chemical-free, eco-friendly agricultural produce, botanical pesticides are gaining traction. Neem (*Azadirachta indica*), in particular, has drawn attention [22] for its insecticidal, antifungal, and antibacterial properties [3]. Traditionally valued in Indian agriculture and medicine, neem'spesticidal potential is being re-evaluated in the context of nanotechnology for enhanced efficacy and sustainability.

Neem holds an esteemed position in India's cultural and agrarian history. Known by names such as Indian lilac, margosa, and nimba, it belongs to the Meliaceae family and thrives across dry regions of the Indian subcontinent [4]. The Neem Foundation estimates over 20 million neem trees in India, accounting for 60% of the world's neem population. Its resilience in degraded soils and ability to counter desertification has expanded its cultivation across Asia, Africa, the Americas, and Australia[5]. Neem is culturally sacred in India and has been referenced in0Ayurvedic texts as a "village pharmacy" for its wide-ranging medicinal uses [6].

MATERIALS AND METHODOLOGY [23]*:*

In this study, a variety of laboratory instruments were employed to ensure the accuracy, reliability, and reproducibility of the experimental results. The instruments used include a **laboratory grinder** and **mortar and pestle** for sample preparation, while **rotary evaporators**, **heating mantles**, **hot air ovens**, and **water baths** were used for controlled heating and evaporation processes. **Weighing balances** and **analytical balances** provided precise measurements of samples and reagents. To maintain and monitor appropriate environmental and storage conditions, a **refrigerator (2–8°C)**, **freezer (-20°C)**, and **thermo-hygrometer** were utilized. Analytical procedures involved the use of a **UV spectrophotometer**, **pH meter**, and **muffle furnace** for various testing protocols. Microbial and contamination testing was conducted using specialized equipment such as the **colony counter**, **Total Organic Carbon (TOC) analyzer**, **Endotoxin Testing System (LAL Reader)**, and **media dispenser**. Additionally, a **magnetic stirrer/hot plate** facilitated proper mixing of solutions. The integration of these instruments allowed for comprehensive sample processing, precise measurement, and rigorous analysis throughout the study

The plant's pesticidal effects stem from bioactive compounds like **azadirachtin, nimbin**, and **salannin**[7].However, conventional neem formulations face limitations such as poor water solubility, UV instability, and inconsistent potency [8]. These drawbacks reduce field effectiveness and suitability for sensitive crops like medicinal plants.

To overcome these challenges, researchers have developed **neem-based nanoemulsions**—stable colloidal dispersions with droplet sizes between 20–200 nm. These nanoemulsions, consisting of oil and water stabilized by surfactants, improve solubility, penetration, and stability of neem extracts[9]. Their features include enhanced bioavailability, reduced environmental impact, and compatibility with both hydrophilic and lipophilic substances[10].

In agriculture, nanoemulsions serve as carriers for pesticides, herbicides, and fungicides, enabling site-specific delivery and minimizing runoff and drift [11]. Neem oil nanoemulsions, in particular, improve the pesticidal action of azadirachtin by enhancing cuticular penetration and residual efficacy [12].This technology is especially valuable for **medicinal crops**, which demand residue-free pest control due to the risk of contamination and alterations in active phytochemicals[13]. Neem nanoemulsions ensure biocompatibility, support organic certification, and help maintain therapeutic quality without disrupting plant metabolism [14]. Additionally, their antimicrobial properties promote crop vigor by suppressing soil-borne and foliar pathogens.

SELECTION OF PLANT

The plant neem (*Azadirachta indica*) was selected for study. Its leaves were collected and Identification of neem leaves was carried out, As shown in **Figure 1**.

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JCHR (2025) 15(6), 01-07 | ISSN:2251-6727

Figure 1: Collection of the plant Neem (*Azadirachta indica*)

SELECTION OF PLANT:

The completely shade dried material was coarsely powdered and allowed soxhlet for successive extraction with ethanol as shown in **figure 2.** The obtained liquid extracts were subjected to Rotary evaporator and subsequently concentrated under reduced pressure (invaccum at 40°C) and evaporated to dryness and stored at4°C in air tight bottle (Waks, A et,al.2019).

Figure 2: Leaf extract of the plant Neem (*Azadirachta indica*)

Ethanol Extract:

50g of dried leaf powder of *Azadirachta indica* were takenin a separate container. To this 250 ml of ethanol wasadded and kept for 24 h with periodic shaking. Filteredand the filtrate was collected. The procedure was repeatedthree times. The collected filtrates were pooled (Waks, A et,al.2019).

PHYTOCHEMICAL SCREENING:

- **Detection of alkaloids:**Mayer's test, Hager's test, Dragendroff's test
- **Detection of carbohydrates:**Molisch's test, Fehling's test, Barfoed's test
- **Test for flavonoids:**Alkaline reagent test, Ferric chloride test
- **Test for steroids and triterpenoids:**Liebermann-Burchard's test, Salkowski test
- **Detection of proteins and amino acids:**Millon's test, Ninhydrin test
- **Detection of tannins:**Ferric chloride test, Test for chlorogenic acid
- **Detection of glycosides:** Borntrager's test, Keller-Killiani's test

FORMULATION OF NANO-EMULSION

Drugs: Neem extract (125mg)

Polymers : Non Dependable variable

Dependable variable: Drug release study and Drug loading efficiency

Formulation Table: Design expert ®

F Code	Neem extract (mg)	Ethyl cellulose (mg)	Hydroxy propyl Methylcellulose (mg)	Entrapment Efficiency%
F1	125	100	150	66.53
F2	125	150	200	81.73
F3	125	200	200	81.28
F4	125	100	100	70.55
F5	125	150	100	72.93
F6	125	100	150	66.53
F7	125	200	150	89.23
F8	125	150	100	72.93
F9	125	150	200	81.73
F10	125	100	200	68.03
F11	125	200	100	80.36
F12	125	200	150	80.63

Table 1: List of composition for nanoemulsion formulation

Synthesis of nanoemulsions

It will be necessary to carry out solubility experiments to determine which oil, surfactant, and co-surfactant will be the most appropriate for the formulation as compositing

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JCHR (2025) 15(6), 01-07 | ISSN:2251-6727

shown in **table 1** and stepwise formulation process shown in **figure 3.** To ensure simple removal, an organic solvent that will be both volatile and capable of dissolving the medicine will be selected. A suitable organic solvent, such as chloroform or dichloromethane, will be used to dissolve the active substance.The oil phase will be added to the solvent and drug mixture and thoroughly mixed to ensure complete dissolution of the drug. A mixture of surfactant and co-surfactant commonly referred to as mix, will be prepared in the appropriate proportion. A pseudo-ternary phase diagram will be used to determine the ratio.The oil-solvent mixture will gradually be added to the aqueous phase containing the oil. The coarse emulsion will then be moved to a rotary evaporator, and the organic solvent will be evaporated. Ultrasonication, either with a probe or a bath sonicator, will be utilized to decrease the size of the droplets and achieve an emulsion that is nano-sized.The necessary droplet size (less than 200 nm) will be obtained by optimizing the time of ultrasonication, which will be between five and fifteen minutes. The nanoemulsion will be filtered via a membrane filter with a pore size of 0.22 µm to eliminate any contaminants or large particles.Thenanoemulsion will be stored in amber glass vials at a temperature between 4 and 8 degrees Celsius. Under conditions of reduced pressure and regulated temperature (between 30 and 40 degrees Celsius), the organic solvent will be evaporated completely, resulting in the formation of a stable emulsion. To create a coarse emulsion, the components will be mixed together while being stirred constantly [21].

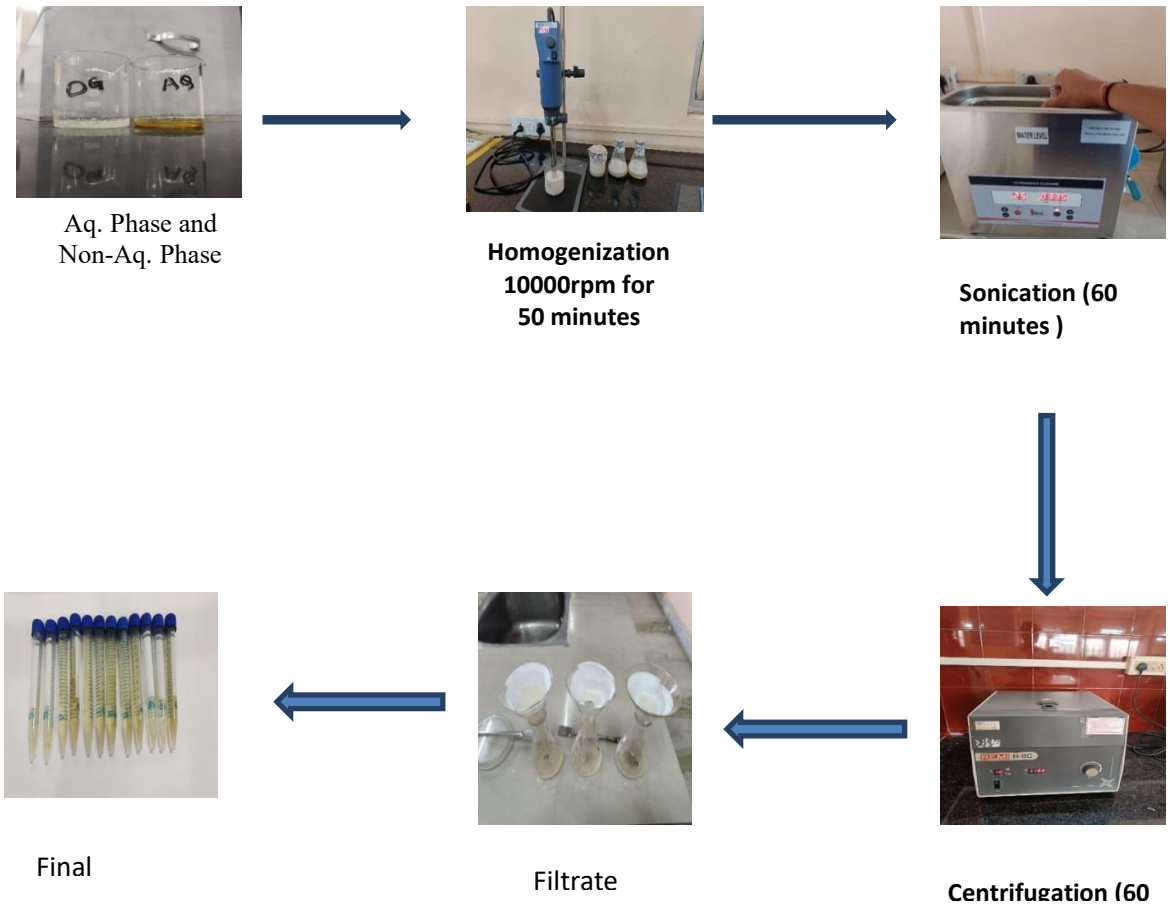

Figure 3: Step wise repressions of nanoemulsion preparation

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JCHR (2025) 15(6), 01-07 | ISSN:2251-6727

Characterization of Nanoemulsions and Antimicrobial Screening includes:

- Percent Drug Yield
- Percent Drug Loading and Entrapment Efficiency (EE)
- Particle Size Analysis
- Zeta Potential MeasurementScanning Electron Microscopy (SEM)
- In Vitro Drug Release StudiesAgar Disc Diffusion
- Method for Antimicrobial Activity
- Determination of Minimum Inhibitory Concentration (MIC) by MicrodilutionAssa

RESULT:

Thepotentialtherapeuticpropertiesof *Azadirachta indica*havemadeit remainlargelyunexplored in the realm of antioxidant studies. Many other plant species are the subjects of scientific scrutinyintotheirateoxidantattributes,leavingbehind*A zadirachta indica*.Thisproject,therefore, seeks to shed light on the antioxidant potential of Azadirachta indicaby investigating its extracts and constituents to find out their capacity to combat oxidative stress. Therefore, rigorous experimentation is being pursued to unmask any advantageous role that *Azadirachta indica*may possess in terms of its antioxidant activity, contributing to a wider understanding of its medicinal value.

PHARMACOGNOSTICALSTUDIES:12

OrganolepticCharacteristics:

The organoleptic characteristics of *Azadirachta indica* are as follows: the color is creamy white to light brown; the taste is mildly sweet; the odour is not distinct; and the texture is fibrous and tough

PhysiochemicalParameters:

The analytical evaluation of *Azadirachta indica* revealed the following physicochemical parameters: total ash content was 17.36% w/w, acid insoluble ash was 3.7% w/w, water soluble ash was 9.48% w/w, alcohol soluble extractive was 3.24% w/w, water soluble extractive was 3.67% w/w, loss on drying was 8.97% w/w, and the pH was recorded as 6.55

PHYTOCHEMICALEVALUATION:

Extraction:

The powdered stem of *Azadirachta indica* was subjected to solvent extraction using ethanol through cold maceration to preserve the integrity of active constituents that might degrade under heat. The ethanolic extract obtained from this process was then dried, weighed, and the yield recorded. The percentage yield of the ethanolic extract of *Azadirachta indica* was found to be 13.4%. This extract was subsequently used for phytochemical screening and antioxidant activity evaluation.

Preliminaryphytochemicalevaluation:

A general qualitative phytochemical screening of the ethanolic extract of *Azadirachta indica* was conducted to identify the presence of various chemical constituents. The results revealed the presence of alkaloids, as confirmed by positive results in Mayer's, Wagner's, and Dragendorff's tests. Carbohydrates were detected through Benedict's, Fehling's, and Molisch's tests, while Seliwanoff's and Barfoed's tests showed negative results. Glycosides were indicated by a positive Borntrager's test, whereas the magnesium–hydrochloric acid reduction test was negative. Cardiac glycosides were present, as both the Bromine water and Killer-Killani tests yielded positive results. Proteins and amino acids were confirmed through positive responses in Millon's, Biuret, Ninhydrin, and heat tests. Flavonoids were detected using the alkaline reagent and lead acetate tests, though the ferric chloride test was negative. Phenolic compounds were confirmed by both iodine and ferric chloride tests. Tannins were partially present, as shown by a positive bromine water test and a negative ferric chloride test. Among phytosterols, the sulphur test was positive while Salkowski's test was negative. Saponins were present as indicated by a positive foam test. Finally, anthraquinones were absent, as the Borntrager's test gave a negative result.

TotalPhenolicContent(TPC)of*Azadirachta indica*:

Thecrudeextractwasquantifiedforphenoliccontentbyu singfolinciocalteumethod.Gallic acidwasusedasastandardandstandardcalibrationcurve wasplottedconcentrationversus absorbance. The

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phenolic contentofthe crude extractwas calculated and expressed as Gallic acid equivalentpergram extract.**Figure4** and **table 2** represent standard calibration plot of Gallic acid and flavonoid content of crude extracts respectively. The results showed that 100µl/ml concentration of the extract contains significantly higher phenoliccontent(51.82±2.25GAE/gm)ascomparedtooth erconcentrationsofcrudeextracts.

Sl.No.	**Sample**	**GAEmg/g**
1.	100µl/mlconcentrationofsamplesolution	51.82458±2.252

Table 2:TotalPhenolicContentresultoftheextract

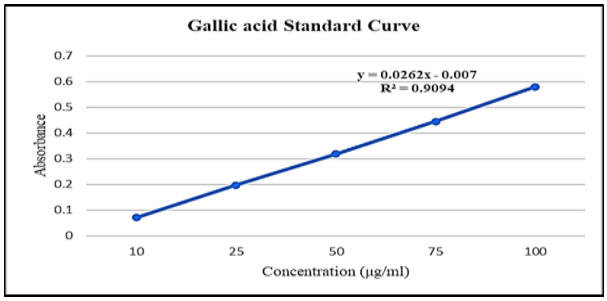

Figure4: CalibrationcurveforStandardGallicacid.

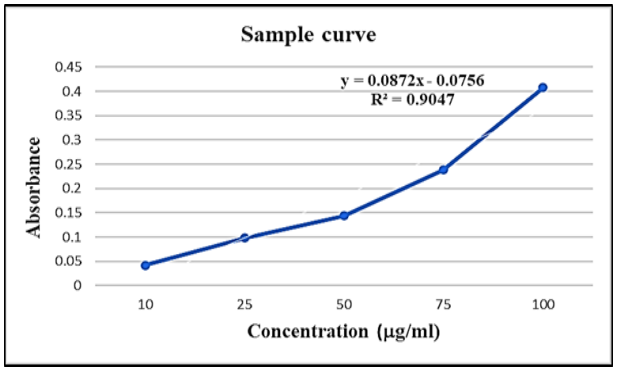

Figure 5:SamplecurveforTPC.

TotalFlavonoidContent(TFC)of*Azadirachta indica*:

All the crude extracts were quantified for flavonoid content by using aluminium chloride method. Quercetin was used as a standard and standard calibration curve was plotted, concentration versus absorbance. The flavonoid content of all crude extract was calculated and expressedasquercetinequivalentpergramextract**Figure 5** representsstandard calibration plot of quercetin whereas **table 3** indicates the flavonoid content of crude extracts.The results showed that 100µl/ml concentration extract contains significantly higher flavonoid content (58.28±1.86QEmg/gm) as compared to other concentrations of crude extract.

Sl.No.	Sample	QEmg/g
1.	100 µl/mlconcentrationofExtract	58.28146±1.862

Table 3:TotalFlavonoidContentresultoftheextract

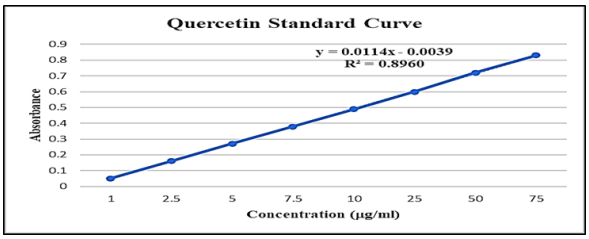

Figure 6:CalibrationcurveforStandardQuercetin

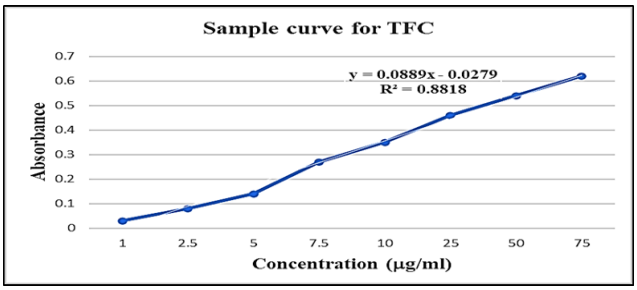

Figure 7:SamplecurveforTFC.

InVitroAntioxidant activity:

DPPHscavengingactivity:

In vitro activity of the ethanolic extract of*Azadirachta indica*was carried out using the DPPH method.Ascorbic acid was used as a standard. In 320 µg/ml concentration, the extractshowed the highestantioxidantactivity which is 75.61% inhibition.Ascorbic acid at320µg/ml concentration shows 82.17% inhibition.DPPHisastablefreeradical,whenan antioxidant treatswithDPPHtheelectronis pairedoffandtheDPPHsolutionisdecolorized.Thescav

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engingactivityofthe antioxidant for the bleaching of the colour stoichiometrically depends on the number of electrons taken up. The strong scavenging capacity of the extracts of *Azadirachta indica*on DPPH was possibly due to the hydrogen-donating ability of the polyphenolic compounds presentin the extract given in **table 4** and **figure 8.**

Table 4:InvitroantioxidantactivityofEthanolicextractof*Azadirachta indica*

Sl. No.	Concentration (g/ml)	Absorbance of Control	Absorbance ofStandard	% inhibition	Absorbance Of Sample	% Inhibition
1	10	0.324	0.212	34.56	0.231	28.70
2	20		0.187	42.28	0.201	37.96
3	40		0.155	52.16	0.178	45.06
4	80		0.117	63.88	0.142	56.17
5	160		0.084	74.10	0.114	64.81
6	320		0.056	82.17	0.093	71.30

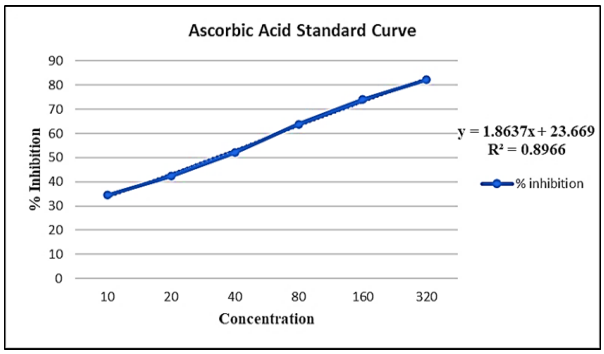

Figure 8:CalibrationcurveforStandardAscorbicacid

Antibacterial Activity

Disc Diffusion Assay

Preliminary screening for bioactivity of the extract on agar plates inoculated with a confluent lawn of bacterial cells proved that growth of all the strains was inhibited though to a varied degree. As tabulated in Table 11, diameter of ZOI reports higher inhibition in B. cereus, E. coli, and S. aureus at 17.7, 18.7, and 17.7 mm, respectively as compared to P.aeruginosa at 10.3 mm. **Figure 9** is representative of the effectiveness of the extract in S. aureus. Additionally, this antibacterial efficiency was retained and enhanced in the extract as evident from Figure:7 for E. coli and corroborated by diameter of ZOI measured for B. cereus, E. coli, P. aeruginosa, and S. aureus at 18.7, 20, 13, and 20 mm respectivelyin**Table 5.**

MIC and MBC

Disc diffusion assay results were further validated by determining the least inhibitory effect of the extract as MIC and the concentration of least biocidal agent required to kill 99.9% of bacteria as MIB when cultured on bacterial media. MIC and MBC values ranged from 390 to 780 µg/mL as represented in **Table 5** and**figure 9.**

TABLE 5.Determination of diameter of Zone of Inhibition (ZOI), Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of AI-AgNPs and AI-AgNPs-PF127 nanoemulsion tested against bacterial species.

Nameofthebacterialspecies	ZOIforextract (mm)	ZOIforAI-AgNPs-PF127 hydrogel (mm)	MICforAI-AgNPs(µg/mL	MBCforAI-AgNPs(µg/mL

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			)	)
Bacilluscereus	17.7±1.24	18.7±0.94	390	390
Escherichiacoli	18.7±1.15	20.0±1.0	780	780
Pseudomonasaeruginosa	10.3±0.50	13.0±0.46	780	780
Staphylococcusaureus	17.7±0.47	20.0±0.47	390	390

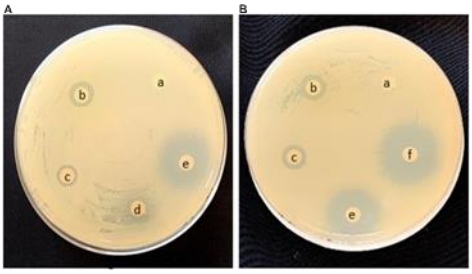

Figure 9:Antimicrobial effectinDiscdiffusionassayof (A)*S. aureus*and (B)*E. coli*(a) control,(b) rifampicin,(c) $AgNO_3$,(d) extract, (e) and (f)

Percentage Entrapment efficiency

Table 6. List of Percentage Entrapment efficiency

Sl no.	Formulation	% Entrapment Efficiency
1	F1	66.53
2	F2	81.73
3	F3	81.28
4	F4	70.55
5	F5	72.93
6	F6	66.53
7	F7	89.23
8	F8	72.93
9	F9	81.73
10	F10	68.03
11	F11	80.36
12	F12	80.63

All the batches showed entrapment efficiency in between 66-90%. The resultant entrapment efficiency is an indication that the method can be appropriate for technology transfer that is production on large scale. The optimized batch showed entrapment efficiency was found to be 89.23%. as shown in **table 6.**

In-vitro Drug Release Kinetics:

The *in-vitro* drug release kinetics of the optimized formulations was analyzed using the software named "DD Solver". The results were highlighted in bold values and indicate that the Korsmeyer-Peppas model provided the best fit for the formulations F7. This suggests that the release mechanism follows a combination of diffusion and erosion processes, consistent with the characteristics of the Korsmeyer-Peppas model shown in **table 7** and **figure 10 to 14.**

Table 7: List of the *in-vitro* Kinetics Models:

Formulation	Zero Order (r^2)	First order (r^2)	Higuchi (r^2)	Hixson-Crewel (r^2)	Korsmeyer-Peppas (r^2)	Release exponent (n)
F1	0.7561	0.9426	0.9896	0.9128	**0.9908**	0.471
F2	0.7627	0.9551	0.9889	0.9217	**0.9893**	0.481
F3	0.8805	0.9926	0.9797	0.9777	**0.9904**	0.599
F4	0.5921	0.9203	0.9605	0.8543	**0.9790**	0.397

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F5	0.9334	0.9900	0.9651	0.9930	**0.9938**	0.677
F6	0.9628	0.9496	0.9246	0.9644	**0.9813**	0.791
F7	0.9473	0.9675	0.9534	0.9737	**0.9939**	0.711
F8	0.9034	0.9512	0.9636	0.9539	**0.9798**	0.633
F9	0.9348	0.9604	0.9536	0.9700	**0.9843**	0.692
F10	0.8581	0.9323	0.9519	0.9220	**0.9602**	0.591
F11	0.9732	0.9252	0.8761	0.9458	**0.9741**	0.947
F12	0.9389	0.9811	0.9643	0.9858	**0.9890**	0.687

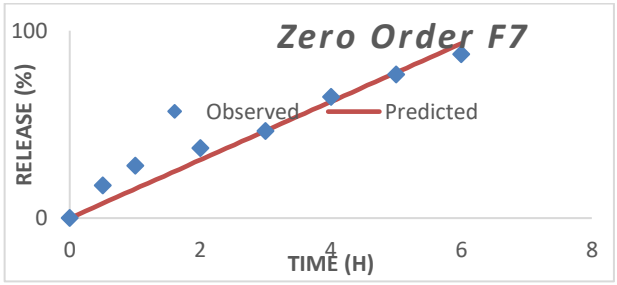

Figure 10: Graph Plot of zero order *In-vitro* Kinetics Model using DD Solver for F7

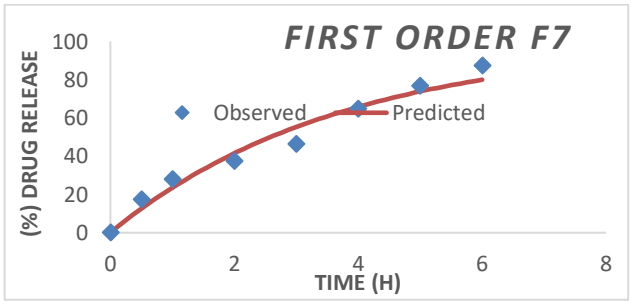

Fig.11: Graph Plot of 1st order *In-vitro* Kinetics Model using DD Solver for F7

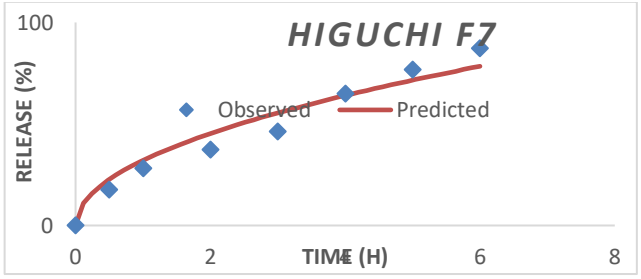

Figure.12: Graph Plot ofhiguchi*In-vitro* Kinetics Model using DD Solver for F7

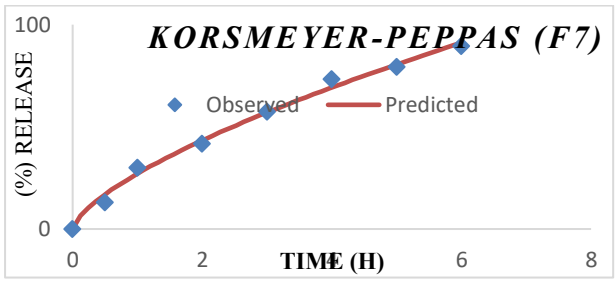

Figure 13: Graph Plot of kprmeyerpeppas*In-vitro* Kinetics Model using DD Solver for F7

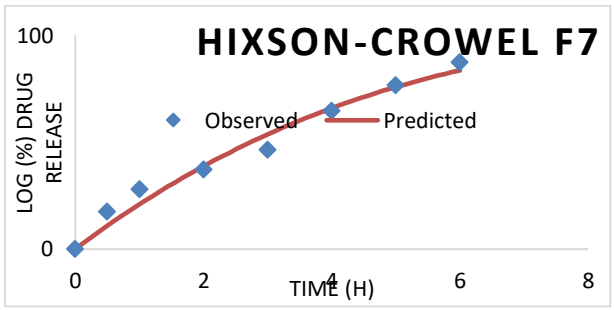

Figure 14: Graph Plot of Hixson crowel*In-vitro* Kinetics Model using DD Solver for F7

Particles size analysis

Table 8: list particle size Analysis

Formulation	Particles size (nm)	Peak size (nm)	PDI
F7	386	889	0.44
	366	820	0.85
	398	921	0.74

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Figure 15: Images of Particles Size of F7

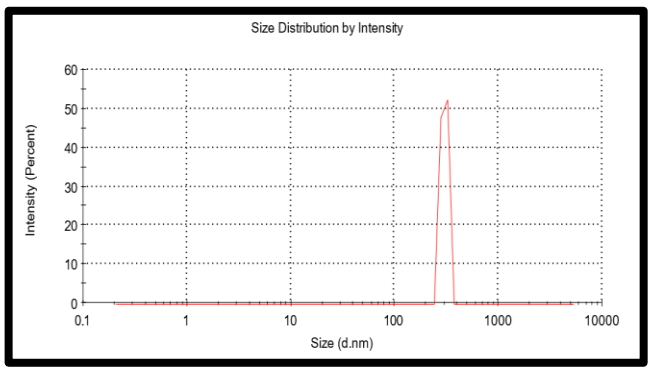
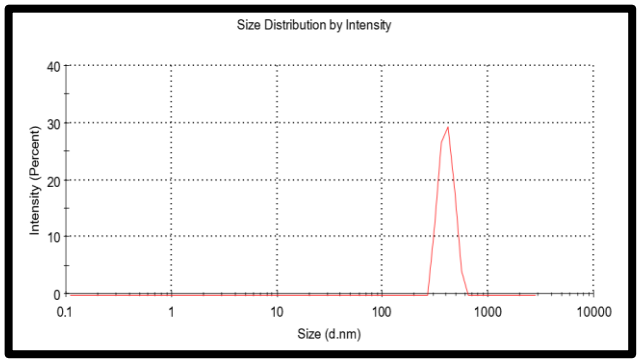


Figure 16: Images of Particles Size of F7:



The particle size analysis of formulation code F7 was conducted to evaluate the uniformity and distribution of the nanoparticles. The results, as presented in **Figure 15, Figure 16,** and the corresponding **table 8,** indicate that the average particle size of F7 formulations ranged between 366 nm to 398 nm, with peak sizes varying from 820 nm to 921 nm.Thepolydisparity index (PDI) values observed were 0.44, 0.85, and 0.74, indicating a moderately broad size distribution, particularly in the second and third measurements. A PDI value below 0.3 generally indicates a monodisperse system.

Scanning electron microscope (SEM):

Based on formulation optimization, including % yield, drug loading, entrapment efficiency, and in-vitro drug release experiments, the F7 formulation was shown to be consistent and optimal. As a result, the F7 formulation was studied using Scanning Electron Microscopy (SEM) to determine the surface shape of the nanoemulsions shown in **figure 17.** The SEM image indicated that the nanoemulsions of the Formulation F7 were smooth and spherical in structure. This nanoscale size confirms the successful formation of polymeric nanoemulsions, which is a critical prerequisite of achieving enhanced drug bioavailability and therapeutic efficacy.

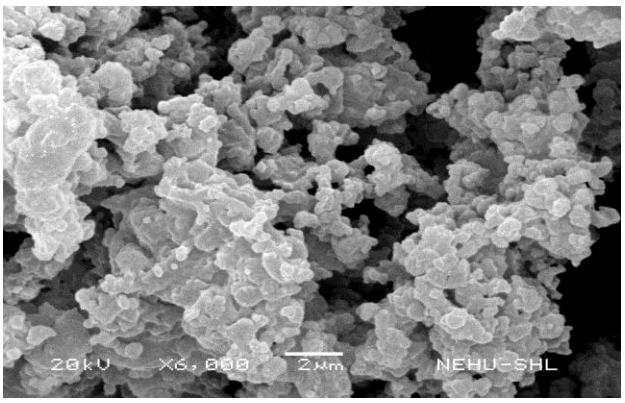


Figure 17: Images of SEM of nanoemulsions of F7

Fitting of Data and Model

Independent variables demonstrate that the model was significant for all the response variables. It was observed that independent variables X1 (polymer concentration) and X2 (HPH pressure) had a positive effect on the entrapment efficiency and drug loading of the nano-formulation that was nanoemulsions was achieved. The statistical evaluation was performed by using ANOVA. The results were showed in below **table 9.**The coefficients in the regression equation that contain more than one factor term are called interaction terms. This demonstrates that the link between variables and responses is not necessarily linear. When more than one element is changed at the same time and at various levels in a formulation, the reactions might vary.

Response: Entrapment Efficiency

Table 9: Model Comparison for Drug Loading Prediction

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	**0.0027**		**0.7451**	**0.5830**	**Suggested**
2FI	0.7608		0.6708	0.1381	

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Quadratic	0.8233		0.5180	-1.1031	Aliased

3D surface plot analysis:

Three dimensional surface plots were generated by the Design Expert® software are presented in **figure 17.**depicts response surface plot of Polymer Conjugate Concentration (X1) and HPH Pressure (X2) on entrapment efficiency. The 3-D surface image shows a linear response, which indicates with the increase in the polymer concentration the entrapment efficiency increases, as more the polymer available more will be the entrapment efficiency. Here, two design batches i.e. F2 and F7 showed maximum entrapment efficiency i.e. 81.73% and 89.23% respectively. But as seen in first response Surface graph being a nanoparticle formulation considering the least particle size is also a crucial factor. So, the Design Batch with least particle size and maximum entrapment efficiency is selected. Therefore, F7 is considered as an optimized Batch.

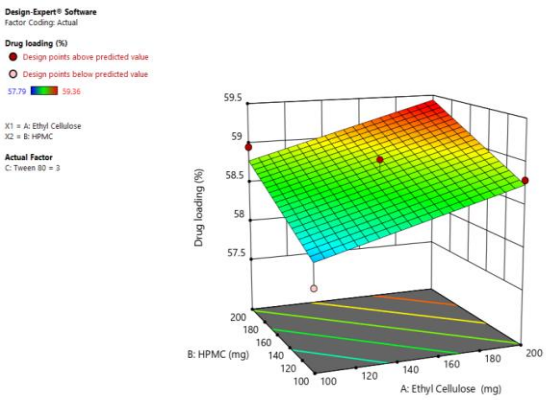

Figure 18: 3D surface plot analysis response surface plot of Polymer Conjugate Concentration (X1) and HPH Pressure (X2) on entrapment efficiency

DISCUSSION:

Based on a thorough understanding and evaluation of the processes and facilities of a sterile drug manufacturer, a comprehensive microbial surveillance scheme was established in this study. This scheme encompassed routine monitoring, microbial identification, contamination tracking, and risk assessment [15]. The distribution and diversity of microflora across the entire production chain were systematically examined. Over a period of three months, a total of 292 bacterial isolates were recovered from various sources, covering all areas of the production process and cleanrooms of different classifications. The widespread presence of microbial contaminants underscores a high contamination risk and highlights the urgent need to optimize the surveillance program, including enhanced cleaning and disinfection strategies.In this study, MALDI-TOF MS was employed as the primary method for microbial identification, supplemented by sequencing. This approach enabled rapid and efficient characterization of the environmental flora and facilitated the development of a fast, effective surveillance protocol [16]. MALDI-TOF MS is widely recognized as a simple, high-throughput proteomic tool for the identification of a broad spectrum of bacterial species. Taking database coverage into account, the system achieved identification accuracies of 75.8% at the species level and 95.4% at the genus level, supporting its suitability as a frontline tool for routine environmental monitoring.However, using only the commercial database, genus- and species-level identification rates dropped to 77.4% and 60.0%, respectively. Performance was notably lower for Bacillus spp., with just 35% genus-level and 30% species-level identification accuracy—despite the commercial database containing over 100 Bacillus species. These results fall short of the 90%+ species-level accuracy typically reported in clinical settings [17][18]. This discrepancy likely stems from insufficient representation of environmental isolates in the commercial MALDI-TOF MS database. Even when species are included, strain diversity may be limited. Environmental isolates from cleanroom settings are subject to higher stress due to stringent cleaning and disinfection protocols, making them harder to identify than clinical strains [19].

Our findings are consistent with those who reported successful identification of only 8% of bacterial isolates from spacecraft cleanrooms using manufacturer-supplied databases, even under rigorous microbial reduction practices. These results underscore the need to expand MALDI-TOF MS commercial databases with spectra from pharmaceutical cleanroom isolates. Collaboration between MALDI-TOF MS developers and pharmaceutical manufacturers is essential to improve identification performance. Furthermore, considering

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regional differences in manufacturing environments, product types, and production processes, facilities may harbor unique microbial strains. Developing customized, in-house reference databases using locally isolated strains presents an effective strategy to enhance the accuracy and utility of MALDI-TOF MS systems for environmental monitoring [20].

CONCLUSION:

In conclusion, the development and application of neem extract-based nanoemulsion represent a significant advancement in the field of agricultural biotechnology, particularly in the realm of sustainable pest management. By leveraging the natural bioactive compounds of neem and incorporating them into a nanoemulsion system, this approach markedly enhances the pesticidal efficacy of neem extracts. The nano-sized formulation ensures improved solubility, better stability, and increased bioavailability of the active constituents, resulting in a more potent and efficient delivery of pesticidal action against a broad spectrum of agricultural pests.

Moreover, the use of neemnanoemulsion aligns well with the principles of environmental safety and ecological balance. Unlike conventional chemical pesticides, which are often associated with harmful residues, soil degradation, non-target toxicity, and disruption of beneficial organisms, neem-based nanoformulations exhibit minimal toxicity to non-target species, including humans, and do not leave behind harmful residues in the soil or crops. This makes them particularly suitable for the cultivation of medicinal and aromatic plants, where purity, safety, and compliance with quality standards are critically important.

The outcomes of this research not only demonstrate the potential of neemnanoemulsion as a viable alternative to synthetic pesticides but also contribute to the growing body of evidence supporting the integration of green technologies in agriculture. The formulation offers a cost-effective, eco-friendly, and sustainable pest control strategy that can help address global challenges such as pesticide resistance, environmental pollution, and food safety concerns.

Importantly, this study sets the stage for further research and innovation in the area of nano-biopesticide development. To realize the full potential of neem-based nanoemulsions in agricultural practices, it is imperative to conduct long-term field trials across diverse agro-climatic conditions. Additionally, comprehensive toxicological studies and regulatory assessments will be necessary to ensure safe, standardized, and scalable implementation. By doing so, neemnanoemulsion can be effectively positioned as a key component in the future of sustainable and organic farming systems.

Future Recommendation

Neem Extracts Pesticide as Biodegradable Nanoemulsion for the Purpose of Cultivation of Various Medicinal Crops" should focus on large-scale field trials across diverse medicinal plant species to validate its practical efficacy and crop safety under varying agro-climatic conditions. Efforts must also be directed toward enhancing formulation stability, extending shelf life, and ensuring residue-free application in compliance with pharmacopeial standards, as medicinal crops demand high purity and safety. Additionally, in-depth toxicological and ecological studies are essential to confirm the nanoemulsion’s safety for non-target organisms, soil health, and beneficial microbes. Establishing standardized regulatory frameworks and promoting awareness through farmer training and integration into organic farming practices will further support the adoption of this eco-friendly and sustainable pest control solution in medicinal agriculture.

Acknowledgments

The author would like to thank all co-authors for their continuous support and also expresses gratitude to SAGE University for providing a suitable work environment to carry out this project.

Conflicts of Interest

The authors declare no conflicts of interest.

Funding

No funding was received for this research.

Ethics Statement

This project did not involve any animal or human subjects; therefore, ethical approval was not required.

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