https://doi.org/10.48047/AFJBS.6.12.2024.5502-5518

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

FORMULATION, EVALUATION AND PHYSICAL STABILITY STUDY OF *MORINGA OLEIFERA* L SEED OIL NANOEMULSION FOR ANTIMICROBIAL ACTIVITIES

Sayyad Mohd Umar Bokhari*, Md Sarfaraz Alam

HIMT College of Pharmacy, Plot No: 8, Institutional Area, Knowledge Park I, Greater Noida, Uttar Pradesh 201301.

***Corresponding Author** Sayyad Mohd Umar Bokhari

Research Scholar, HIMT College of Pharmacy, Greater Noida, Uttar Pradesh- 201301

Email: smubokhari786@gmail.com

Article History
Volume 6, Issue 12, 2024
Received: 15 June 2024
Accepted: 10 July 2024
Doi:
10.48047/AFJBS.6.12.2024.5502-5518

ABSTRACT

It's well known that synthetic antimicrobial drugs can cause adverse reactions such immunosuppression, toxicity, and hypersensitivity. The continued evolution of multi-resistant strains necessitates the continuous discovery of novel medications. The aforementioned adverse effects reduce the therapeutic efficacy of the currently prescribed antimicrobial drugs, demanding the search for alternative treatments. Due to its natural origins and negligible side effects, the field of herbal therapy has experienced exponential growth over the past several decades. The current goal of the work is to create an effective nanoemulsion of *Moringa oleifera* L. Seed Oil for antibacterial purposes. Titration technique was used to create the nanoemulsion, which was then evaluated for thermodynamic stability, pH, conductivity, viscosity, Refractive index, particle size distribution, and particle size analysis. Studies of the antimicrobial capabilities of gram positive and gram negative bacteria, as well as fungi, were conducted *in vitro*. According to ICH guidelines, nanoemulsion stability testing was conducted. Moringa seed oiltahen as oil phase, Tergitol 15-S-20 (surfactant), and transcutol P (co-surfactant) are used to formulate a nanoemulsion percentage composition of Oil:Smix(1:1):Water was 15:33:52. The following values were discovered to be 6.12± 0.124 for pH, 29.35 ±1.67 mP for viscosity, 1.404 ±0.024 for refrective index, and 10^{-4} s cm^{-1} for conductivity. The average particle size, polydispersity index, and zeta potential were determined to be 61.50 ± 0.512 nm, -28.4 mV±0.302, respectively. The stability study confirmed that the optimized nanoemulsion was sufficiently stable for3 months at room temperature. It was shown that the nanoemulsion of Moringa oleifera oil and Moringa seed oil had bactericidal and fungicidal properties with MICs of 0.110 g mL-1 and 0.123 g mL-1, respectively. As a result of the facts above, M. oleifera oil nanoemulsion could be a potential antimicrobial.

Keys words: Nanoemulsion, Moringa seed oil, ICH guidelines, Antimicrobial activity, MIC

INTRODUCTION

Due to the frequency of microbial diseases, antimicrobial agents are necessary; *M. oleifera* has been proven to be a successful antimicrobial agent. Bacillus subtilis, Staphylococcus aureus, and Vibrio cholera are just a few of the bacteria that *M. oleifera*leaves extract are effective against, according to studied by Foud et al [1]. The presence of pterygospermin, moringine, and benzyl isothiocyanate in the seed was thought to be the reason for its antibacterial properties [2]. Numerous commonly prescribed antimicrobial medications are known to have side effects such immunosuppression, toxicity, and hypersensitivity that endanger the general public's health [3]. The more modern, broad-spectrum antibiotics are also expensive, making them inaccessible for the underprivileged. Since strains that are multi-resistant to antibiotics and antifungals are constantly changing, as noted by Silver et al., there is a constant need for the identification of novel medications.The aforementioned adverse effects decrease the therapeutic efficiency of the antimicrobial medications now provided, forcing the search for alternate disease treatments [4]. The practise of herbal medicine has grown significantly during the past several decades. Due to their natural origins and absence of unfavourable side effects, a variety of higher plants have been utilised to treat human illnesses since the dawn of humanity [5]. This popularity is growing in both developing and developed countries. In light of this, scientists have examined higher plants for a range of biological traits, including antibacterial[6] and antifungal effects (Eilert et al., 1980; 1981; Omer and Elnima, 2003; Saadabi, 2006; Saadabi et al., 2006; 2007; 2009). Here, *M. oleifera* seed oil is taken into account for researching its antibacterial action in light of *M. oleifera* leaves extract's antimicrobial activity. However, the oil had a drawback in that it was difficult for it to reach the dipper area or skin. Oil nano conversion may thus be the best way to address the aforementioned problem. The term "nanotechnology" refers to technological advancements that take place on the nano scale, which is typically between 100 and 1000 nm. There has been a rise in the use of nanotechnology in pharmaceuticals and medicine during the past several years [7]. Nanoemulsions are a subcategory of scattered particles. O/W NEs are oil-in-water (o/w) emulsions with mean droplet sizes between 100 and 1000 nm. Droplet diameters typically vary from 100 to 500 nm. The current goal of the work is to create a stable nanoemulsion of *Moringa oleifera L* Seed Oil and assess its stability as well as its anti-microbial capability by *in vitro* method.

MATERIALS AND METHODS

Natural Aroma Products Pvt. Ltd. of New Delhi, India provided a sample of moringa seed oil (MSO). We bought ethanol, PEG 400, Tween 80, Tween 20, and Tween 20 from Merck (India). Diethylene glycol monoethyl ether (Transcutol P), caprylo caproyl macrogol-6 glycerides (Labrasol), and plurololéique samples were generously donated by Gattefosse (Mumbai, India). All of the chemicals that were left were of the analytical grade.

FORMULATION AND DEVELOPMENT OF NANOEMULSION

Screening of Excipients

Solubility/miscibility of the medication in oil, surfactant, and co-surfactant are important factors to consider when choosing components for nanoemulsions. Greater drug loading and more stable nanoemulsions are made possible by this. Using a vortex mixer (Nickel- Electro Ltd., Oldmixon Crescent, UK), a 5-mL stoppered vial of oil containing a 1:1 combination of each surfactant and cosurfactant was mixed individually to determine the miscibility of moringa. In order to reach equilibrium, as described by Azeem et al., the combined vial was then held at 370°C + 10°C in an isothermal shaker (Nirmal International, New Delhi, India) for 72 hours. The equilibrated samples were taken out of the shaker and centrifuged for 15 minutes at 3000 rpm. Several

surfactants, such as Tween 20, Tween 80, Labrasol, and Tween 60, and co-surfactants, such as ethanol, Transcutol P, plurololequie, PEG 200, and PEG 400, were combined with moringa oil in a 1:1 ratio (oil: surfactant/co-surfactant) [8]. Visual checks for miscibility were conducted. For further investigation, only the mixtures with a 1:1 ratio of clear or transparent were taken into account.

Phase Studies

Based on solubility/miscibility tests, Tergitol 15-S-20, Transcutol-P, and Moringa oil were selected as the oil phase, surfactant, and co-surfactant, respectively. To avoid surface-active pollutants, double-distilled water was used for the aqueous phase. Surfactants were mixed (S_{mix}) in a variety of weight ratios (1:0, 1:1, 2:1, 3:1, 4:1, and 5:1) with increasing quantities of surfactant compared to co-surfactant. There are sixteen different ratios of oil and S_{mix}(1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3.5, 1:3, 3:7, 1:2, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1) that were made in order to accurately highlight the boundaries of the phases produced in the phase diagrams. Pseudoternary phase diagrams were created utilising the aqueous phase titration technique to identify the nanoemulsion's current zone. Slow titration with the aqueous phase was used for each weight ratio of oil and S_{mix}, and visual observations were made for oil-in-water (o/w) nanoemulsions that were transparent and flow able with ease. The aqueous phase, the oil phase, and the combination of surfactant and co-surfactant at a predetermined weight ratio (S_{mix}ratio) were employed to represent the physical states of the nanoemulsion on a Phase three component called phase diagramor pseudoternary phase diagram [9].

Selection of Formulations

The pseudoternary phase diagrams showing the biggest nanoemulsion region were used to choose a variety of formulations covering the whole range of nanoemulsion occurrence in the phase diagrams with minimal surfactant and maximum water concentration. Selections of formulations were subjected to various physical stability tests [10].

Formulation of nanoemulsions

The choice of many nanoemulsions with variable percentages of oil, water, and Smix was made possible by the pseudo ternary phase diagrams displaying the greatest nanoemulsion area. With minimal surfactant and the highest water concentrations indicating the existence of nanoemulsions, several oil% compositions were chosen to roughly cover the whole range of nanoemulsion occurrence in phase diagrams. After adding the proper quantity of water and surfactant to the oil phase, a clear and transparent liquid—a nanoemulsion—was created. The prepared nanoemulsions were carefully kept at room temperature and packaged.

Thermodynamic stability testing of nanoemulsions

The following thermodynamic stability tests were performed on the nanoemulsions in order to identify the stable nanoemulsion and to exclude the unstable nanoemulsions.

I. Freeze thaw cycle

For 24 hours, nanoemulsions were stored in a deep freezer (at -20 o C). The nanoemulsions were taken out after 24 hours and left at room temperature. Within two to three minutes, the thermodynamically stable nanoemulsions took on their initial shape. 2–3 of these cycles were performed. [11].

II. Centrifugation studies

Following a freeze-thaw cycle, nanoemulsions were subjected to centrifugation tests in which they were spun for 30 minutes at 5,000 rpm. The stable formulations lacked turbidity or phase separation.

III. Heating cooling cycle

Six cycles between 4 °C and 40 °C, with a 48-hour storage period, were carried out. Further research was done on those formulations that remained stable at these temperatures ([12].

Characterization of nanoemulsion

Viscosity

The viscosity of the nanoemulsion was assessed using the Brookfield DV III ultra V6.0 RV cone and plate Rheometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA) at temperature 25±0.3 °C.

Refractive index

The refractive index (RI) of the nanoemulsion formulation was determined using a precision standard testing Abbes type refractrometer from Equipment Corporation in India.

pH

The sample's pH was evaluated using a Cyberscan pH-meter from Singapore's Eutech Instruments. Each time the pH of the nanoemulsion was measured, the pH-meter was calibrated. The pH-meter probe was placed inside the beaker once the components had been added, and the measurement was taken. In order to investigate the pH variations of the formulations after certain durations at various temperatures, the pH of the freshly created formulations was measured.

Conductivity

A conductometer (Cyberscan, Eutech Instrument, Singapore) was used to measure conductivity. The conductometer probe was placed into the beaker after the sample (2 g) had been added. It was observed that freshly made formulations have measured conductivity.

Particle size analysis

Using a Nano ZS90 from Malvern Instrument in the UK, photon correlation spectroscopy was used to estimate the average size and polydispersity index of the nanoemulsion droplets. on the principle of dynamic light scattering. The technique known as dynamic light scattering (DLS) is used to gauge the sizes of the particles in colloidal solutions. In DLS, the sample is subjected to a laser beam, and various levels of scattered light are generated depending on the size of the particles. It is possible to determine the particle size and diffusion coefficient by looking at these intensity variations. Standard disposable polystyrene cuvettes with tiny volumes were utilised for the cell. At 25^0C, all measurements were taken[13].

Transmission electron microscopy

Optimized formulations were examined using a TEM CM-10 (Philips, Netherlands) transmission electron microscope. A drop of 2% phospho-tungestic acid (PTA) nanoemulsion was tested for 30 seconds on a grid covered with carbon. After being covered with a cover slip, the dried coated grid was set up on a slide and examined using a TEM running at 60–80 KV at different magnifications (1550x, 2150x, 4600x, 21500x, and 44000x). The use of TEM allowed for the creation of a "positive" image of the nanoemulsions and their surroundings [14].

CollectionofPathogenicmicroorganisms

Stain samples for *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Salmonella typhi* (ATCC 27736), *Pseudomonas aeruginosa* (ATCC 25566), and *Candida albicans* (ATCC 10231) experiments were taken.

Determination of Antibacterial Activity

The antibacterial activity was evaluated using the disc diffusion technique. At 37 °C, Mueller-Hinton agar 18-hour culture plates were used for the experiment. The bacterial suspension was modified to obtain a bacterial cell density of $1x10^8$ UFC/mL using a sterile saline solution. A sterile brush dipped in each bacterial solution was applied to the plates in order to achieve a consistent microbial growth on both the test plates and the control plates. 10 µl of *Moringa*

oleifera oil and 10 µl of the *Moringa oleifera* oil nanoemulsion were added to the sterile disc. All of them were placed on top of 20 mL plates that contained microbial contamination. After 15 minutes at room temperature, plates were incubated for 24 hours at 37° C. The inhibitory zones were measured in millimetres. A standard disc containing ciprofloxacin (5-µg /disc, MASTDISCSTM, Mast Diagnostics Ltd.) was used as a reference control for bacterial inhibition, and fluconazole (5-µg /disc, MASTDISCSTM, Mast Diagnostics Ltd.) was used as a positive control for fungal inhibition. Following the completion of each experiment in triplicate, the mean zone of inhibition diameter was calculated. The effect was classified as either "sensitive" or "resistant" using a cutoff value of 8 mm [15].

Determination of Minimum Inhibitory Concentration "MIC."

The antibacterial effectiveness of Moringa oleifera oil and the Moringa oleifera oil nanoemulsion with a large inhibitory diameter were evaluated against each bacterium. A 96-well tray filled with Mueller-Hinton broth was used to measure the minimum inhibitory concentration (MIC). The solubility of the Moringa oleifera oil was increased by the addition of Tween 80 (Sigma-Aldrich). From 15% to 5% (v/v), the Moringa oleifera oil nanoemulsion was decreased three times. In a nutshell, 10 µl of bacterial inoculum were added to each well along with one of the serial dilutions of Moringa oleifera oil and the Moringa oleifera oil nanoemulsion. To assess the effects of Moringa oleifera oil and the Moringa oleifera oil nanoemulsion on the tested microorganisms, Mueller-Hinton broth, Moringa oleifera oil, and Moringa oleifera oil nanoemulsion were utilised. Mueller-Hinton broth containing the microorganism inoculate was employed as a positive control and reference basis. MIC was calculated during a 24-hour period of stirring incubation at 37° C [16].

Stability Studies as per ICH Guidelines

In accordance with the recommendations of the International Conference on Harmonisation (ICH), accelerated stability experiments on nanoemulsion were conducted. These were put in a humidity chamber set % RH as specified in ICH guidelines,at4°C, 25°C and 40°C temperature. At 0, 30, 60, and 90 days, samples were taken out. The sample's conductivity, pH, viscosity, droplet size, and refractive index were all measured [17].

Statistical Analysis

The one-way ANOVA test was used in statistical analysis to compare all the parameters between the first study and the 90 days of observation under all storage circumstances. In terms of statistics, a difference was deemed significant when the p value was less than 0.05.

FORMULATION AND DEVELOPMENT OF NANOEMULSION

Criteria for Excipient Selection

The excipients must be non-irritating, non-sensitizing, GRAS (generally recognised as safe), and pharmaceutically acceptable. Since too much surfactant might irritate the skin, safety is an important consideration when selecting a surfactant. Non-ionic surfactants are thought to be less irritating than ionic surfactants to achieve this.

Table 1: Miscibility of moringa seed oil with surfactants and co-surfactant.

Miscibility of moringa seed oil				
S. No.	**With surfactant (1:1)**	**Inference**	**With co-surfactant**	**Inference**
1	Tween 20	Turbid	Ethanol	Turbid

2	Tween 80	Turbid	Transcutol P	Clear
3	Tergitol 15-S-20	Clear	PEG 200	Turbid
4	Unitop 100	Turbid	Pleurololeique	Turbid

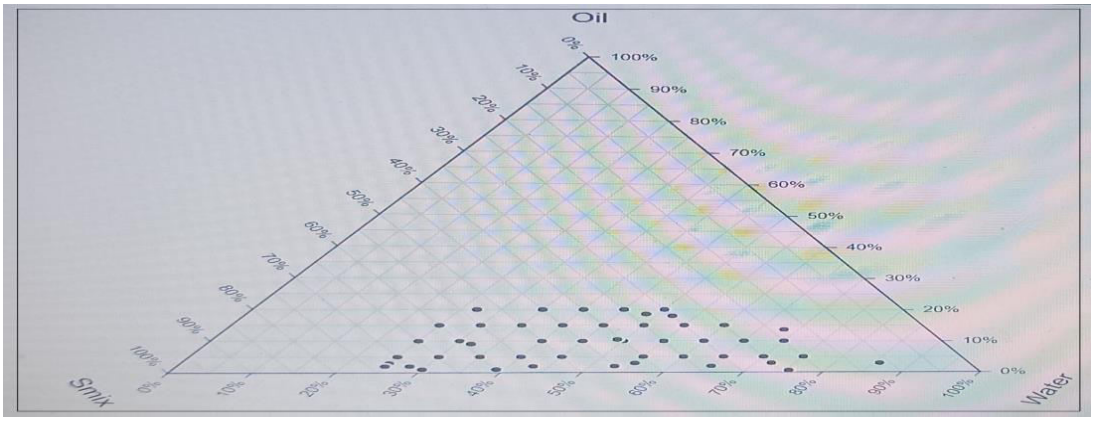
An additional crucial factor for choosing a surfactant with the right HLB value. It is believed that hydrophilic surfactants and cosurfactants favour the interface and require less energy to create stable nanoemulsions. For the creation of o/w nanoemulsions, for instance, an HLB value larger than 10 is required. As a result, it's important to select a surfactant and cosurfactant with the right HLB value (Date et al. 2006). When employed in a 1:1 ratio with Tergitol 15-S-20 as a surfactant and Transcutol P as a co-surfactant, moringa seed oil was shown to be the most miscible. The high HLB value of Tergitol 15-S-20 may aid in the effective emulsification of Moringa seed oil. The stratum corneum's lipophilic domain can be better penetrated thanks to Transcutol P, a particularly effective solubilizing agent. Therefore, Transcutol P was chosen as a co-surfactant, Tergitol 15-S-20 was chosen as a surfactant, and Moringa seed oil was chosen as an oil phase for the production of a nanoemulsion via pseudo ternary phase diagram [18].

Construction of pseudo ternary phase diagrams

One of the early processes, creating a phase diagram, provides the foundation for the nanoemulsion-based drug delivery system, especially when it comes to precisely defining a phase boundary. While just a small amount of free energy is required for the production of an emulsion, it is crucial to carefully consider the thermodynamically spontaneous generation to separate metastable entities [19]. A phase diagram can be used to determine the connection between a mixture's chemical composition and its phase behaviour (Lawrence and Rees, 2000). The phase diagrams were thoroughly examined using Transcutol P, Tergitol 15-S-20, and Moringa seed oil as surfactants and cosurfactants. We assessed the systems' visual clarity and flow characteristics. The ones that did not change the meniscus after being tilted at a 90° angle and were categorised as metastable nanoemulsion gels were not chosen. Pseudo ternary phase diagrams were created utilising the data gathered during the titration after making observations. Separate phase diagrams were created for each ratio of Smix produced in order to categorise the various components of the o/w nanoemulsion. The phase diagrams in Figure 1 are the only ones that show the o/w nanoemulsion area inFigure 1. For S_{mix}(1:1). On the basis of the phase digram several formulations were picked at different locations from the phase diagram to support the dose of the drug. A surfactant called lecthin alone (1:0) was employed to start making pseudo ternary phase. It was discovered that there were relatively few nanoemulsions in the area, and that emulsions made up the majority of the area. Pseudoternary phase diagrams were created after cosurfactant Transcutol P was added in a ratio of 1:1 to the surfactant Tergitol 15-S-20. It was discovered that the area of nanoemulsion existence significantly increased. An even bigger area of nanoemulsion, coupled with some emulsion, gels, or nanoemulsion gels, emerged from an increase in co-surfactant concentration (1:2). The area of the nanoemulsion decreased when co-surfactant concentration increased from 1:2 to 1:3, and then to 1:4, and more area was made up of emulsion and gels. Whether a nanoemulsion zone is large or tiny relies on how well the specific surfactant or surfactant combination can dissolve the oil phase, higher areas of clear, homogeneous solution ie. Nanoemulsions are produced as a result of higher solubilization. When the surfactant (Tergitol 15-S-20) was used alone, it was observed that the oil phase was solubilized to a lesser extent. This indicates that the surfactant was unable to sufficiently lower

the oil droplets' interfacial tension on its own, which prevented it from lowering the system's free energy to the ultra-low level required to create nanoemulsions. The introduction of a cosurfactant caused the interfacial tension to fall to a significantly lower level, which helped the existence of a larger nanoemulsion zone in the phase diagram. With a further increase in co-surfactant from 1:1 to 1:2, which led to an even greater reduction in interfacial tension and free energy, the largest area of nanoemulsion formation was achieved. A further increase in co-surfactant concentration (1:3) compared to before caused the interfacial tension of the interfacial layer to rise, resulting in more gel and less nanoemulsion area.

Figure 1: Pseudoternary phase diagram of S_{mix} 1:1 indicating o/w nanoemulsion region using Moringa seed oil, Tergitol 15-S-20 (Surfactant), Transcutol P (Co-surfactant).



Formulation of nanoemulsions

Several formulations were selected from the nanoemulsion zone of each phase diagram.

When selecting potential formulations with varying compositions of oil, water, and Smix from phase diagrams, the following parameters were taken into account:

1. For sufficient antibacterial actions, the oil content must be high enough.
2. From each phase diagram, a different oil concentration was chosen.
3. For each proportion of oil selected, the phase diagram, which used the lowest concentration of Smix to create its nanoemulsion, provided the formula.
4. The formulation choice was centred on the lowest concentration of the Smix as determined by the phase diagram and (Tables 2 and 3).

[20].

Table 2: Selected formulations from pseudoternary phase diagram of S_{mix} ratio 1:1, 1:2, 1:3. 2:1, 3:1 and 4:1which wasthermodynamically stable nanoemulsions listed in Table.

Nanoemulsion Code no:	Moringa seed oil Volume in (µL)	Tergitol 15-S-20+ Transcutol P (Smix) Volume in (µL)	Distilled Water Volume in (µL)	Volume of Nanoemulsion (µL)
NE1	150	330(Smix 1:1)	520	1000
NE2	200	350(Smix 1:1)	450	1000
NE3	150	550(Smix 1:3)	300	1000
NE4	200	400(Smix 3:1)	300	1000
NE5	250	450(Smix 4:1)	300	1000

Discussion

To ensure that the prepared nanoemulsions would remain stable under various circumstances, thermodynamic stability tests were performed on them. These tests included a freeze-thaw cycle, centrifugation studies, a heating-cooling cycle, and a low-temperature, high-shear, and low-temperature stability test. Nanoemulsions are thermodynamically stable systems that form at a certain concentration of oil, surfactant, and water, and exhibit no phase separation, creaming, or cracking. It is thermostable as opposed to kinetically stable emulsions, which will eventually phase split (Lawrence and Rees, 2000). Centrifugation, freeze-thaw, and heating-cooling stress studies were conducted to confirm the thermodynamic stability of the selected formulations. The formulations that met the requirements for thermodynamic stability were retained for additional study.

EVALUATION OF NANOEMULSION

Characterization of nanoemulsion formulation

Table 3: Viscosity, Refractive index and pH of optimized nanoemulsion NE1

Formulation code	Refractive index	Viscosity (mP) ± SD (n=3)	pH	Conductivity
NE1	1.404 ± 0.024	29.35 ± 1.67 mP	6.12± 0.124	10^{-4} s cm^{-1}

An Abbes type refractometer (Nirmal International, New Delhi, India) was used to measure the nanoemulsion's refractive index at a temperature of 25±0.5 °C. At 25±1 ^{o}C, a pH metre (Accument AB 15, Fisher Scientific, U.S.A.) was used to test the apparent pH of the formulation in triplicate.

Conductivity

The specific conductivity of nanoemulsion NE1was found to 10^{-4} s cm^{-1}.

Transmission electron microscopy

After TEM analysis for the optimized formulation, it was determined that the particles were almost spherical in shape, evenly dispersed, and in the nano range. Due to their spherical form and small size, they are very permeable (Figure 2).

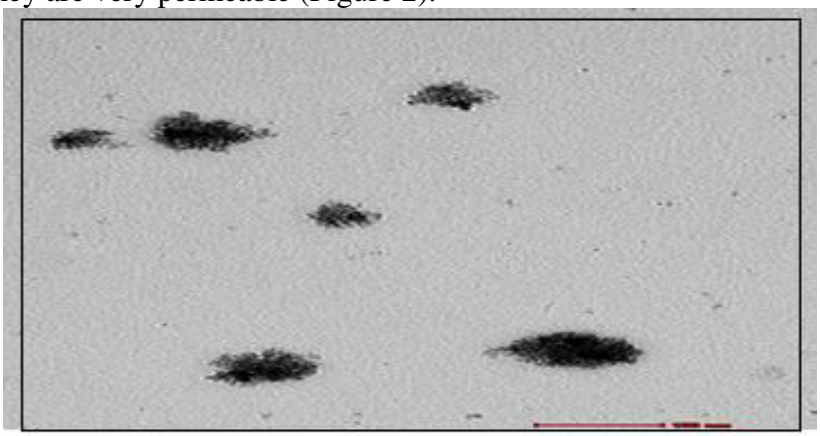
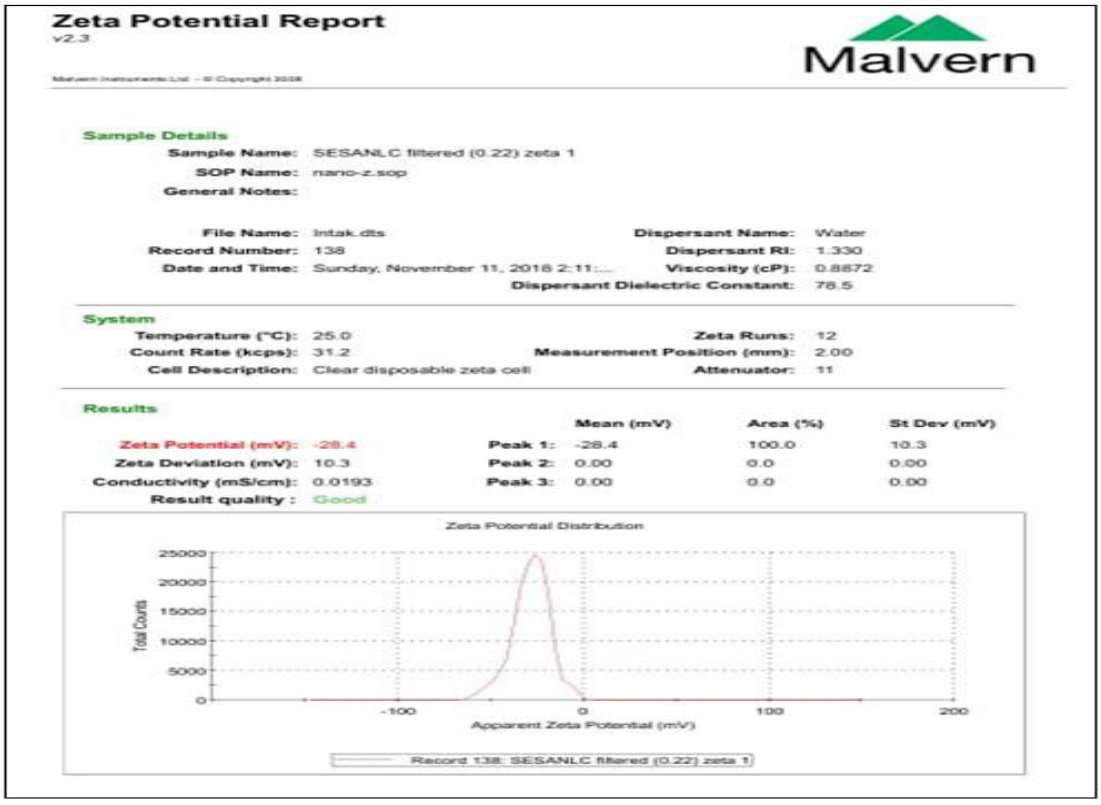


Figure 2: TEM photograph of particle size of nanoemulsion (NE1)

Droplet size and size distribution of nanoemulsion formulation (NE1)

Using photon correlation spectroscopy (Nano ZS90, Malvern Instrument, UK), the average size of the nanoemulsion droplets was found to be 61.50 0.512 nm, and their polydispersity index was found to be 0.302 as well. All of the nanoemulsions had droplet sizes between 50 and 250 nm. The peak in (Figure2) indicates the constrained size distribution of all nanoemulsions.



(a)

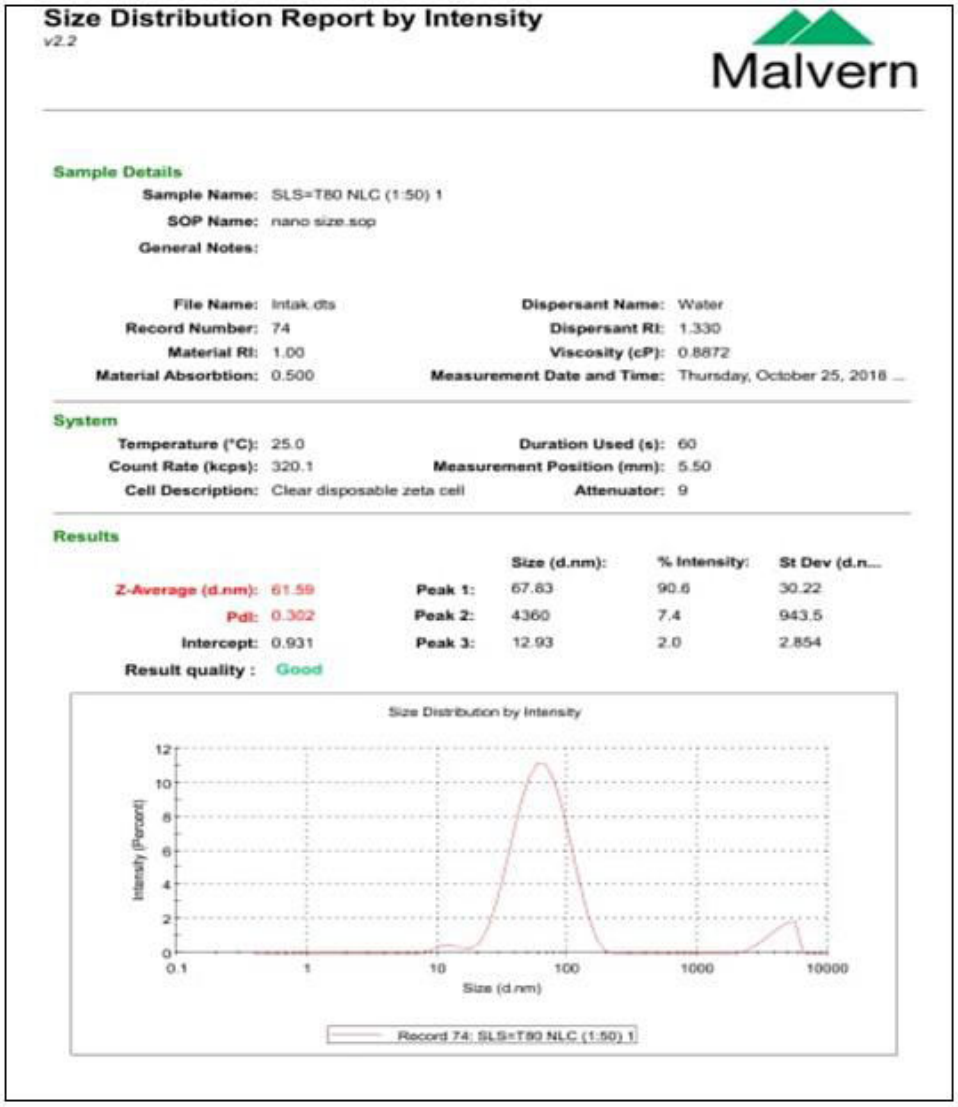


Figure 3: Droplet size distribution (a) and zeta potential (b) of nanoemulsion formulation (NE1)

Discussion

The optimized nanoemulsion (NE1) was reported in this work based on the unique characteristics of the nanoemulsion. The droplet sizes of the nanoemulsion (NE1), which was a colloidal dispersion, were typically between 50 and 200 nm. The polydispersity index (PI), a measurement of droplet size uniformity within the formulation, was also established. The zeta potential of the nanoemulsion (NE1) was found to be -28.4 mV. The nanoemulsion (NE1) formulation has a constrained size distribution (PI = 0.302). Dynamic light scattering (DLS) studies' results and the droplet size estimated by TEM analysis (Figure 2). According to the viscosity measurement, the nanoemulsion (NE1) formulation's viscosity was extremely low, as it was for an o/w emulsion (29.35 ± 1.67 mP). Tergitol 15-S-20, a fatty acid polyhydric alcohol ester with a greater intrinsic viscosity than Transcutol P, a short chain alcohol with a lower intrinsic viscosity, as well as lower oil content, may be the source of the low viscosity, according to Djordjevic et al. (2006). Refractive index, which

indicates the isotropic nature of the formulation and represents the net value of a nanoemulsion's component components. The formulation (NE1)'s average refractive index was found to be 1.404. It was decided to utilize NE1 since it contains less surfactant, while a high surfactant level can irritate.

Antimicrobial Activity.

According to Table 8, different stains responded differently to *Moringa oleifera* oil and the *Moringa oleifera* oil nanoemulsion. All species were poisoned by the combination of *Moringa oleifera* oil and *Moringa oleifera* oil nanoemulsion. The strength of the *Moringa oleifera* oil's and *Moringa oleifera* oil nanoemulsion's antibacterial activity against these selected pathogens varied. The results (Table 8) show that the antibacterial activity of *Moringa oleifera* oil nanoemulsion is superior to that of *Moringa oleifera* seed oil.

Table 4: Antimicrobial Activity of *Moringa oleifera* oil and *Moringa oleifera* oil nanoemulsion against different Pathogens.

Antimicrobial Activity	**Zone of Inhibition(mm)**				
	S.aureus	***E.coli***	***S.typhi***	***P.aerginosa***	***C.albican***
***Moinga oleifera*oil(oil only)**	7.90±0.86	5.32±0.86	8.13±0.57	6.97±0.43	12.02±0.34
(Water &Smix)	2.09±0.71	2.90±0.33	3.03±0.63	2.58±0.61	3.59±0.234
Nanoemulsion (oil+water +Smix)					
i. 5% by wt.	11.56±0.123	10.98±0.28	14.01±0.56	13.09±0.64	16.82±0.55
ii. 10% by wt.	13.01±0.561	13.24±0.67	16.89±0.441	16.82±0.78	21.05±1.2
iii. 15% by wt.	19.28±0.125	25.25±0.56	17.91±0.678	25.18±0.45	23.45±1.43
Ciprofloxacin 5µg/disc	26.13±0.321	34.22±1.02	19.87±0.723	29.98±0.1.9	-
Fluconazole 5µg/disc	-	-	-	-	33.94±0.1.7
MIC(g/ml^{-1})	0.110	0.110	0.110	0.110	0.123

Four distinct species of bacteria, excluding *Candida albicans, Salmonella typhi, Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa*, were tested for the MIC of *Moringa oleifera* oil and *Moringa oleifera* oil nanoemulsion. The term "MIC" refers to the lowest concentration of *Moringa oleifera* oil and *Moringa oleifera* oil nanoemulsion that prevents inoculum formation. Three concentrations were found to have the greatest effects on the development of all strains. The findings of the 590 nm absorbance measurements in each well

relative to the negative control are shown in Table 4. Furthermore, the *Moringa oleifera* oil nanoemulsion outperformed the Moringa seed oil in terms of antibacterial activity against four isolates of bacteria: *Staphylococcus aureus, Escherichia coli, Salmonella typhi,* and *Pseudomonas aeruginosa* at concentrations of 0.110g.mL^{-1}It can also be deduced that the moringa seed oil nanoemulsion shown more antibacterial activity than the moringa seed oil against all 4 isolates of bacteria, with inhibition zones ranging from 10 to 16 mm at a concentration of 0.123g.mL-1. At a minimum concentration of 15%, the Moringa seed oil nanoemulsion found to be absolutely suitable to halt the growth of all cultivated strains. Also noticed were the bactericidal and fungicidal activities of the nanoemulsion of *Moringa oleifera* oil and Moringa seed oil, with MICs of 0.110 g mL-1 and 0.123 g mL^{-1}, respectively (Table 8).

Stability Study

Stability studies demonstrate how various environmental conditions, including as temperature, humidity, and light, may affect a dosage form's quality over time. A perfect medicinal product has to be fully characterized physically, chemically, and microbiologically before research can begin and for the whole period of the intended shelf life. Accelerated stability testing for the nanoemulsions were carried out for three months in accordance with ICH guidelines. Improved nanoemulsions underwent accelerated stability tests at4°C, 25^{0}C and 40°C, at specified % RH as per ICH guidelines. Over the course of three months, the formulation was assessed based on droplet size, viscosity, pH, and RI. The study found that after three months, there had been no appreciable change in any of the characteristics. While the viscosity and pH slightly dropped, the droplet size and refractive index marginally rose (Table 5).

Table 5: Droplet size, Viscosity, RI, and pH of the optimized nanoemulsion during storage.

Time (months)	**Temp. (°C)**	**Mean droplet size (nm) ± SD (n=3)**	**Mean Viscosity (mP) ± SD (n=3)**	**RI ± SD (n=3)**	**pH ± SD (n=3)**
0	4.0 ± 2	61.59± 0.512	29.35 ± 1.67 mP	1.404± 0.024	6.12± 0.124
1	4.0 ± 2	60.89± 0.762	30.45 ± 1.56 mP	1.403± 0.023	6.13± 0.134
2	4.0 ± 2	61.90± 0.812	31.34 ± 1.86 mP	1.405± 0.042	6.14± 0.111
3	4.0 ± 2	61.52± 0.514	32.12 ± 1.56 mP	1.403± 0.012	6.11± 0.115
0	25 ± 2	61.59± 0.512	29.35 ± 1.67 mP	1.404± 0.024	6.12± 0.124
1	25 ± 2	61.82± 0.745	29.12 ± 1.34 mP	1.405± 0.036	6.14± 0.165

2	25 ± 2	61.95± 0.834	29.23 ± 1.45 mP	1.407± 0.076	6.12± 0.1234
3	25 ± 2	62.02± 0.456	29.11 ± 1.81 mP	1.409± 0.054	6.10± 0.1123
0	40 ± 2	61.59± 0.512	29.35 ± 1.67 mP	1.404± 0.024	6.12± 0.124
1	40 ± 2	62.23± 0.721	29.01 ± 1.87 mP	1.406± 0.056	6.12± 0.156
2	40 ± 2	62.97± 0.843	28.93 ± 1.56 mP	1.407± 0.067	6.10± 0.1245
3	40 ± 2	63.32± 0.476	28.13 ± 1.31 mP	1.411± 0.045	5.98± 0.115

Conclusion: Nanoemulsions may easily penetrate through the cell membranes due to their small size and lipophilic nature, which ultimately increases availability inside the cell. As a result, *Moringa oleifera* oil nanoemulsion may enhance the dosage form's quality and shelf life while reducing the growth of infectious germs and fungi. Despite the possibility of using nanoemulsion to suppress pathogenic microbes is beneficial, there are still a number of concerns that need to be examined in terms of the safety profile for human usage.

Acknowledgment: Author's are thankful to HIMT group of institution, Greater Noida, U.P for providing facilities to complete this research work.

Conflict of Interest: None declared.

References:

1. Fouad EA, Abu Elnaga ASM, Kandil MM. Antibacterial efficacy of *Moringa oleifera* leaf extract against pyogenic bacteria isolated from a dromedary camel (*Camelus dromedarius*) abscess. *Vet World*. 2019;12(6):802-808. doi:10.14202/vetworld.2019.802-808.
2. Padla EP, Solis LT, Levida RM, Shen CC, Ragasa CY. Antimicrobial isothiocyanates from the seeds of Moringa oleifera Lam. *Z Naturforsch C J Biosci*. 2012;67(11-12):557-564. doi:10.1515/znc-2012-11-1205.
3. Sheikh A, Yeasmin F, Agarwal S, et al. Protective effects of Moringa oleifera Lam. leaves against arsenic-induced toxicity in mice. *Asian Pac J Trop Biomed*. 2014;4(Suppl 1):S353-S358. doi:10.12980/APJTB.4.201414B44.
4. Patil SV, Mohite BV, Marathe KR, Salunkhe NS, Marathe V, Patil VS. Moringa Tree, Gift of Nature: a Review on Nutritional and Industrial Potential. *Curr Pharmacol Rep*. 2022;8(4):262-280. doi:10.1007/s40495-022-00288-7.
5. Paikra BK, Dhongade HKJ, Gidwani B. Phytochemistry and Pharmacology of *Moringa oleifera Lam. J Pharmacopuncture*. 2017;20(3):194-200. doi:10.3831/KPI.2017.20.022.

6. Jahan S, Shahjahan M, Rasna SS, et al. Antibacterial Effect of Moringa (Moringa oleifera) Leaf Ethanolic Extract Against Staphylococcus aureus and Escherichia coli. *Mymensingh Med J*. 2022;31(4):976-982.
7. Ogier J, Arnauld T, Doris E. Recent advances in the field of nanometric drug carriers. *Future Med Chem*. 2009;1(4):693-711. doi:10.4155/fmc.09.48.
8. Azeem A, Rizwan M, Ahmad FJ, et al. Nanoemulsion components screening and selection: a technical note. *AAPS PharmSciTech*. 2009;10(1):69-76. doi:10.1208/s12249-008-9178-x.
9. Wang B, Cai T, Liu Q, et al. Preparation and evaluation of spirulina polysaccharide nanoemulsions. *Int J Mol Med*. 2018;42(3):1273-1282. doi:10.3892/ijmm.2018.3717.
10. Sarfaraz Alam M, Ali MS, Zakir F, et al. Enhancement of Anti-Dermatitis Potential of Clobetasol Propionate by DHA [Docosahexaenoic Acid] Rich Algal Oil Nanoemulsion Gel. *Iran J Pharm Res*. 2016;15(1):35-52.
11. Ali MS, Alam MS, Alam N, Siddiqui MR. Preparation, characterization and stability study of dutasteride loaded nanoemulsion for treatment of benign prostatic hypertrophy. *Iran J Pharm Res*. 2014;13(4):1125-1140.
12. Ali MS, Alam MS, Alam N, Anwer T, Safhi MM. Accelerated Stability Testing of a Clobetasol Propionate-Loaded Nanoemulsion as per ICH Guidelines. *Sci Pharm*. 2013;81(4):1089-1100. Published 2013 Apr 7. doi:10.3797/scipharm.1210-02.
13. Aodah AH, Hashmi S, Akhtar N, et al. Formulation Development, Optimization by Box-Behnken Design, and In Vitro and Ex Vivo Characterization of Hexatriacontane-Loaded Transethosomal Gel for Antimicrobial Treatment for Skin Infections. *Gels*. 2023;9(4):322. Published 2023 Apr 11. doi:10.3390/gels9040322.
14. Alam MS, Ali MD, Ahmad S, et al. Evaluation of wound healing potential and biochemical estimation of sage oil nanoemulsion on animal model. *Pak J Pharm Sci*. 2021;34(4):1385-1392.
15. Elbhnsawi NA, Elwakil BH, Hassanin AH, et al. Nano-Chitosan/*Eucalyptus* Oil/Cellulose Acetate Nanofibers: Manufacturing, Antibacterial and Wound Healing Activities. *Membranes (Basel)*. 2023;13(6):604. Published 2023 Jun 15. doi:10.3390/membranes13060604.
16. Ahmed M, Marrez DA, Abdelmoeen NM, et al. Proximate Analysis of *Moringa oleifera* Leaves and the Antimicrobial Activities of Successive Leaf Ethanolic and Aqueous Extracts Compared with Green Chemically Synthesized Ag-NPs and Crude Aqueous Extract against Some Pathogens. *Int J Mol Sci*. 2023;24(4):3529. Published 2023 Feb 9. doi:10.3390/ijms24043529.
17. Shahid M, Hussain A, Khan AA, et al. Ketoconazole-Loaded Cationic Nanoemulsion: *In Vitro-Ex Vivo-In Vivo* Evaluations to Control Cutaneous Fungal Infections. *ACS Omega*. 2022;7(23):20267-20279. Published 2022 May 30. doi:10.1021/acsomega.2c02219.
18. Gul U, Khan MI, Madni A, et al. Olive oil and clove oil-based nanoemulsion for topical delivery of terbinafine hydrochloride: *in vitro* and *ex vivo* evaluation. *Drug Deliv*. 2022;29(1):600-612. doi:10.1080/10717544.2022.2039805.
19. Panagiotou T, Fisher RJ. Enhanced Transport Capabilities via Nanotechnologies: Impacting Bioefficacy, Controlled Release Strategies, and Novel Chaperones. *J Drug Deliv*. 2011;2011:902403. doi:10.1155/2011/902403.
20. Mandal S, Vishvakarma P. Nanoemulgel: A Smarter Topical Lipidic Emulsion-based Nanocarrier. Indian J of Pharmaceutical Education and Research. 2023;57(3s):s481-s498.

21. Mandal S, Jaiswal DV, Shiva K. A review on marketed Carica papaya leaf extract (CPLE) supplements for the treatment of dengue fever with thrombocytopenia and its drawback. International Journal of Pharmaceutical Research. 2020 Jul;12(3).
22. Bhandari S, Chauhan B, Gupta N, et al. Translational Implications of Neuronal Dopamine D3 Receptors for Preclinical Research and Cns Disorders. *African J Biol Sci (South Africa)*. 2024;6(8):128-140. doi:10.33472/AFJBS.6.8.2024.128-140
23. Tripathi A, Gupta N, Chauhan B, et al. Investigation of the structural and functional properties of starch-g-poly (acrylic acid) hydrogels reinforced with cellulose nanofibers for cu2+ ion adsorption. *African J Biol Sci (South Africa)*. 2024;6(8): 144-153, doi:10.33472/AFJBS.6.8.2024.141-153
24. Sharma R, Kar NR, Ahmad M, et al. Exploring the molecular dynamics of ethyl alcohol: Development of a comprehensive model for understanding its behavior in various environments. Community Pract. 2024;21(05):1812-1826. doi:10.5281/zenodo.11399708
25. Mandal S, Kar NR, Jain AV, Yadav P. Natural Products As Sources of Drug Discovery: Exploration, Optimisation, and Translation Into Clinical Practice. African J Biol Sci (South Africa). 2024;6(9):2486-2504. doi:10.33472/AFJBS.6.9.2024.2486-2504
26. Kumar S, Mandal S, Priya N, et al. Modeling the synthesis and kinetics of Ferrous Sulfate production: Towards Sustainable Manufacturing Processes. African J Biol Sci (South Africa). 2024;6(9):2444-2458. doi:10.33472/AFJBS.6.9.2024.
27. Revadigar RV, Keshamma E, Ahmad M, et al. Antioxidant Potential of Pyrazolines Synthesized Via Green Chemistry Methods. African J Biol Sci (South Africa). 2024;6(10):112-125. doi:10.33472/AFJBS.6.10.2024.112-125
28. Sahoo S, Gupta S, Chakraborty S, et al. Designing, Synthesizing, and Assessing the Biological Activity of Innovative Thiazolidinedione Derivatives With Dual Functionality. African J Biol Sci (South Africa). 2024;6(10):97-111. doi:10.33472/AFJBS.6.10.2024.97-111
29. Mandal S, Bhumika K, Kumar M, Hak J, Vishvakarma P, Sharma UK. A Novel Approach on Micro Sponges Drug Delivery System: Method of Preparations, Application, and its Future Prospective. Indian J of Pharmaceutical Education and Research. 2024;58(1):45-63.
30. Mishra, N., Alagusundaram, M., Sinha, A., Jain, A. V., Kenia, H., Mandal, S., & Sharma, M. (2024). Analytical Method, Development and Validation for Evaluating Repaglinide Efficacy in Type Ii Diabetes Mellitus Management: a Pharmaceutical Perspective. Community Practitioner, 21(2), 29–37. https://doi.org/10.5281/zenodo.10642768
31. Singh, M., Aparna, T. N., Vasanthi, S., Mandal, S., Nemade, L. S., Bali, S., & Kar, N. R. (2024). Enhancement and Evaluation of Soursop (Annona Muricata L.) Leaf Extract in Nanoemulgel: a Comprehensive Study Investigating Its Optimized Formulation and Anti-Acne Potential Against Propionibacterium Acnes, Staphylococcus Aureus, and Staphylococcus Epidermidis Bacteria. Community Practitioner, 21(1), 102–115. https://doi.org/10.5281/zenodo.10570746
32. Khalilullah, H., Balan, P., Jain, A. V., & Mandal, S. (n.d.). Eupatorium Rebaudianum Bertoni (Stevia): Investigating Its Anti-Inflammatory Potential Via Cyclooxygenase and Lipooxygenase Enzyme Inhibition - A Comprehensive Molecular Docking And ADMET. Community Practitioner, 21(03), 118–128. https://doi.org/10.5281/zenodo.10811642
33. Mandal, S. Vishvakarma, P. Pande M.S., Gentamicin Sulphate Based Ophthalmic Nanoemulgel: Formulation and Evaluation, Unravelling A Paradigm Shift in Novel

Pharmaceutical Delivery Systems. Community Practitioner, 21(03), 173-211. https://doi.org/10.5281/zenodo.10811540

34. Mishra, N., Alagusundaram, M., Sinha, A., Jain, A. V., Kenia, H., Mandal, S., & Sharma, M. (2024). Analytical Method, Development and Validation for Evaluating Repaglinide Efficacy in Type Ii Diabetes Mellitus Management: A Pharmaceutical Perspective. Community Practitioner, 21(2), 29–37. https://doi.org/10.5281/zenodo.10642768
35. Singh, M., Aparna, T. N., Vasanthi, S., Mandal, S., Nemade, L. S., Bali, S., & Kar, N. R. (2024). Enhancement and Evaluation of Soursop (Annona Muricata L.) Leaf Extract in Nanoemulgel: a Comprehensive Study Investigating Its Optimized Formulation and Anti-Acne Potential Against Propionibacterium Acnes, Staphylococcus Aureus, and Staphylococcus Epidermidis Bacteria. Community Practitioner, 21(1), 102–115. https://doi.org/10.5281/zenodo.10570746
36. Gupta, N., Negi, P., Joshi, N., Gadipelli, P., Bhumika, K., Aijaz, M., Singhal, P. K., Shami, M., Gupta, A., & Mandal, S. (2024). Assessment of Immunomodulatory Activity in Swiss Albino Rats Utilizing a Poly-Herbal Formulation: A Comprehensive Study on Immunological Response Modulation. Community Practitioner, 21(3), 553–571. https://doi.org/10.5281/zenodo.10963801
37. Mandal S, Vishvakarma P, Bhumika K. Developments in Emerging Topical Drug Delivery Systems for Ocular Disorders. Curr Drug Res Rev. 2023 Dec 29. doi: 10.2174/0125899775266634231213044704. Epub ahead of print. PMID: 38158868.
38. Abdul Rasheed. A. R, K. Sowmiya, S. N., & Suraj Mandal, Surya Pratap Singh, Habibullah Khallullah, N. P. and D. K. E. (2024). In Silico Docking Analysis of Phytochemical Constituents from Traditional Medicinal Plants: Unveiling Potential Anxiolytic Activity Against Gaba, Community Practitioner, 21(04), 1322–1337. https://doi.org/10.5281/zenodo.11076471
39. Pal N, Mandal S, Shiva K, Kumar B. Pharmacognostical, Phytochemical and Pharmacological Evaluation of Mallotusphilippensis. Journal of Drug Delivery and Therapeutics. 2022 Sep 20;12(5):175-81.
40. Singh A, Mandal S. Ajwain (Trachyspermumammi Linn): A review on Tremendous Herbal Plant with Various Pharmacological Activity. International Journal of Recent Advances in Multidisciplinary Topics. 2021 Jun 9;2(6):36-8.
41. Mandal S, Jaiswal V, Sagar MK, Kumar S. Formulation and evaluation of carica papaya nanoemulsion for treatment of dengue and thrombocytopenia. Plant Arch. 2021;21:1345-54.
42. Mandal S, Shiva K, Kumar KP, Goel S, Patel RK, Sharma S, Chaudhary R, Bhati A, Pal N, Dixit AK. Ocular drug delivery system (ODDS): Exploration the challenges and approaches to improve ODDS. Journal of Pharmaceutical and Biological Sciences. 2021 Jul 1;9(2):88-94.
43. Shiva K, Mandal S, Kumar S. Formulation and evaluation of topical antifungal gel of fluconazole using aloe vera gel. Int J Sci Res Develop. 2021;1:187-93.
44. Ali S, Farooqui NA, Ahmad S, Salman M, Mandal S. Catharanthus roseus (sadabahar): a brief study on medicinal plant having different pharmacological activities. Plant Archives. 2021;21(2):556-9.
45. Mandal S, Vishvakarma P, Verma M, Alam MS, Agrawal A, Mishra A. Solanum Nigrum Linn: An Analysis Of The Medicinal Properties Of The Plant. Journal of Pharmaceutical Negative Results. 2023 Jan 1:1595-600.

46. Vishvakarma P, Mandal S, Pandey J, Bhatt AK, Banerjee VB, Gupta JK. An Analysis Of The Most Recent Trends In Flavoring Herbal Medicines In Today's Market. Journal of Pharmaceutical Negative Results. 2022 Dec 31:9189-98.
47. Mandal S, Vishvakarma P, Mandal S. Future Aspects And Applications Of Nanoemulgel Formulation For Topical Lipophilic Drug Delivery. European Journal of Molecular & Clinical Medicine.;10(01):2023.
48. Chawla A, Mandal S, Vishvakarma P, Nile NP, Lokhande VN, Kakad VK, Chawla A. Ultra-Performance Liquid Chromatography (Uplc).
49. Mandal S, Raju D, Namdeo P, Patel A, Bhatt AK, Gupta JK, Haneef M, Vishvakarma P, Sharma UK. Development, characterization, and evaluation of rosa alba l extract-loaded phytosomes.
50. Mandal S, Goel S, Saxena M, Gupta P, Kumari J, Kumar P, Kumar M, Kumar R, Shiva K. Screening of catharanthus roseus stem extract for anti-ulcer potential in wistar rat.
51. Shiva K, Kaushik A, Irshad M, Sharma G, Mandal S. Evaluation and preparation: herbal gel containing thuja occidentalis and curcuma longa extracts.
52. Vishvakarma P, Kumari R, Vanmathi SM, Korni RD, Bhattacharya V, Jesudasan RE, Mandal S. Oral Delivery of Peptide and Protein Therapeutics: Challenges And Strategies. Journal of Experimental Zoology India. 2023 Jul 1;26(2).
53. Mandal, S., Tyagi, P., Jain, A. V., & Yadav, P. (n.d.). Advanced Formulation and Comprehensive Pharmacological Evaluation of a Novel Topical Drug Delivery System for the Management and Therapeutic Intervention of Tinea Cruris (Jock Itch). Journal of Nursing, 71(03). https://doi.org/10.5281/zenodo.10811676