



Formulation and Characterization of Nano Emulsion from *Limolina Acidissima*

Vaidhyathan Baskaran², Mahalakshmi Manikandan¹, Manasvi Senthil¹, Karthick Raja Srinivasan¹, Swathi Govindaraj^{1*}

¹Department of biotechnology, Bannari Amman Institute of Technology, Sathyamangalam, Erode – 638401

²Department of biotechnology, Prathyusha Engineering College, Tiruvallur – 602025

(Received: 05 December 2025 Revised: 15 January 2026 Accepted: 10 February 2026)

KEYWORDS

Nanoemulsions,
Emulsifying agent,
Metastable solids,
Brownian motion

ABSTRACT:

An emulsion is an opaque, heterogeneous system made up of two immiscible liquid phases, usually oil and water, with one phase scattered throughout the other as microscopic or colloidal-sized droplets. These systems display the characteristic traits of metastable colloids, such as Brownian motion, reversible phase changes caused by droplet interactions, and irreversible transitions resulting in emulsion disintegration. Emulsifiers—surfactants that reduce interfacial tension and can form structured interfaces—help to form and stabilize emulsions. Emulsions have a wide spectrum of internal dynamics and mechanical properties, which change considerably with dilution or concentration. Their ability to disperse hydrophobic substances in a continuous aqueous phase makes them useful in a variety of applications, including painting, road surfacing, lubrication, and paper coating, where they also help eliminate the need for organic solvents by allowing for the homogeneous mixing of immiscible fluids. Emulsions are essential in a variety of industries, including food, cosmetics, medicines, and petroleum, allowing the development of new products and procedures that would otherwise be impossible. To achieve the intended functionality and stability in emulsions, variables such as emulsifier selection, droplet size and distribution, and ambient conditions must be carefully controlled.

INTRODUCTION

The concepts of mini-emulsions, nanoemulsions, ultrafine emulsions, and submicron emulsions are commonly used in scientific literature to describe emulsions with nanometer-scale droplet sizes. The functioning and practical value of these systems are heavily reliant on their physicochemical stability, as emulsion droplets tend to agglomerate over time, causing phase separation. To address this, emulsifiers—surface-active chemicals that diminish interfacial tension between immiscible liquid phases—are used to prevent droplet aggregation and improve stability. Because of their nanoscale dimensions, nanoemulsions are highly resistant to sedimentation and creaming, frequently appearing optically clear or translucent, making them ideal for both fundamental research and a wide range of industrial applications. The present emphasis on producing oil-in-water (O/W) and water-in-oil (W/O) nanoemulsions originates from their superior properties over conventional emulsions, including increased thermodynamic stability, higher bioavailability, and greater visual and functional clarity. Nanoemulsions are often created using high-energy input methods such as high-pressure homogenization, ultrasonication, and

microfluidization to assure the generation of uniformly small and stable droplets.

Nanoemulsions are a very promising area of study and development because their nanoscale droplet size dramatically improves the solubilization, bioavailability, and targeted administration of active chemicals. Nanoemulsions are often made using high-energy emulsification processes such as high-shear stirring, high-pressure homogenization, and ultrasonication, with the most efficient equipment producing the smallest, most uniform droplets by delivering energy quickly and uniformly. These adaptable systems allow for a wide range of dose forms, including liquids, creams, sprays, gels, aerosols, and foams, as well as a variety of administration routes such as topical, oral, intravenous, intranasal, pulmonary, and ocular.

Nanoemulsions are superior aqueous carriers for hydrophobic chemicals in industries such as cosmetics and insecticides, providing increased solubilization capacity over simple micellar solutions and greater kinetic stability over coarse emulsions. The small droplet size makes a significant contribution to long-term physical stability by reducing frequent destabilizing



events like creaming, sedimentation, and coalescence. Furthermore, surfactant qualities determine emulsification type: when the major surfactant is hydrophilic, oil-in-water (O/W) emulsions prevail, whereas lipophilic surfactants favor water-in-oil (W/O) systems. Notably, emulsifiers' polar head groups are a more effective barrier to droplet coalescence than their hydrophobic tails, increasing emulsion stability.

As a consequence, nanoemulsion technology can successfully synthesize oil-in-water (O/W) emulsions with relatively large internal phase volumes. Nanoemulsions also overcome a number of problems associated with traditional pharmaceutical crystallization techniques, resulting in enhanced drug delivery outcomes. Furthermore, nanoemulsions serve as fundamental building blocks for the manufacture of compartmentalized nanoparticles and encapsulated oil droplets, which broadens their application in advanced formulations. Nanoemulsions are produced using advanced procedures that manufacture uniformly fine and stable droplets at the nanoscale. Prominent methods include high-pressure homogenization, which uses intense pressure to force liquids through confined gaps, and microfluidization, which uses microchannels to create highly uniform emulsions.

Ultrasonication uses high-frequency acoustic waves to break down droplets into nanosized particles, whereas phase inversion methods use temperature or compositional parameters to invert the continuous and dispersed phases. Another extensively used strategy is spontaneous emulsification, which allows droplets to self-assemble under certain conditions without the need for external energy. Collectively, these improved techniques are critical for manufacturing nanoemulsions with remarkable stability and optimal performance across a wide range of applications.

Nanoemulsions feature various benefits that greatly increase their utility in modern medication delivery systems. They promote quick and uniform absorption, reducing inter-individual variability and resulting in more consistent and predictable therapeutic effects. Nanoemulsions make it possible to formulate poorly water-soluble pharmaceuticals into stable aqueous dosage forms by significantly enhancing lipophilic component solubilization. This augmentation results in enhanced bioavailability, which improves therapeutic efficacy and patient response. Furthermore, nanoemulsions provide excellent variety in

administration methods, including oral, intravenous, and topical distribution, broadening their applicability across a wide range of therapeutic settings.

Nanoemulsion-based drug delivery methods provide quick and efficient penetration of the active pharmaceutical ingredient, considerably increasing therapeutic efficacy. Because of their improved thermodynamic stability when compared to conventional formulations, these systems are ideal for long-term use. Nanoemulsions' intrinsic stability and optimal delivery qualities make them ideal for the development of sustained and controlled-release medication platforms, assuring consistent, long-term, and dependable therapeutic effects.

2. OBJECTIVES AND METHODOLOGY

2.1 Objective:

The purpose of the research is to create a stable nanoemulsion by extracting essential oils from wood apple leaves to inhibit biofilm formation on walls. The goal is to isolate and screen biofilm-producing bacterial strains from contaminated wall surfaces. The study also includes the synthesis and characterisation of a nanoemulsion made using wood apple extract and surfactants like Tween 20 and PEG. Furthermore, the study aims to test the formed emulsions' inhibitory effectiveness against the identified biofilm-producing bacterial strains.

2.2 Methodology

The chart below (Figure 1: Methodology Chart) provides a comprehensive overview of the end-to-end process undertaken throughout the entire project.

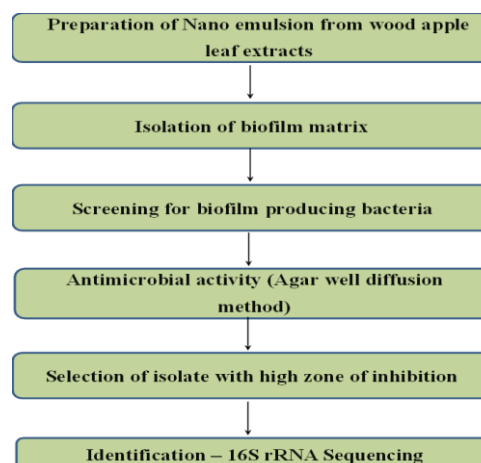


Figure 1: Methodology Chart) provides a comprehensive overview of the end-to-end process.

2.3 Experimental procedure

2.3.1 Collection of plant sample

The leaves of *Limonia acidissima* (Wood apple) were collected from various locations in and around Sathyamangalam.

Extraction of wood apple extract:

2.3.2 Maceration method

Maceration extraction is a conventional and simple procedure for isolating chemicals from plants, spices, or other materials by immersing them in a suitable solvent—usually a high-proof alcohol such as ethanol—for a set period of time. During the maceration process, the solvent dissolves the desired phytochemicals from the plant matrix, producing a concentrated solution known as extract. In this investigation, 10 grams of crushed wood apple leaves were placed in a beaker containing 50 mL of hexane and agitated in an orbital shaker for about 48 hours. Following this interval, the extract was filtered through filter paper to eliminate solid residues, and the filtrate was collected and saved for later use. Figure 2 depicts the obtained extract.



Figure 2: Extraction of bioactive compounds by Maceration method

2.3.3 Soxhlet Method Extraction:

Soxhlet extraction is a continuous extraction method used to separate bioactive chemicals from solid or semi-solid materials. Franz von Soxhlet established it in 1879, and 1921

it has since become a common approach in chemistry and materials research. In this technique, 10 grams of pulverized wood apple leaves were wrapped in filter paper and placed in the main chamber of the Soxhlet extractor. The extraction solvent was hexane (150 mL), which was placed in the distillation flask. The extraction was carried out at a regulated temperature of 60°C ($\pm 5^\circ\text{C}$). Three extraction cycles were run, each lasting 20 minutes. Upon completion of the cycles, the extract was collected and stored for further use. and the resulting extract is shown in Figure 3.

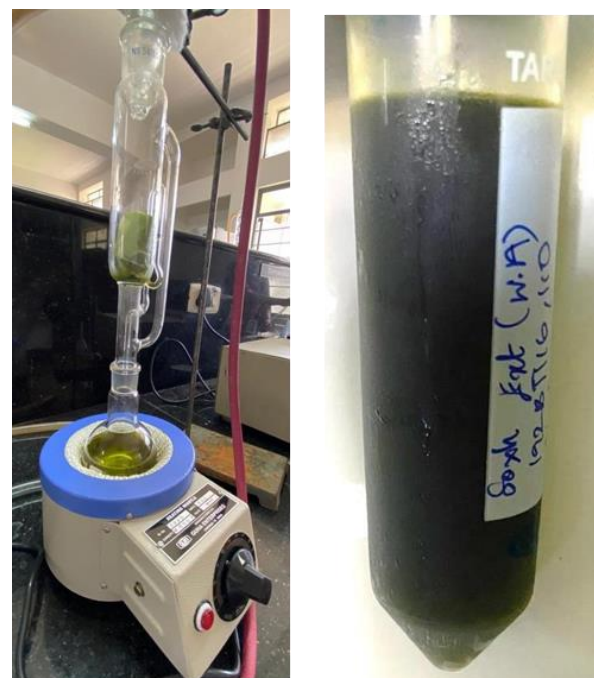


Figure 3: Soxhlet Extraction Setup and Extraction of bioactive compounds by Soxhlet method

2.3.4 FORMULATION OF NANOEMULSION

Nano emulsion(O/W) formulation:

The extracted plant oils were then used to create oil-in-water (O/W) nanoemulsions using various surfactant compositions (Tween 20 and PEG) and coconut oil. Surfactants help to make the hydrophobic and hydrophilic phases compatible, allowing for the creation of a stable emulsion. Five unique nanoemulsions were created by systematically altering the plant oil-surfactant ratios. The emulsification procedure was carried out utilizing ultrasonic waves at a constant frequency of 90 Hz.

Formulation – 1

In this work, nanoemulsions were made from wood apple



extract produced using two separate extraction methods: maceration and Soxhlet extraction. Formulation was carried out using samples from both processes, which included Smix (a surfactant and solvent mixture) and oil. Surfactants were added to help stabilize the emulsions. Table 4.1 presents the standard formulation utilized in this study. The extract concentration was adjusted for each sample—1 mL for the first, 2 mL for the second, and so on—while the oil volume and Smix concentration

remained constant at 1 mL and 2 mL, respectively. Two surfactants, Tween 20 and PEG, were tested, and the total volume was set to 10 mL. Each emulsion was then subjected to ultrasonication for three different durations: 15, 20, and 25 minutes. Following sonication, the emulsions were analyzed for physical, mechanical, and thermal stability. Table 4.3.2 presents the standard formulation, also referred to as Formulation-1.

TABLE 4.3.2 Standard formulation table

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
	(mL)				
Wood apple leaf Extract	1	1	1	2	2
Oil	2				
Smix (1:1)	1	2	3	4	5
Distilled Water	Make upto 12ml				

Formulation - 2

According to the results of Formulation 1, Tween 20-based nanoemulsions outperformed those stabilized with

PEG. As a result, Samples 1 and 2 from Formulation-1 were chosen as standard references. Based on these observations, Formulation-2 was created, as shown in Table 4.3.3 below.

TABLE 4.3.3 Formulation 2

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
	(mL)				
Wood apple leaf Extract	1	2	3	4	5
Oil	1				
Smix (1:1)	2				
Distilled Water	Make upto 10ml				

Formulation 2's base composition was derived from Formulation 1 (Samples 1 and 2), which served as a reference for extract concentrations. The first three samples included 1 mL of extract each, while the fourth and fifth samples comprised 2 mL. In this formulation,

the oil concentration was increased to 2 mL and held constant across all samples. The Smix concentration was gradually adjusted based on the number of samples, with the total volume set to 12 mL for each formulation. All



samples were then exposed to ultrasonication for three distinct times: 15, 20, and 25 minutes.

STABILITY TESTS

Physical stability:

Nanoemulsions are intrinsically thermodynamically unstable systems due to their small droplet size and large surface area, which generate substantial interfacial energy. This intrinsic instability can result in a variety of destabilizing processes. The stability of nanoemulsions can be assessed through visual inspection, also known as physical stability evaluation, which involves detecting changes in their physical appearance over time.

Mechanical stability:

Mechanical stability of nanoemulsions is defined as their capacity to withstand changes in droplet size or structure when subjected to mechanical forces such as shear, agitation, or centrifugation. This mechanical resilience can be assessed using a variety of ways, including visual inspection and stress testing.

Thermal Stability:

Thermal stability of nanoemulsions refers to their capacity to retain structural integrity and physical attributes when subjected to high temperatures. This property is critical for nanoemulsions designed for high-temperature applications, such as in the food or petroleum industries. Thermal stability can be assessed using a variety of methods, including visual inspection, droplet size analysis, and turbidity measurements. Visual examination is frequently used as the first step in assessing thermal instability, which might manifest as phase separation, color changes, or coalescence. Droplet aggregation or flocculation may also occur, indicating impaired stability.

2.4 CHARACTERIZATION OF NANOEMULSIONS

Fourier Transform Infrared Spectroscopy (FTIR) is a sophisticated analytical technique for determining and quantifying the chemical composition of a sample. It works by monitoring the interaction of infrared radiation with the sample, with the absorption of specific wavelengths representing the vibrational excitation of chemical bonds. By evaluating the frequency and intensity of these absorption bands, FTIR provides specific information on the types of chemical bonds and functional groups present in the material, allowing for exact molecular characterization. X-ray diffraction (XRD) analysis is a strong technique for determining the atomic or molecular structure of crystalline materials. It works on the idea that when X-rays are directed at a crystalline substance, they interact with the atomic lattice, resulting in a distinct diffraction pattern. This pattern offers detailed information on the spatial arrangement and periodicity of atoms within the crystal, allowing for exact structural analysis. The zeta potential is an important metric that measures the electrical charge present on the surface of droplets in a nanoemulsion. It has a significant impact on the stability, physicochemical characteristics, and biological activity of nanoemulsions, influencing whether they aggregate or remain dispersed.

3. RESULT AND DISCUSSIONS

3.1 FORMULATION 1: Comparative study – Surfactant as Tween20 VS Surfactant as PEG

The results showed that the emulsion with tween created a better and more stable emulsion than the emulsion with PEG. So we determined that PEG does not contribute to increased stability and used Tween 20 instead. The results for nanoemulsions with tween20 as surfactant have been interpreted as a table, which is provided below as Tables.

Table.5.1.1 Nano emulsions done with variations of sonication time using Tween20 as surfactant with their mechanical stability after centrifugation.

RPM	SET 1 (15min)					SET 2 (20min)					SET 3 (25min)				
	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
2000	-	-	-	-	-	-	-	*	*	*	*	*	*	*	*
3000	-	-	-	*	*	-	-	*	*	*	*	*	*	*	*



4000	-	-	-	*	*	-	*	-	*	*	*	*	*	*	*
5000	-	-	-	*	*	-	*	*	*	*	*	*	*	*	*
6000	-	-	-	*	*	-	*	*	*	*	*	*	*	*	*

Here, (-) indicates no phase separation, (*) indicates phase separation. "S" indicates Sample

Table.5.1.2 Nanoemulsions done with variations of sonication time using PEG as surfactant with their mechanical stability after centrifugation.

RPM	SET 1 (15min)					SET 2 (20min)					SET 3 (25min)				
	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
2000	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
3000	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
4000	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
5000	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
6000	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

Here, (-) indicates no phase separation, (*) indicates phase separation. "S" indicates Sample.

1.1 FORMULATION 2:

• Comparative study – Extraction method Maceration VS Soxhlet method.

This formulation was designed using Formulation 1, specifically the steady findings reported in Sets 1 (S1, S2, and S3) and 2 (S1), which exhibited no phase separation. A comparison study was carried out to assess the interaction of Tween 20 with extracts produced using two different extraction methods: maceration and Soxhlet.

This comparison did not include Formulation 1, as PEG-based emulsions did not develop. These findings led to the development of a new formulation, Formulation 2. The experimental results using Formulation 2 showed that the Soxhlet-extracted materials produced more stable nanoemulsions with much less phase separation than those extracted using the maceration process. The table below presents the results of the mechanical stability test conducted on samples of Formulation 2 using Tween 20 as the surfactant. It details the mechanical stability of the nanoemulsions after centrifugation.

Table 5.2.1 illustrates the mechanical stability of nanoemulsions prepared with varying sonication times using the maceration extract, assessed post-centrifugation.

RPM	SET 1 (15min)					SET 2 (20min)					SET 3 (25min)				
	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
2000	-	-	-	-	*	-	-	*	*	*	-	-	*	*	*
3000	-	-	-	*	*	-	-	*	*	*	-	-	*	*	*
4000	-	-	-	*	*	-	*	-	*	*	*	*	*	*	*
5000	-	-	-	*	*	-	*	*	*	*	*	*	*	*	*
6000	-	-	-	*	*	-	*	*	*	*	*	*	*	*	*

Table.5.2.2. Nanoemulsions done with variations of sonication time using Soxhlet extract with their mechanical stability after centrifugation.

RPM	SET 1 (15min)					SET 2 (20min)					SET 3 (25min)				
	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
2000	-	-	-	-	*	-	-	*	*	*	-	-	*	*	*
3000	-	-	-	*	*	-	-	*	*	*	-	-	*	*	*
4000	-	-	-	*	*	-	-	*	*	*	-	-	*	*	*
5000	-	-	-	*	*	-	-	*	*	*	-	-	*	*	*
6000	-	-	-	*	*	-	-	*	*	*	-	-	*	*	*

Physical stability

The visual observations of nanoemulsions are shown below. Figure 5.3.1 exhibits nanoemulsions prepared with Tween 20, whereas Figure 5.3.2 depicts nanoemulsions prepared with PEG. Both sets were created using the Formulation 1 technique.



Figure.5.3.1 Tween20 SAMPLE- After sonication

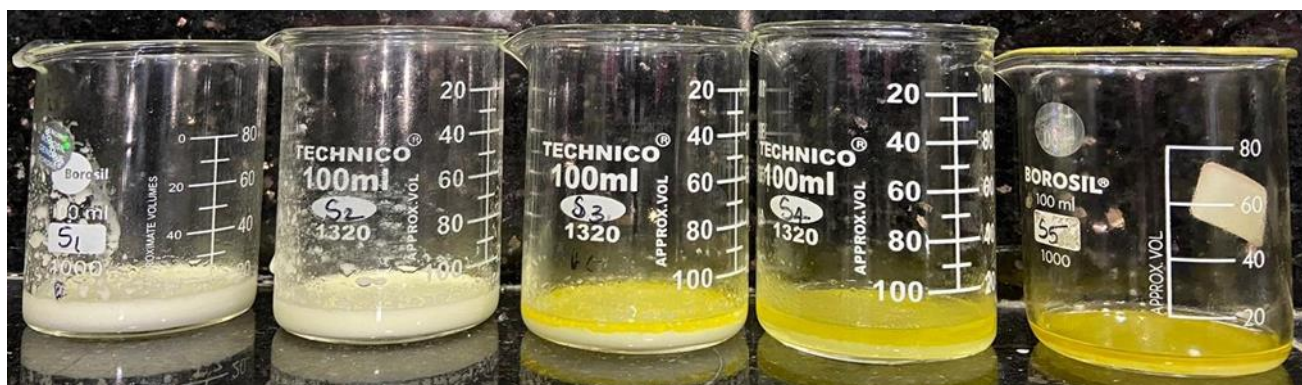


Figure.5.3.2 PEG SAMPLE - After sonication



1.2 Mechanical stability

• Comparative study – Surfactant as Tween20 VS Surfactant as PEG

The image clearly indicates phase separation in PEG emulsions, whereas Tween 20 emulsions did not exhibit phase separation in multiple samples. PEG-based nanoemulsions demonstrated consistent phase separation across all formulations. As a result, Tween 20 was chosen for future investigation because Tween 20-based nanoemulsions were more stable than those based on PEG.

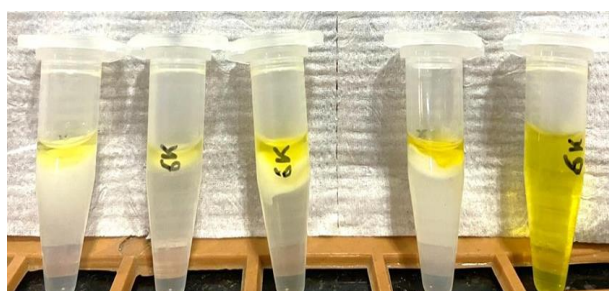


Figure.5.4.1 Tween20 sample - After Centrifugation

Figure.5.4.2 PEG sample - After Centrifugation

1.3 Characterization of Nanoemulsions.

1.3.1 FTIR :

The below figure 5.5.1 shows the spectrum analysis done for the sample SET 1 – S1.

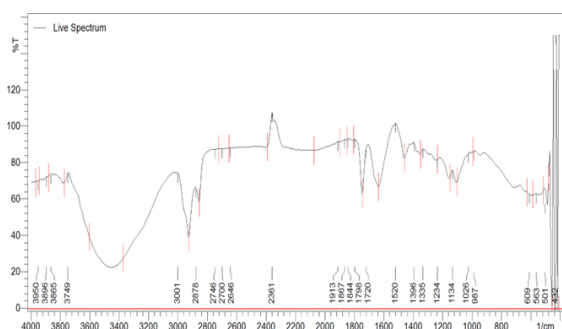


Figure.5.5.1 FTIR Spectrum analysis for SET 1 – S1

Figure 5.5.1 depicts the FT-IR analysis of the produced nanoemulsion, which exhibited numerous different absorption bands matching to the sample's various functional groups. Peaks at 3001 cm^{-1} indicate C-H stretching vibrations in alkene groups, while 2878 cm^{-1} corresponds to C-H stretching in alkanes. Peaks between 2746 and 2700 cm^{-1} indicated C-H stretching of aldehyde groups, while the band at 2646 cm^{-1} suggested free O-H stretching vibrations. The peak at 1913 cm^{-1} represents

C-H bending in aromatic compounds, while the absorption at 1798 cm^{-1} indicates C=O stretching of conjugated acid halide groups. Furthermore, the band at 1720 cm^{-1} indicated C=O stretching of aldehydes.

The spectrum showed peaks at 1520 cm^{-1} , representing N-O stretching in nitro compounds, and 1396 cm^{-1} , indicating O-H bending in carboxylic acids. The absorption at 1335 cm^{-1} showed O-H bending in alcohol groups, while the peak at 1234 cm^{-1} was due to C-O stretching in alkyl aryl ethers. Additionally, the bands at 1134 cm^{-1} and 1026 cm^{-1} corresponded to C-N stretching vibrations of amine groups. The peak at 987 cm^{-1} was attributed to C=C bending of alkenes, while the band at 609 cm^{-1} was associated with C-I stretching in halo compounds. Collectively, these spectrum signatures confirm the presence of many functional groups, indicating the nanoemulsion's complex chemical composition.

The below figure 5.5.2 shows the spectrum analysis done for the sample SET 1 – S2.

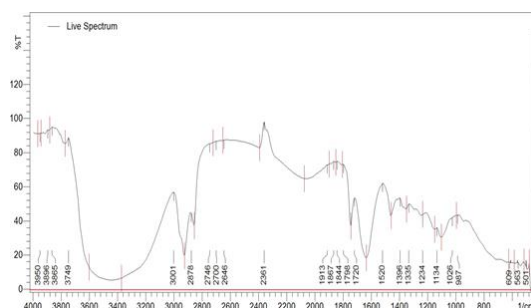


Figure.5.5.2 FTIR Spectrum analysis for SET 1 – S2

The FT-IR analysis shown in Figure 5.5.2 was carried out to identify the functional groups contained in the produced nanoemulsion. The peak at 3001 cm^{-1} represents C-H stretching vibrations in alkene groups, while the band at 2878 cm^{-1} indicates C-H stretching in alkane groups. Absorptions at 2746 and 2700 cm^{-1} are due to C-H stretching of aldehyde groups. The band at 2646 cm^{-1} indicates free O-H stretching vibrations. The peak at 1913 cm^{-1} indicates C-H bending in aromatic compounds, while the absorption at 1798 cm^{-1} represents C=O stretching in conjugated acid halides.

The band at 1720 cm^{-1} indicates C=O stretching of aldehyde groups. The 1520 cm^{-1} peak is caused by N-O stretching of nitro compounds. Furthermore, the band at 1396 cm^{-1} suggests O-H bending of carboxylic acids, whereas the absorption at 1335 cm^{-1} is related to O-H bending of alcohol groups. The peak at 1026 cm^{-1}



indicates C-N stretching vibrations of amine groups. The absorption at 987 cm^{-1} is attributable to C=C bending of alkene groups, whereas the band at 609 cm^{-1} is assigned to C-I stretching in halo compounds. These spectrum characteristics confirm the nanoemulsion's complicated molecular composition. Comparing the two FTIR spectrum the incorporation of oil into the nanoemulsion based on the surfactant interactions with absence of few chemical bonds clearly confirms that the emulsion have been done in a perfect manner.

CONCLUSION:

The plant materials were extracted using both steam distillation (Soxhlet method) and maceration procedures. To improve the stability of the nanoemulsions, two alternative surfactants, Tween 20 and Polyethylene Glycol (PEG), were tested for their efficiency in stabilizing the formulations. Surfactants improve nanoemulsion stability by facilitating optimal contact between the oil and water phases. Nanoemulsions were created by combining extracted oils, surfactants, oil, and water in variable amounts and subjecting them to various sonication times to improve stability. Comprehensive stability tests were performed to determine their physical and mechanical robustness. These results highlight the potential of Limolina acidissima-based nanoemulsions as stable carriers for bioactive compounds in pharmaceutical and cosmetic applications. The nanoemulsions were characterized using Fourier Transform Infrared Spectroscopy (FTIR) to identify functional groups based on characteristic absorption peaks. Further examination into thermal and mechanical stability revealed that only emulsions in SET 1, notably samples S1 and S2, demonstrated substantial stability.

REFERENCES:

- Amer A. and Mehlhorn H. (2006). "Repellency effect of forty-one essential oils against Aedes, Anopheles and Culex mosquitoes". Parasitol Res. 99:479-4903.
- Ariharan V. N., Kalirajan K. and Nagendra Prasad P. (2015) "FT-IR studies on three different traits of Vilvam leaves" Journal of Chemical and Pharmaceutical Research, 7(1):214- 219.
- Ashoka M , Nupur P , Dharitri M , Mamta B , Debasish S , Swetlina J , Debabrata D , Nirupama C (2015) "Study of Biofilm in Bacteria from Water Pipelines" Journal of Clinical and Diagnostic Research. Mar, Vol-9(3): DC09-DC11
- Aubrun O S, Simonnet J T, Alloret F L (2004) "Nano emulsions: a new vehicle for skincare products, colloid interface sciences", 108- 109, 2004,145-149.
- Barnard D R., (2000), "Repellents and toxicants for personal protection":W.H.O. – Geneva.
- Beer D D , Stoodley P. (2006) "Microbial biofilms". Prokaryotes, 1: 904 9377.
- Bhupen K, Somi B, Anil K S (2013) "Plant essential oils as mosquito repellent" International Journal of Research and Development in Pharmacy and Life Sciences. December- January, 2013, Vol. 3, No.1, pp 741-747ISSN: 2278-0238.
- Bruna F, Murbach T A, Lidiane N B, Isabella S P and Ary Fernandes J (2014) "Antimicrobial activity of essential oils" Journal of Essential Oil Research, Vol. 26, No. 1, 34–40.
- Burt S. (2004). "Essential oils: their antibacterial properties and potential applications in food-A review". Int J Food Microbial; 94:223-253.
- Hasim S. Falah R, Ayunda D and Faridah D N (2015) "Potential of lemongrass leaves extract (Cymbopogon citratus) as prevention for oil oxidation" Journal of Chemical and Pharmaceutical Research, 7(10):55-60.
- Hamieh A, Olama Z, Khawaja G and Holail H (2015) "Bacterial Diversity and Biofilm Formation in Drinking Water Distribution System in Lebanon" International Journal of Current Microbiology and Applied Sciences ISSN: 2319-7706 Volume 4 Number 5 pp. 976-990
- Hassan W , Gul S, Rehman S, Noreen H, Shah Z, Mohammadzai I and Zaman B (2016) "Chemical Composition, Essential Oil Characterization and Antimicrobial Activity of Carum copticum" Vitamins & Minerals Volume 5 • Issue 2 • 1000139 ISSN:2376-1318
- Jefferson K K (2004) "Mini review what drives bacteria to produce a biofilm" FEMS Microbiol. Lett., 236: 163 173.
- Kaustubh D S and Vidya S T (2015) "Isolation and Characterization of Biofilm Forming Bacteria from Oral Microflora" International Journal of Current Microbiology and



Applied Sciences Special Issue-2: 118-127

15. Kumar S, Wahab N. and Wankoo R. (2011) "Bioefficacy of Mentha piperita essential oil against dengue fever mosquito Aedes aegypti" Asian Pac J Trop Biomed. 2011 Apr; 1(2):85-8. doi: 10.1016/S2221-1691(11)60001-4.
16. P. P. Ram, G. Varun, S. Balram, C. Deepak, R. Veerma, and B. Anil, "Extraction and isolation of marmelosin from Aegle marmelos, synthesis and evaluation of their derivative as antidiabetic agent," Der Pharmacia Lettre, vol. 4, no. 4, pp. 1085–1092, 2012.
17. Monroe D (2007). "Looking for chinks in the armor of bacterial biofilms" . PLoS Biol., 5(11): e307.
18. M. Kalia, P.K. Singh, V.K. Yadav, B.S. Yadav, D. Sharma, S.S. Narvi, et al., Structurebased virtual screening for identification of potential quorum sensing inhibitors against LasR master regulator in Pseudomonas aeruginosa,