

DESIGN AND STABILITY TESTING OF MORINGA OLEIFERA SEED OIL NANOEMULSION FOR ANTIMICROBIAL USE

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ABSTRACT

Aim: The aim of the present investigation is to study the design and stability testing of moringa oleifera seed oil nanoemulsion for antimicrobial use. **Material & Methods-** *Moringa oleifera* seed oil was analyzed for organoleptic properties like color, odor, and appearance. About 2g of oil and 25ml of alcoholic potassium hydroxide was taken in flask. The resultant mixture was heated for 1h; to which 1ml of 1% phenolphthalein was added and titrated with 0.5N HCl. About 2g of oil and 10ml of carbontetrachloride were taken in flask and 20ml of Wij's solution (1.5% iodine monochloride in 98% acetic acid) was added and kept in dark for 30min. Fifteen ml of potassium iodide and 100ml of water added in above solution. The resultant solution was titrated with 0.1M sodium thiosulphate solution using starch as an indicator. About 1g of oil, 20ml acetic acid-chloroform (2:3), 1g potassium iodide was taken in test tube and boiled. This mixture was transferred to 20 ml of 5% potassium iodide solution. The resultant solution was titrated with 0.1N sodium thiosulphate solution using starch as an indicator. *Moringa oleifera* seed oil was run over TLC plate using silica gel-G as stationary phase. After the sample has been applied on the plate, solvent mixture is drawn up the plate via capillary action. The qualitative evaluation of the plate was done by determining the migrating behavior of the separated substances given in the form of R_f value. The high energy emulsification technique was used in the formulation of the nanoemulsion gel. After adding a surfactant and cosurfactant mix solution to the oil phase, MSO was added and agitated with a magnetic stirrer until the mixture was uniform. distilled water was gradually added by titration while the mixture was constantly agitated until a transparent nanoemulsion was achieved. Table 1 shows the adjusted percentages of the nanoemulsion gel components compared in the Nanoemulsion gel containing MOSO: a physicochemical study Test for organoleptic Phase separation, variations in colour, smell, and clarity were all carefully noted. **Results:** The color and odor of *Moringa oleifera* seed oil found to be pale yellow and characteristic respectively. The Refractive Index of *M. oleifera* seed oil (1.48) was in close agreement with values reported for conventional oils from soybean (1.46- 1.47) and palm kernel (1.49- 1.41). The high refractive index of this oil seems to confirm the high number of carbon atoms in their fatty acids. Refractive index also increases as the double bond increases. The saponification value indicates average molecular weight of fatty acid contents as glyceride in oil. Generally higher number of saponification value of oil used in manufacturing of soap. Iodine value is reflection of unsaturated degree of fats and oil and therefore, the high value; indicate high number of unsaturated double bonds. Peroxide value is an index of rancidity, thus low peroxide value indicates resistance of the oil to peroxidation during storage. The peroxide value of *Moringa oleifera* seed oil is low (7.48 mEq/Kg) compared to the maximum acceptable value of 10 meq KOH/g set by the Codex Alimentarius Commission for ground nut seed oils. The oil is thus stable and would not easily go rancid. **Conclusion-** These discoveries feature the promising capability of MSO nanoemulsion as compelling effective specialists for battling bacterial contaminations, making ready for future exploration on improving plans and investigating their clinical applications.

KEYWORDS: Design, Stability Testing, *Moringa Oleifera*, Seed Oil, Nanoemulsion, Antimicrobial Use.

INTRODUCTION

The Moringaceae family tree, *Moringa oleifera*, goes by several names, including kelor, benzolive, marango, drumstick tree, and sajna. Vitamins A, B, C, E, phenolics, caortenoids, and carotene are among the bioactive substances that have been documented.^[1] Oil extracted from *Moringa oleifera* seeds is semi-solid, smells somewhat like bitter almonds, and has a yellowish brown colour. The percentage composition of mixed fatty acids in seed oil is as follows: palmitic acid—1.04%, stearic acid—3.58, arachidic acid—3.44, behenic acid—7.09%, palmitoleic acid—2.38%, and linoleic acid—1.83%. To protect against degradation-induced damage, you may employ the oil's antioxidant components. In order to prevent ageing and a host of degenerative disorders, the body's antioxidant molecules act as a countermeasure against free radicals.^[2]

Nanotechnology is a controlled delivery widely utilized in pharmaceuticals, cosmetics, and food industries to optimize the dispersion of active ingredients.^[3] Nanoparticle preparations such as solid lipids, nanogels, polymers, liposomes, and nanoemulsion have been shown to prevent external degradation of herbal medicines and increase their bioavailability.^[4] Among these, nanoemulsion is a promising carrier for the delivery of lipophilic compounds, improving the permeation of active ingredients.

Nanoemulsion also increases the surface area and penetration of active substances.^[5] In this context, nanoemulsion has been identified as a potential carrier for delivering lipophilic compounds in *Moringa oleifera* seed oil to increase the permeation of active ingredients. This preparation consists of oil and water phases, which are stabilized by the presence of surfactant and cosurfactant as important components.^[6] Surfactants lower interfacial tension by reducing the repulsive force of immiscible liquid.^[7] Meanwhile, co surfactants prevent aggregation and increase pH stability.^[8]

The novelty and originality of this study lie in the use of nanoemulsion to deliver *Moringa oleifera* Seed Oil (MOSO). In light of the aforementioned, MOSO was formulated into nanoemulsion at different concentrations, characterized, and evaluated for its antimicrobial activity.

MATERIAL AND METHODS

Materials

Moringa oleifera seeds were procured from local market.

Methods

Collection and authentication

Fresh *Moringa oleifera* seeds were authenticated by Senior Botanist, Dept. of Botany, College of Horticulture, Ahmednagar (M.S.) India.

Extraction of oil

Moringa oleifera seeds were air dried for a week and

placed in hot air oven at 40°C (Lab Star, Mumbai), ground and sieved through #40 and #60 to get a coarse powder. Extraction of seeds was carried out by Soxhlet extraction using petroleum ether (60-80 °C) as solvent.^[9]

Preliminary physicochemical study^[10]

1. Organoleptic properties

Moringa oleifera seed oil was analyzed for organoleptic properties like color, odor, and appearance.

2. Saponification value

About 2g of oil and 25ml of alcoholic potassium hydroxide was taken in flask. The resultant mixture was heated for 1h; to which 1ml of 1% phenolphthalein was added and titrated with 0.5N HCl.

3. Iodine value

About 2g of oil and 10ml of carbontetrachloride were taken in flask and 20ml of Wij's solution (1.5% iodine monochloride in 98% acetic acid) was added and kept in dark for 30min. Fifteen ml of potassium iodide and 100ml of water added in above solution. The resultant solution was titrated with 0.1M sodium thiosulphate solution using starch as an indicator.

4. Peroxide value

About 1g of oil, 20ml acetic acid-chloroform (2:3), 1g potassium iodide was taken in test tube and boiled. This mixture was transferred to 20 ml of 5% potassium iodide solution. The resultant solution was titrated with 0.1N sodium thiosulphate solution using starch as an indicator.

5. Identification of oil actives

Moringa oleifera seed oil was run over TLC plate using silica gel-G as stationary phase. After the sample has been applied on the plate, solvent mixture is drawn up the plate via capillary action. The qualitative evaluation of the plate was done by determining the migrating behavior of the separated substances given in the form of R_f value.^[11]

Analytical methods

1. UV spectroscopy

The ultra-violet absorption spectrum of *Moringa oleifera* seed oil in n-hexane was obtained using UV-Visible spectrophotometer (Shimadzu 1650) in the range of 400-200 nm.^[12]

1. Infrared spectroscopy

Oil-exipients interaction study

FT-IR spectroscopy used to determine the molecular interaction between oil and excipients. FT-IR measurement of oil was taken at ambient temperature. The blend of *Moringa oleifera* seed oil and other excipients used for final formulation were analyzed by FT-IR spectrophotometer (FT-IR 8400-S; Shimadzu, Japan). Drop of oil and blend placed on the thin polymeric film were scanned over a wave number range of 4000 to 400cm⁻¹ in FT-IR instrument and spectral analysis was done.^[13]

Nano emulsion gel formulation

The high energy emulsification technique was used in the formulation of the nanoemulsion gel. After adding a surfactant and cosurfactant mix solution to the oil phase, MSO was added and agitated with a magnetic stirrer until the mixture was uniform. distilled water was gradually added by titration while the mixture was

constantly agitated until a transparent nanoemulsion was achieved. Table 1 shows the adjusted percentages of the nanoemulsion gel components compared in the Nanoemulsion gel containing MOSO: a physicochemical study Test for organoleptic Phase separation, variations in colour, smell, and clarity were all carefully noted.

Table 1: Formulas for nanoemulsion gel including moringa seed oil.

Composition of Nanoemulsion	Formula 4 (%w/w)	Formula 5 (%w/w)	Formula 6 (%w/w)
Moringa Seed Oil	5	10	15
Tween 80	35	35	35
Sorbitol	25	25	25
distilled water ad	100	100	100

Physical and chemical analysis of nanoemulsion gel containing MOSO Assessment of organoleptic Acidity evaluation

In a 25°C laboratory, pH values were measured using a digital pH meter. Three measurements were taken from a single sample. Prior to doing any measurements, we calibrated the pH meter using pH7.01, 4.01, and 10.01.

Shear force

At room temperature (25°C±2°C), the viscosity of the nanoemulsion gel was measured using the NDG-8s viscometer. The viscosity was measured in three independent experiments at two distinct spindle speeds.^[7] Determination of individual particle sizes in nanoemulsion gels.

Particle sizes were measured using the FRITSCH Laser Particle Size Analyzer

As a preliminary step, one measurement was made before the centrifugation test began. The nanoemulsion gel preparation was spun at 3750 rpm for 5 hours after being inserted into the centrifugation tube.

Comprehensive Antimicrobial Analysis Materials

Moringa oleifera seed oil (MSO), Tween 80, Sorbitol, Carbopol 940, TEA, and distilled water. All chemicals used were of analytical grade.

Apparatus and Conditions

Analytical balance, magnetic stirrer, sonicator, viscometer, pH meter, centrifuge, and particle size analyzer were employed for the formulation and physicochemical evaluation.

Formulation

The nanoemulsion gels were formulated using high-energy emulsification methods. MSO (5%, 10%, and 15%) was mixed with a solution containing surfactants and cosurfactants (Tween 80 and Sorbitol), followed by the gradual addition of distilled water until a translucent nanoemulsion was formed. The final nanoemulsion gel formulations were evaluated for antimicrobial efficacy.

Antimicrobial Testing

The antimicrobial activity of each formulation was evaluated using the agar well diffusion method to determine the inhibition zones against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Additionally, MIC values were determined using serial dilution techniques.

RESULTS AND DISCUSSION

Physicochemical properties of *Moringa oleifera* seed oil

Table 2: Physicochemical characteristics of *Moringa oleifera* seed oil.

Characteristics	Observation
% yield of oil in petroleum ether	24%
Color	Pale yellow
Odor	Characteristic
Refractive index	1.48
Saponification value	192.3mgKOH/g
Iodine value	84.5gI ₂ /100g
Peroxide value	7.48mEq/Kg

The color and odor of *Moringa oleifera* seed oil found to be pale yellow and characteristic respectively. The Refractive Index of *M. oleifera* seed oil (1.48) was in close agreement with values reported for conventional oils from soybean (1.46- 1.47) and palm kernel (1.49-1.41). The high refractive index of this oil seems to confirm the high number of carbon atoms in their fatty acids.^[26] Refractive index also increases as the double bond increases.^[27] The saponification value indicates average molecular weight of fatty acid contents as glyceride in oil. Generally higher number of saponification value of oil used in manufacturing of soap. Iodine value is reflection of unsaturated degree of fats and oil and therefore, the high value; indicate high number of unsaturated double bonds. Peroxide value is an index of rancidity, thus low peroxide value indicates resistance of the oil to peroxidation during storage. The peroxide value of *Moringa oleifera* seed oil is low (7.48 mEq/Kg) compared to the maximum acceptable value of 10 meq KOH/g set by the Codex Alimentarius Commission for ground nut seed oils.^[28] The oil is thus

stable and would not easily go rancid.

Analytical methods UV- spectroscopy

As shown in Fig 1 *Moringa oleifera* seed oil containing

oleic acid exhibited absorption maxima at 240 nm; whereas palmitic acid and stearic acid showed absorbance at 281 nm and 411 nm respectively in n-Hexane.

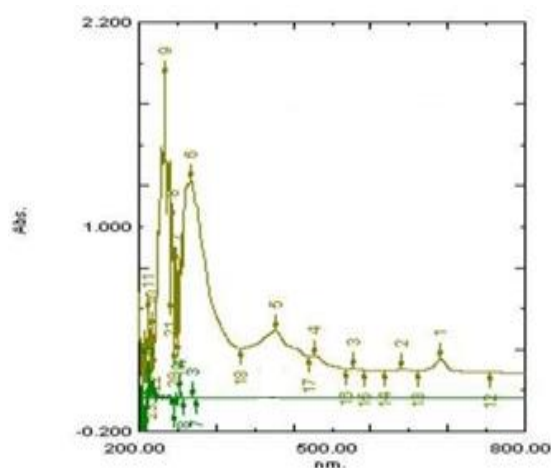


Fig. 1: UV spectra of *M.oleifera* seed oil in n-Hexane.

Infrared spectroscopy

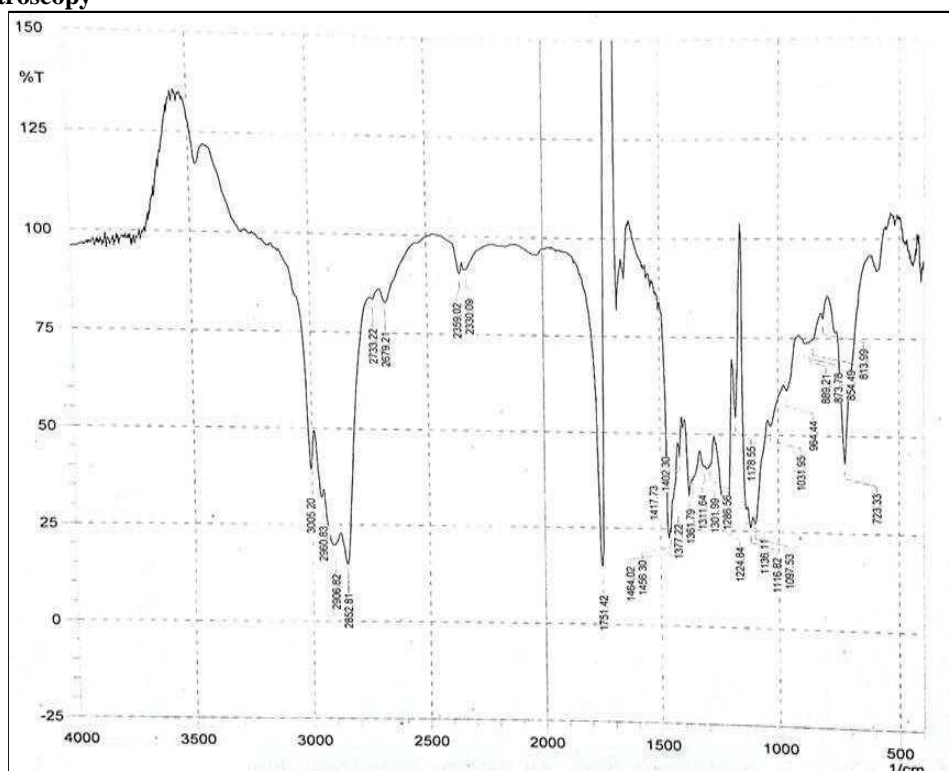


Fig. 2: IR Spectrum of *Moringa oleifera* seed oil.

IR spectral analysis of *Moringa oleifera* seed oil has shown peak $3200\text{--}2800\text{cm}^{-1}$ C-H alkane stretch, $1450\text{--}1375\text{cm}^{-1}$ CH_3 bending, $1600\text{--}1900\text{cm}^{-1}$ C-C alkane, $1000\text{--}650\text{cm}^{-1}$ alkene out of plane bend, $1850\text{--}1650\text{cm}^{-1}$ C=O carbonyl stretch.

Organoleptic test

According to the results of the observation and stability test that was carried out over the course of a period of

twelve weeks, the nanoemulsion demonstrated a degree of stability that was sufficient during the whole of that time period. Indicators that may be used to identify whether or not a medication that has been manufactured is stable include changes in colour and smell, as well as phase separation. As a result of the inner phase's aggregate formation—which has a higher propensity to rise to the surface—creaming developed in the preparation. This made it possible to make the creaming.

This process is responsible for the formation of creaming. Creaming is described by Sinko⁹ as the separation of an emulsion into two layers, one of which has a larger concentration of drop grains, or the dispersed phase, than the other layers. In order to make cream, the creaming process is necessary. It is possible for the sedimentation velocity to become negative when the dispersed phase's density is lower than the continuous phase's. This might happen. No matter the MSO concentration, no coarse grains were included in the production of this nanoemulsion gel.

pH measurement

For the purpose of determining the pH level of the MSO nanoemulsion, a digital pH meter was used for a period of twelve weeks. Documentation of the pH values of three distinct formulations was carried out after a period of twelve weeks at room temperature, and the results are shown in Table.

Table 3: The effect of storage on pH value of MOSO nanoemulsion.

The effect of storage on pH value of MOSC nanocomposites:													
Formula	Time (week)												
	0	1	2	3	4	5	6	7	8	9	10	11	12
F1	6.96	6.96	6.93	6.86	6.76	6.66	6.60	6.46	6.46	6.33	6.23	5.90	5.90
F2	7.00	6.96	6.93	6.93	6.86	6.76	6.73	6.60	6.53	6.43	6.30	6.10	6.10
F3	7.03	7.00	7.00	6.93	6.93	6.90	6.83	6.73	6.56	6.53	6.36	6.20	6.13

Viscosity test

In order to determine the viscosity of the nanoemulsion, an NDG 8-second viscometer equipped with the appropriate spindle number was used. This procedure was carried out for a period of twelve weeks while the temperature was kept at room temperature. A graph that illustrates the change in viscosity of the nanoemulsion is shown in Figure, which also includes the data that was obtained from the viscosity test results.

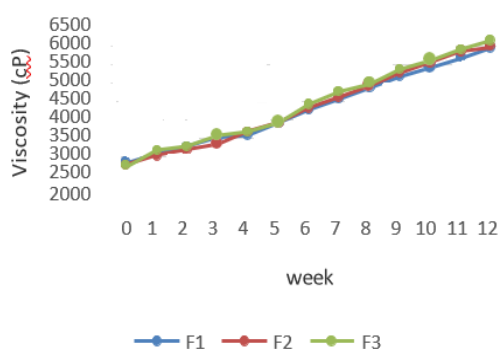


Fig. 3: The effect of storage on viscosity of MOSO nanoemulsion.

For the purpose of determining the viscosity of the Nanoemulsion preparation, it was subjected to analysis at room temperature for a period of twelve weeks. During this time, the temperature at room temperature was rather low. Following the facts presented here, it is possible to draw the conclusion that the viscosity of the nanoemulsion preparation will increase as the temperature at which it is stored falls. However, it is also important to note that storing the nanoemulsion at room temperature results in an increase in the gel's viscosity. This is something that should be taken into consideration. There is a connection between this and the concept that the length of time that the preparation is stored will lead to an increase in the viscosity of the

substance. The increase, on the other hand, is not very significant either.

Nanoemulsion particle size measurement

This table displays the findings of the particle size analysis that was performed. According to the findings of the centrifugation test shown in Figure, all of the formulae are stable, and there is no phase separation. This indicates that all of the formulas are stable in the face of the gravitational force that has been encountered for a period of one year. The centrifugation test is used to characterize the stability of the dose since it determines the influence of the gravity of the Earth that is comparable to one year⁸. Following testing on all three formulations, F1 through F3 did not exhibit any signs of separation. Due to the fact that gravity has an impact, this demonstrates that these three formulae remain constant for a period of one year.

Table 4: The effect of storage on particle size analyzing.

Formula	Distribution of particle size(nm)		
	0week(nm)	6week(nm)	12week(nm)
F1	51.11	128.42	241.81
F2	64.28	279.86	338.52
F3	75.75	354.36	414.62

Comprehensive Antimicrobial Analysis

The following table presents the comparative antimicrobial activity of the different nanoemulsion formulations containing Moringa seed oil, based on inhibition zone measurements and MIC values.

Table 5: Comprehensive Antimicrobial Analysis.

Bacteria	F1 Inhibition Zone (mm)	F2 Inhibition Zone (mm)	F3 Inhibition Zone (mm)	F1 MIC (Åµg/mL)	F2 MIC (Åµg/mL)	F3 MIC (Åµg/mL)	F2vs F1 Efficacy (%)	F3vs F1 Efficacy (%)
<i>Staphylococcus aureus</i>	12.5	14	15.5	25	20	15	12	24
<i>Escherichiacoli</i>	10	11.5	13	50	40	30	15	30
<i>Pseudomonas aeruginosa</i>	8.5	9.5	11	100	80	60	11.76	29.41

This data demonstrates that increasing the concentration of Moringa seed oil improves the antimicrobial efficacy. Inhibition zone measurements indicate that the F3 formulation, which contains 15% MSO, consistently produced the largest zones of inhibition against all three bacteria, while the F1 formulation (5% MSO) showed the lowest activity. MIC values further corroborate the enhanced antimicrobial efficacy of higher MSO concentrations, with the lowest MIC values observed in F3 for all three bacterial strains. F2 demonstrated moderate efficacy, showing a 12%–15% improvement over F1 across bacterial strains.

CONCLUSIONS

The study demonstrates that stable formulations with significant antimicrobial activity can be produced by effectively incorporating *Moringa oleifera* seed oil (MSO) into nanoemulsion- based delivery systems. The nanoemulsion gels showed great physicochemical strength more than 12 weeks, with no significant changes in variety, smell, or stage detachment. According to the analysis of the viscosity and particle size, higher concentrations of MSO resulted in an increase in viscosity and a gradual increase in particle size over time, but these changes had no effect on the stability as a whole. Antimicrobial testing revealed that formulations with higher MSO concentrations, particularly the 15% formulation (F3), were more effective against bacterial strains like *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*. The increased antimicrobial potential of higher MSO concentrations is further supported by the decreased MIC values and the expanded inhibition zones. These discoveries feature the promising capability of MSO nanoemulsion as compelling effective specialists for battling bacterial contaminations, making ready for future exploration on improving plans and investigating their clinical applications.

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