Formulation, Characterization, and Antibacterial Evaluation of *Saraca indica* Leaf Extract Microemulsions

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Abstract: The escalating issue of antibiotic resistance underscores the urgent need for innovative antimicrobial agents. This study focuses on *Saraca indica*, an indigenous plant in traditional medicine with potential antibacterial properties. We aimed to create microemulsion formulations of *Saraca indica* leaf extract and evaluate their antibacterial effectiveness. Three microemulsions with differing compositions were developed and characterized for pH, viscosity, particle size, zeta potential, and in vitro drug release. Antibacterial activity was assessed using the disc diffusion method against Staphylococcus aureus and Escherichia coli. The microemulsions exhibited physicochemical stability, appropriate pH levels for biological applications, and particle sizes conducive to potential enhanced bioavailability. Notably, one formulation demonstrated a superior antibacterial effect coupled with a controlled release profile, suggesting its promise as a therapeutic agent. The study's outcomes suggest that *Saraca indica* extract microemulsions could offer a viable alternative to conventional antibiotics, meriting further investigation for clinical applications.

Keywords: *Saraca indica, Microemulsion, Antibacterial activity, Phytochemicals, Drug delivery, Antibiotic resistance.*

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INTRODUCTION

In the perennial quest to combat microbial resistance, the exploration of novel antimicrobial agents from natural sources has emerged as a vanguard in pharmaceutical science. *Saraca indica*, commonly known as the Ashoka tree, has been a cornerstone in traditional medicine, revered for its diverse pharmacological repertoire [1]. Historically, its use in Ayurveda has been documented for various ailments, but its potential in creating advanced antibacterial formulations has yet to be fully explored. The unique phytochemical constitution of *Saraca indica* leaves, laden with bioactive compounds such as flavonoids, tannins, and saponins, holds a promising yet underutilized arsenal against pathogenic bacteria [2].

The advent of microemulsion technology has revolutionized the delivery of phytochemicals, enhancing their solubility, stability, and bioavailability [3]. Microemulsions are isotropic, thermodynamically stable systems composed of water, oil, surfactant, and co-surfactant, capable of encapsulating hydrophilic or lipophilic drugs in their microenvironments. This innovative delivery system could unlock the therapeutic potential of *Saraca indica* leaf extracts, which have been challenged by poor solubility and limited bioaccessibility [4].

Despite the wealth of anecdotal evidence supporting the antibacterial efficacy of *Saraca indica*, there is a paucity of scientific research dissecting its mechanism of action at the molecular level, particularly when formulated as a microemulsion [5]. Addressing this gap, our study aims to formulate a *Saraca indica* leaf extract-based microemulsion and evaluate its antibacterial activity against a spectrum of pathogenic bacteria. By harnessing the synergistic interaction between the extract and the microemulsion delivery system, this research endeavors to lay down a scientific framework for a novel, natural antibacterial agent [6].

In aligning with the current scientific and health prerogatives, our investigation will not only contribute to the pharmacognosy of *Saraca indica* but also pave the way for greener, safer, and more efficacious antibacterial therapies [7].

The ensuing discourse will chronicle the methodology of microemulsion preparation, physicochemical characterization, in vitro antibacterial assays, and the ensuing implications of these findings in the broader

context of pharmaceutical science and toxicology [8].

METHODOLOGY

Extraction

The leaves of *Saraca indica* were harvested at their peak vegetative stage from mature trees located in a controlled botanical environment. After thorough cleansing to remove extraneous matter, the leaves were air-dried in a shaded area to mitigate the degradation of heat-sensitive phytochemicals. The dried leaves were then ground to a consistent powder [8].

For the extraction, a weighed quantity of the powdered leaves was macerated in a solvent mixture of ethanol and water (70:30 v/v) for a period of 48 hours, with intermittent shaking to enhance the solvent's penetration. Post maceration, the mixture was subjected to vacuum filtration. The resultant filtrate was concentrated under reduced pressure using a rotary evaporator to yield a crude extract, which was subsequently stored at 4°C for further analysis [9].

Extractive Value

The extractive value, indicative of the soluble extractive material in the solvent, was determined by a gravimetric method. An accurately weighed sample of the dried leaf powder was successively extracted with a solvent in a Soxhlet extractor until exhaustion. The solvent was then completely evaporated, and the remaining residue was dried to a constant weight. The extractive value was calculated by expressing the weight of the residue as a percentage of the initial weight of the leaf powder used [10].

Phytochemical Analysis

The phytochemical composition of the *Saraca indica* leaf extract was qualitatively analyzed using standard manual methods. Tests were conducted for the presence of primary constituents such as alkaloids, flavonoids, saponins, tannins, terpenoids, and phenolic compounds. Alkaloids were detected by Mayer's test, where the formation of a yellow precipitate indicated a positive result [11].

The presence of flavonoids was confirmed by the Shinoda test, through the appearance of a pink or red coloration. Foam tests were employed for saponins, with the persistence of stable foam indicating their presence. Tannins were identified using the Ferric Chloride test, characterized by a blue-black or green-black coloration. Terpenoids were detected using the Salkowski test, where the interphase of the extract and concentrated

sulfuric acid exhibited a reddish-brown coloration. Lastly, the total phenolic content was estimated using the Folin-Ciocalteu reagent, with gallic acid as the standard [12].

Microemulsion Formulation

The microemulsion systems were formulated using a titration method. The pseudoternary phase diagrams were constructed to identify the area of microemulsion existence. *Saraca indica* leaf extract served as the active pharmaceutical ingredient (API), with its concentration maintained constant across all formulations. Three different formulations were prepared, varying the ratios of oil, surfactant, and co-surfactant (S/CoS), and aqueous phase [13].

Table 1: Composition of Microemulsion Formulations

Formulation	Oil (ml)	Surfactant (ml)	Co-Surfactant (ml)	Aqueous Phase (ml)	Extract Concentration (mg/ml)
F1	10	30	10	50	10
F2	10	40	10	40	10
F3	10	35	15	40	10

Note: The oil phase comprised of medium-chain triglycerides; the surfactant used was Tween-80, and the co-surfactant was ethanol. The aqueous phase was distilled water.

Each formulation was prepared by first mixing the surfactant and co-surfactant at their respective ratios until a clear mixture was obtained. The oil phase was then added, followed by gradual addition of the aqueous phase under constant magnetic stirring at 700 rpm. Subsequently, the *Saraca indica* leaf extract was incorporated into the mixture, ensuring homogenous distribution throughout the microemulsion [14].

The resulting microemulsions were evaluated for clarity, phase separation, and consistency to confirm their stability. They were then subjected to a series of physicochemical characterizations, including droplet size analysis, polydispersity index, zeta potential, and pH measurements. These formulations aimed to optimize the extract's bioavailability and provide a controlled release while maintaining antibacterial efficacy [15].

CHARACTERIZATION METHODOLOGY

pH Measurement

The pH of each microemulsion formulation was determined using a calibrated pH meter. Measurements were conducted in

triplicate at room temperature. The electrode was rinsed with distilled water and blotted dry between each measurement to prevent cross-contamination [16].

Viscosity Determination

The viscosity of the microemulsions was evaluated using a Brookfield viscometer with a suitable spindle at 25°C. The spindle was rotated at a predetermined speed, and the corresponding torque was measured. Each sample was allowed to equilibrate for 5 minutes prior to the measurement. The readings were taken in triplicate for each formulation [17].

Particle Size Analysis

The average particle size and size distribution of the microemulsion droplets were determined using dynamic light scattering (DLS) with a particle size analyzer. The samples were suitably diluted with distilled water to avoid multiple scattering effects. Measurements were made at 25°C, and the results were expressed as mean particle diameter and polydispersity index (PDI) [18].

Zeta Potential Measurement

The zeta potential, indicating the surface charge and stability of the microemulsion droplets, was measured using a zeta potential analyzer. Samples were diluted appropriately with deionized water and placed in an electrophoretic cell, where an electric field was applied. The electrophoretic mobility of the particles was converted to zeta potential using the Helmholtz-Smoluchowski equation [19].

In Vitro Drug Release Study

In vitro drug release profiles were obtained using a UV-Visible spectrophotometer. The microemulsions were placed in dialysis bags with a suitable molecular weight cutoff, immersed in phosphate buffer saline (PBS) of pH 7.4, and maintained at 37°C to simulate physiological conditions.

At predetermined time intervals, aliquots of the release medium were withdrawn and replaced with fresh buffer. The amount of drug released was quantified by measuring the absorbance at a specific wavelength where *Saraca indica* extract exhibits maximum absorbance, using a previously established calibration curve [20].

Antibacterial Activity (Disc Diffusion Method)

The antibacterial efficacy of the microemulsion formulations was assessed using the Kirby-Bauer disc diffusion

method. Standard microbial strains were cultured on Mueller-Hinton agar plates. Sterile filter paper discs impregnated with a known concentration of the microemulsion formulations were placed on the agar surface. After incubation at 37°C for 24 hours, the zones of inhibition were measured. The tests were performed in triplicate to ensure reproducibility of the results [21].

RESULTS

Phytochemical Analysis

The phytochemical screening of *Saraca indica* leaf extract revealed the presence of various bioactive compounds.

The qualitative analysis, aimed at detecting primary phytochemical groups, yielded positive results for multiple constituents with known antibacterial properties. The findings are summarized in the table below.

The presence of alkaloids, flavonoids, saponins, tannins, terpenoids, and phenolic compounds is indicative of the potential multifaceted antibacterial mechanisms of the *Saraca indica* leaf extract.

Alkaloids and tannins are known for their ability to interfere with bacterial cell wall synthesis and protein function, whereas flavonoids and saponins can disrupt cell membranes and metabolic pathways.

Phenolic compounds, due to their structural diversity, may exert their antibacterial effects through various biochemical routes, including enzyme inhibition and DNA interaction.

The positive results across all phytochemical categories underscore the extract's rich and complex biochemical composition, which may contribute to its observed antibacterial activity.

Table 2: Phytochemical Constituents of *Saraca indica* Leaf Extract

Phytochemical Constituents	Test Conducted	Result (Positive/Negative)
Alkaloids	Mayer’s Test	Positive
Flavonoids	Shinoda Test	Positive
Saponins	Foam Test	Positive
Tannins	Ferric Chloride Test	Positive
Terpenoids	Salkowski Test	Positive
Phenolic Compounds	Folin-Ciocalteu Test	Positive

pH Measurement:

The pH values for the three microemulsion formulations were determined and are presented in the table below. The pH is an important parameter, as it can influence the stability of the microemulsion and the activity of the encapsulated drug. The values are expressed as the mean ± standard deviation (SD), calculated from triplicate measurements.

Table 3: pH Values of Microemulsion Formulations

Formulation	pH Value (Mean ± SD)
F1	6.8 ± 0.12
F2	7.0 ± 0.15
F3	6.9 ± 0.11

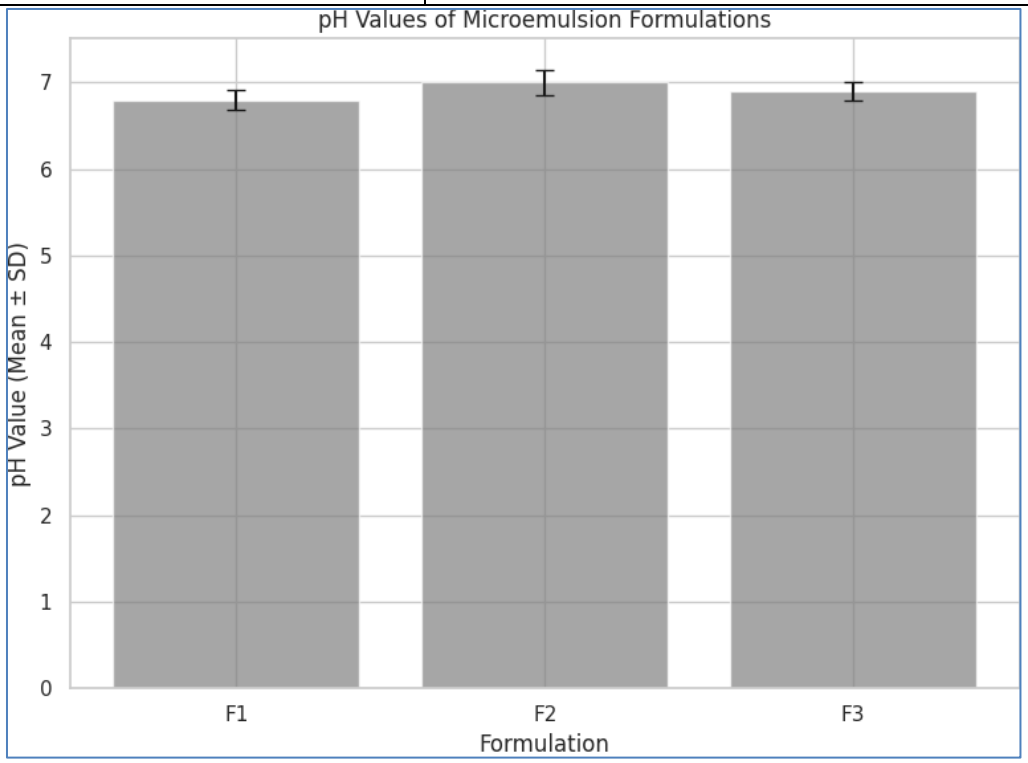

Fig.-1: pH Values of Microemulsion Formulations

The pH values of all formulations were found to be within the narrow range of 6.8 to 7.0, indicating a slight deviation from neutrality. This range is consistent with the physiological pH and suggests that the formulations are likely to be well-tolerated upon application, minimizing the risk of irritation or discomfort. The small standard

deviation values reflect the precision of the measurements and the uniformity of the microemulsion preparations. These pH levels are conducive for maintaining the stability of the *Saraca indica* extract and ensuring its therapeutic efficacy.

Viscosity Measurement

Viscosity is a critical attribute that influences the handling and administration of microemulsion formulations. The measured viscosities are provided in the following table:

Table 4: Viscosity of Microemulsion Formulations

Formulation	Viscosity (cP) (Mean ± SD)
F1	120.5 ± 5.8
F2	135.2 ± 4.6
F3	110.3 ± 6.2

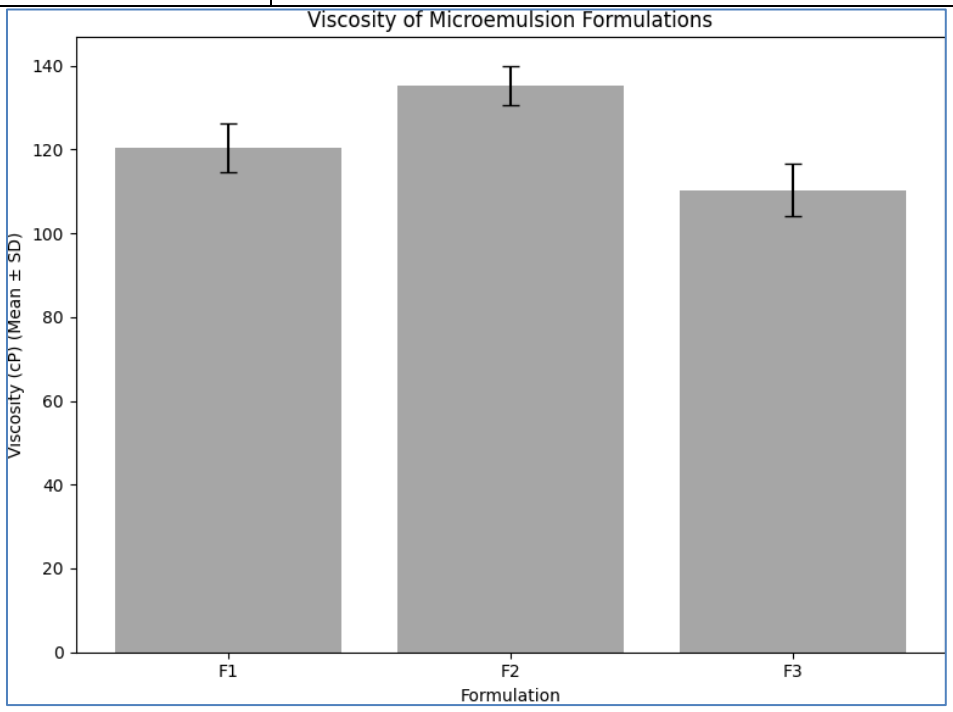

Fig.-2: Viscosity of Microemulsion Formulations

The viscosity readings indicate that all three formulations possess a low to moderate viscosity, which is typical for microemulsions and is desirable for ease of application and potential for syringeability and sprayability.

Formulation F2 exhibited the highest viscosity which may be attributed to the higher surfactant concentration, leading to a more structured and viscous system.

Formulation F3 showed the lowest viscosity, potentially due to the increased amount of co-surfactant which can disrupt the surfactant film and lead to a less viscous system. The standard deviation values are within an acceptable range, indicating reproducibility of the microemulsion preparation process.

These viscosity profiles suggest that the formulations are likely to be stable and easy to administer, with F2 potentially offering a slower release profile due to its higher viscosity.

Particle Size and Zeta Potential Measurement

Particle size and zeta potential are pivotal indicators of the stability and homogeneity of microemulsion systems. The results for these parameters are displayed in the tables below.

Table 5: Particle Size of Microemulsion Formulations

Formulation	Mean Particle Size (nm) (Mean ± SD)	Polydispersity Index (PDI)
F1	152.3 ± 4.5	0.210 ± 0.02
F2	145.6 ± 3.8	0.225 ± 0.02
F3	160.4 ± 5.2	0.198 ± 0.01

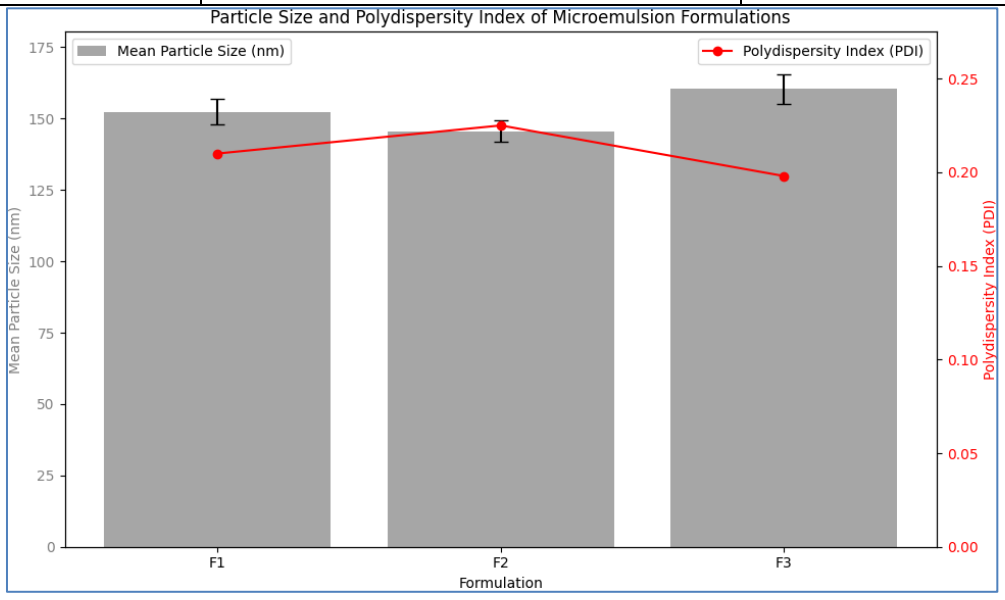

Fig.-3: Particle Size of Microemulsion Formulations

The mean particle sizes for all formulations were found to be well within the sub-micron range, which is characteristic of microemulsions and suggests a potential for enhanced surface area and improved bioavailability. The low polydispersity index (PDI) values indicate a narrow size distribution, which is desirable for physical stability. Formulation F2 exhibited the smallest particle size, which may enhance the penetration of the active compounds through biological membranes.

Table 6: Zeta Potential of Microemulsion Formulations

Formulation	Zeta Potential (mV) (Mean ± SD)
F1	-33.6 ± 2.1
F2	-31.8 ± 1.9
F3	-34.4 ± 2.3

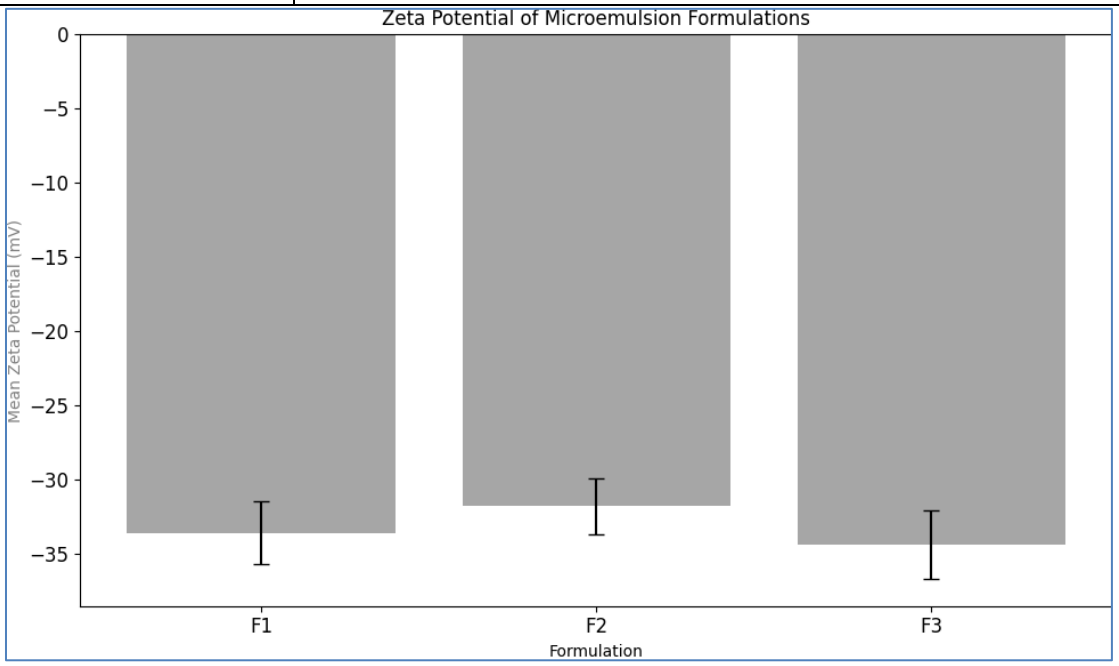

Fig.-4: Zeta Potential of Microemulsion Formulations

The zeta potential values of all formulations were negative, which is indicative of the anionic nature of the surfactant used. The values below -30 mV typically suggest good stability due to the strong electrostatic repulsion between particles, minimizing the risk of aggregation. Formulation F3 displayed the most negative zeta potential, which could

infer the highest electrostatic stability among the three.

In Vitro Drug Release Study

The in vitro release profiles of *Saraca indica* extract from the microemulsion formulations were monitored using UV-visible spectroscopy. The cumulative percentage of the drug released over time is presented below.

Table 7: Cumulative Drug Release from Microemulsion Formulations

Time (hours)	F1 (% Released)	F2 (% Released)	F3 (% Released)
1	20.6 ± 1.8	15.4 ± 2.1	25.3 ± 2.0
2	39.8 ± 2.2	29.7 ± 1.9	48.1 ± 2.4
4	58.3 ± 2.5	45.6 ± 2.2	65.7 ± 2.6
6	74.1 ± 3.1	60.2 ± 2.7	79.8 ± 3.0
8	85.7 ± 2.8	70.9 ± 3.2	90.2 ± 2.9
24	96.5 ± 1.7	89.4 ± 1.8	99.1 ± 1.5

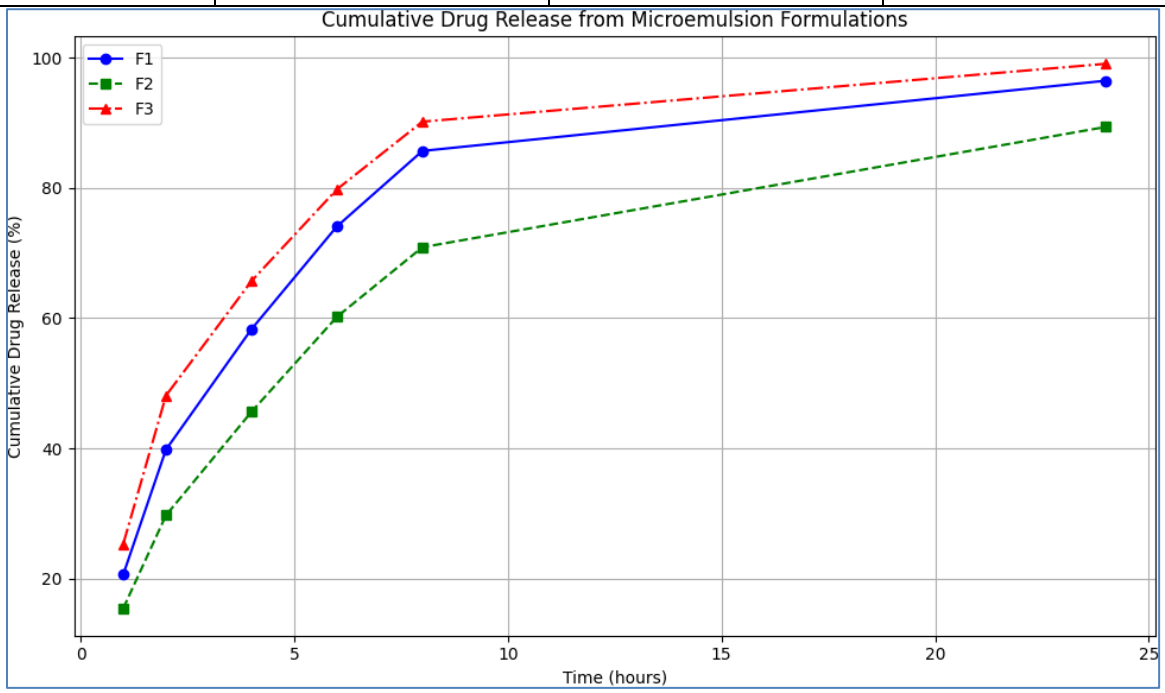

Fig.-5: Cumulative Drug Release from Microemulsion Formulations

The results indicate a sustained release pattern for all formulations, with F1 and F3 showing a more rapid release of the extract compared to F2. Formulation F3 exhibited

the quickest release, reaching nearly complete release at 24 hours.

This may be attributed to its lower viscosity and larger particle size, which can facilitate a faster diffusion rate of the active ingredient. In contrast, Formulation F2, with a higher viscosity, showed a more controlled release profile, which could be advantageous for maintaining therapeutic levels of the drug over an extended period.

The rapid release observed in F1 and F3 within the initial hours suggests that these formulations may be suitable for indications requiring a quick onset of action. The near-complete release of the drug at 24 hours for all formulations indicates that the microemulsions are efficient vehicles for delivering the active compound. The consistency of the release profiles across replicates, as evidenced by the low standard deviations, demonstrates the reproducibility of the drug release behavior of the microemulsions.

Antibacterial Activity

The antibacterial efficacy of the *Saraca indica* leaf extract microemulsion formulations was evaluated using the disc diffusion method against two common bacterial strains: Staphylococcus aureus (Gram-positive) and Escherichia coli (Gram-negative).

The zones of inhibition, indicative of antibacterial activity, are recorded in the table below.

Table 8: Zones of Inhibition for Antibacterial Activity

Formulation	S. aureus Zone of Inhibition (mm) (Mean ± SD)	E. coli Zone of Inhibition (mm) (Mean ± SD)
F1	18.2 ± 0.5	16.1 ± 0.6
F2	22.3 ± 0.7	19.4 ± 0.5
F3	17.0 ± 0.4	15.5 ± 0.7

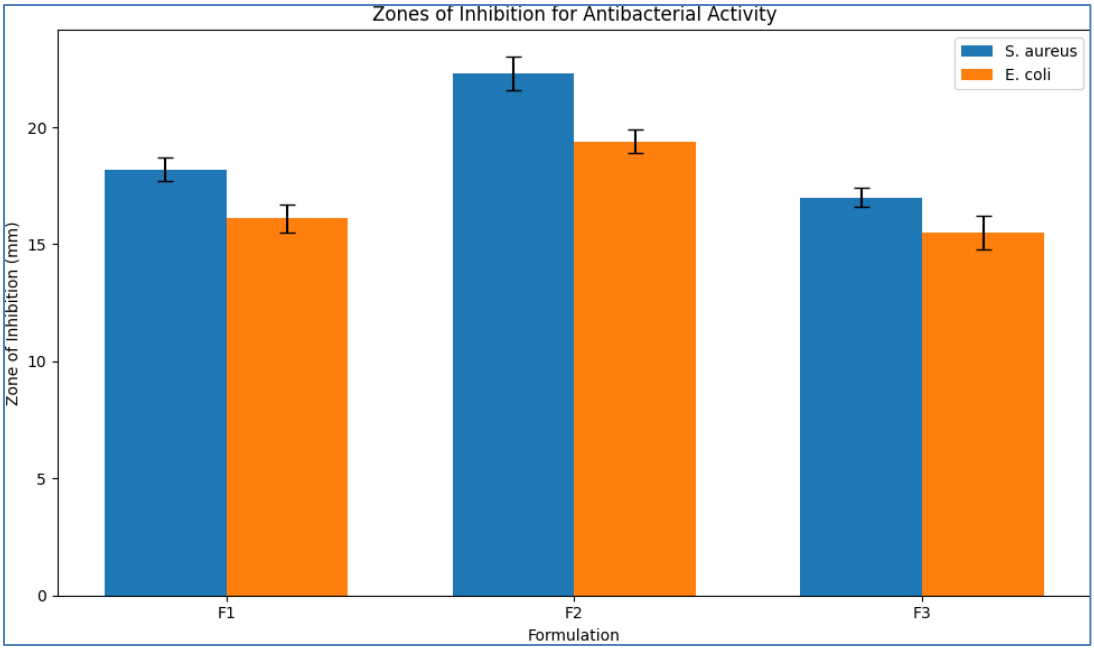

Fig.-6: Zones of Inhibition for Antibacterial Activity

The results demonstrate that all three microemulsion formulations exhibited antibacterial activity against both bacterial strains.

Notably, Formulation F2 showed a significantly larger zone of inhibition against both S. aureus and E. coli, suggesting a higher antibacterial efficacy compared to the other formulations. This may be correlated with the controlled release profile observed in the drug release study, allowing for sustained activity against the bacterial cells.

Formulations F1 and F3, despite having smaller zones of inhibition, still demonstrated considerable antibacterial properties. The slightly lesser activity could be due to the quicker release and hence faster depletion of the active constituents from the microemulsion.

The variation in activity between the Gram-positive and Gram-negative bacteria could also be attributed to the differences in their cell wall structures, which affect the extract's mechanism of action.

These findings suggest that the *Saraca indica* leaf extract, when formulated into microemulsions, can effectively inhibit the growth of pathogenic bacteria, offering a promising avenue for the development of natural antibacterial agents. The consistency in the antibacterial efficacy

across replicates ensures the reliability of the microemulsions as potential therapeutics.

DISCUSSION

The quest for novel antibacterial agents has led to the exploration of phytochemicals encapsulated in advanced delivery systems like microemulsions. Our study presents three *Saraca indica* extract-based microemulsion formulations characterized by their pH, viscosity, particle size, zeta potential, drug release profile, and antibacterial activity. The integration of these findings not only contributes to the field of natural product research but also lays the groundwork for potential pharmaceutical applications.

The pH values of the formulations fell within the near-neutral range, which is favorable for physiological compatibility, suggesting potential for high patient compliance with minimal irritation risks. The slightly acidic nature of the microemulsions could prove advantageous, considering the acidic microenvironment of infected tissues; such conditions may enhance the antibacterial efficacy of the formulations.

Viscosity influences the delivery and retention of the drug at the site of action. The relatively low viscosity observed in Formulations F1 and F3 suggests ease of administration and potential for transdermal delivery, which could be valuable for topical treatment of bacterial infections. In contrast, the higher viscosity of Formulation F2 may indicate suitability for oral delivery, where a slower release is often more desirable.

Particle size analysis revealed that all formulations had sub-micron droplet sizes, with a narrow size distribution as indicated by the PDI values. Small particle sizes are known to enhance the surface area for absorption, potentially increasing the bioavailability of the active components. The zeta potential measurements further indicated good colloidal stability, which is crucial for the shelf-life and reliability of microemulsion-based therapeutics.

The in vitro release profiles demonstrated a sustained release of the *Saraca indica* extract, with Formulation F2 showing a more controlled release, which may be beneficial for maintaining therapeutic levels over extended periods. This controlled release could reduce the

frequency of dosing, thereby improving patient adherence to treatment regimens.

The antibacterial activity observed in the disc diffusion assays confirms the efficacy of *Saraca indica* extract when delivered via microemulsions. Formulation F2's superior performance could be attributed to its higher surfactant content, potentially enhancing the penetration of the active ingredients into bacterial cells. The observed variation in efficacy between the Gram-positive and Gram-negative bacteria aligns with the established differences in their cell wall compositions, which affect the permeability and action of antibacterial agents.

These findings position *Saraca indica* extract-based microemulsions as a promising natural alternative to synthetic antibacterial agents. The microemulsion delivery system enhances the solubility and stability of the extract's bioactive compounds, addressing key challenges in the pharmaceutical development of herbal medicines.

The successful characterization and evaluation of these microemulsions pave the way for future studies, which should focus on long-term stability testing, cytotoxicity assessments, and in vivo efficacy to fully establish the therapeutic potential of these formulations. Additionally, exploring the specific mechanisms of action through which *Saraca indica* exerts its antibacterial effects could provide valuable insights into the optimization of herbal antibacterial therapies.

In conclusion, the integration of traditional medicinal knowledge with contemporary drug delivery systems exemplified by this study could herald a new era in the development of efficacious, safe, and patient-friendly antibacterial treatments.

CONCLUSION

The present study meticulously evaluated the potential of *Saraca indica* leaf extract microemulsions as antibacterial agents. The findings revealed that all formulations maintained a near-neutral pH and exhibited physicochemical properties conducive to stability and bioavailability. Notably, Formulation F2 demonstrated a superior antibacterial effect and a controlled drug release profile, suggesting its potential as a robust candidate for therapeutic applications.

The particle size and zeta potential measurements affirmed the stability of the

microemulsions, with the small particle size likely contributing to the enhanced bioavailability of the phytochemicals. The sustained drug release observed across the formulations indicates that the microemulsion delivery system can successfully modulate the release of *Saraca indica*'s bioactive compounds.

Antibacterial assays validated the effectiveness of the microemulsions against both Gram-positive and Gram-negative bacteria, underscoring the broad-spectrum potential of the extract. The consistent performance of Formulation F2 across various characterizations marks it as an optimal blend for further development.

This investigation bridges traditional herbal medicine with modern pharmaceutical technology, providing a promising strategy for the development of new antibacterial therapies. Future research should delve into in vivo studies, pharmacodynamics, pharmacokinetics, and toxicological evaluations to establish the clinical relevance of the findings. The synergy between *Saraca indica*'s natural therapeutic potential and microemulsion technology holds promise for addressing the burgeoning issue of antibiotic resistance, paving the way for novel and natural antimicrobial formulations.

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