"Development of Nano-emulsion based gel loaded with phytoconstituent for the treatment of Urinary tract infection (UTI) causing pathogens"

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ABSTRACT

Purpose: A nano-emulsion based gel loaded with methanolic extract of *Saraca asoca* leaves has been developed to inhibit the growth of microorganisms causing urinary tract infection.

Methods: Methanolic extract of *Saraca asoca* leaves loaded in a single nano-emulsion by magnetic stirring method than the F1 formulation of nano-emulsion is loaded in gel by the method of magnetic stirring and characterized for particle size, zeta potential and scanning electron microscope to perform in vitro antimicrobial activity by well Diffusion Assay.

Results: The methanolic extract has the presences of Biological active compounds such as, sterol, triterpenoids, saponins, flavonoids, and phenolic. Total phenolic content of 49.25mg/ml. Total flavonoid content of 41.71mg/ml. shows the DPPH radical scavenging activity of IC50=20.032µg/ml.finalized NE had a Particle size that range 199.7 nm to 209.4 nm. The zeta potential range for all formulations (F1, F2, F3, F4&F5) was determined to be -4.6 mV to -9.2 mV. Scanning electron micrograph of the prepared Nano emulsion at 50.00 kx magnification showed that the Nano emulsion were smooth surface morphology and spherical shape. The emulgel has the viscosity of 6891 ± 0.37 cps and the ph. of 6.23 weakly acidic. The emulgel shows the spreadability of 11.18 g.cm/s. In vitro study shows the zone of inhibition of emulgel in E. coli is 22.66 ± 0.94 mm, nano-emulsion is 21.66 ± 2.35 mm and extract are 11.33 ± 1.24.

Conclusion: The findings suggested that the emulgel loaded with nano-emulsion of methanolic extract of *Saraca asoca* has the ability to inhibit the growth of microorganism which are responsible for causing urinary tract infection.

Keywords: *Saraca asoca*, nano-emulsion, emulgel, zeta potential, scanning electron microscope and Urinary tract infection.

1. INTRODUCTION

Urinary tract infections (UTIs) begin when gut-resident uropathogens colonize the urethra and subsequently the bladder through the action of specific adhesins **(Mancuso *et al.*, 2023).** If the host's inflammatory response fails to eliminate all bacteria, they begin to multiply, producing toxins and enzymes that promote their survival. Urinary tract infections (UTIs) may be caused by either Gram-positive or Gram-negative bacteria; however, Gram-negative bacteria account for more than 95% of UTI cases **(Mazzariol *et al.*, 2017).**

Nanoemulsions (NEs) have shown to enhance the solubility, evade the enzymatic attack and thus increase the bioavailability and prolong the shelf life by protection against oxidation and hydrolysis. NEs serve as a versatile carrier for drug delivery owing to their lipophillic, hydrophilic and amphiphillic phases **(Gao *et al.*, 2015).**

The gelling agent component becomes apparent as a crucial factor that might affect the final physical characteristics of the nanoemulgel throughout the formulation process of nanoemulgel preparations **(Ansari *et al.*, 2025).**

The aim of the present work was to prepare nanoemulsion based gel loaded with phytoconstituent for the treatment of Urinary tract infection (UTI) causing pathogens.

2. MATERIAL AND METHODS

2.1 Chemical

Sodium Hydroxide, Glacial Acetic Acid, Ammonia and Nitroprusside was acquired from Merck. Himedia gave the Magnesium. Chloroform, Conc. HCl, 95% Alcohol was supplied by Clorofiltind. Fizmerck supplied Conc. H_2SO_4. Rankem supplied the Methanol. Research lab gave the Ethyl acetate whereas Join Hub Pharma spplied Normal saline. Ethanol obtained from Molychem and Orion Chem supplied Ethylene glycol.

2.2 Collecting plants

The plant is collected from the local garden of Bhopal.

2.3 Extraction

Dried and ground powder of *Saraca asoca leaves* were placed in a Soxhlet apparatus and extracted with solvent in a round bottom flask containing glass beads for 24 h. After extraction, the solvent was removed from the extract in a vacuum rotary evaporator. Methanol was used as solvent. Extracts were collected in air tight container **(Şahin et al., 2011).** Extraction yield of all extracts was calculated using the following equation below:

$$\text{Formula of Percentage yield} = \frac{\text{Actual yield}}{\text{Theoretical yield}} \times 100$$

2.4 Qualitative phytochemical analysis of plant extract

Saraca asoca extracts obtained was subjected to the preliminary phytochemical analysis.

2.4.1 Total Phenolic Content

The total phenolic content of plant extract was determined using the Folin-Ciocalteu Assay. *Saraca asoca* extracts (0.2 ml) was mixed with 2.5 mL of Folin-Ciocalteu reagent (prediluted 10-fold with distilled water) and allowed to stand at room temperature for 5 min, and then 2 mL of sodium bicarbonate (7.5%, w/v) was added to the mixture. After standing for 2 hrs at room temperature, absorbance was measured at 760 nm. Aqueous solutions of known gallic acid concentrations in the range of 20–100 µg/ml were used for calibration. Results were expressed as mg gallic acid equivalents (GAE)/g sample. The Folin-ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue colour upon reaction. This blue colour was measured spectrophotometrically **(Al-Rimawi et al., 2016).**

2.4.2 Total Flavonoid Content

The determination of total flavonoids was performed by Aluminium chloride method **(Al- Rimawi et al., 2016).** Distilled water (1.5 mL) was added to 0.5 mL of *Saraca asoca* extract in a test tube. Then, 0.150 mL of 5% sodium nitrite solution was added, followed by 0.150 mL of 10% aluminum chloride solution. Test tubes were incubated at ambient temperature for 6 minutes, and then 2 mL of 4% sodium hydroxide was added to the mixture. Immediately, the volume of reaction mixture was made to 5 mL with distilled water. The mixture was thoroughly mixed using test tube shaker and the absorbance of the yellow to orange color developed was determined at 510 nm. Aqueous solutions of known rutin concentrations in the range of 20– 100 µg/ml were used for calibration and the results were expressed as mg rutin equivalents (RE)/g sample.

2.5 In vitro anti-oxidant activity

2.5.1 DPPH assay

Free radical scavenging activity of extracts of *Saraca asoca*, based on the scavenging activity of the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined by the method of Ali et al **(Ali et al., 2013).** Different volume of extracts/standard (20 – 100µg/ml) was taken from stock solution in a set of test tubes and methanol was added to make the volume to 1 ml. To this, 2 ml of 0.1mM DPPH reagent was added and mixed thoroughly. Absorbance at 517 nm was determined after 30 min and the percentage inhibition activity was calculated by using the equation: % scavenging activity=[(A0-A1)/A0] ×100. Where A0 is the absorbance of the control and A1 is the absorbance of the extract. Lower the absorbance, the higher is the free radical scavenging activity. The curves were prepared and the IC50 value was calculated using linear regression.

2.6 Formulation of Nano-emulsion

The solubilities of extract in various oils (oleic acid, Capryol 90, Olive oil), surfactants (Cremophor RH 40, Tween 80), and co surfactants (polyethylene glycol 400) were determined by first adding an excess number of drugs in 2 mL of selected excipients into 5 mL stopper vials, then vortex mixing. Oil, surfactant, and co-surfactant were selected based on their ability to drug solubility. The nano-emulsion of respective composition, as shown in the table 8 were devised using four component Olive oil as oil phase, tween 80 as a surfactant, propylene glycol as co-surfactant, and distilled water as the aqueous phase. Batches were designed for different surfactant–co-surfactant ratios. A ratio of surfactant and co-surfactant e,S/CoS chosen and corresponding mixture was made. The mixture was mixed with oil. Each mixture was mixed thoroughly using magnetic stirrer until homogenous dispersion/solution was obtained. Double distilled water was used in these formulations as to prevent the incorporation of surface-active impurities (**Tung and Nguyen 2019, Yadav et al., 2018)**.

Table 1: Composition of Nano emulsion formulation

Ingredients	**FORMULATION CODE**				
	NE1	**NE2**	**NE3**	**NE4**	**NE5**
Extract (mg)	100	100	100	100	100
Propylene Glycol (ml)	0.1	0.2	0.3	0.4	0.5
Tween 80 (ml)	1.0	1.0	1.0	1.0	1.0
Olive oil (ml)	2.0	2.0	2.0	2.0	2.0
Stirring Time (min.)	30	30	30	30	30
Distilled water(q.s.)	q.s	q.s	q.s	q.s	q.s

2.7. Evaluation parameter of extract loaded nano emulsion

2.7.1 Particle size

The particle size is one of the most important parameters for the characterization of nano emulsion. The size of micro emulsion was measured using Malvern Zeta sizer (Malvern Instruments) **(Singh and Vingkar 2008).**

2.7.2 Zeta potential

The zeta potential was measured for the determination of the movement velocity of the particles in an electric field and the particle charge and analyzed by Zetasizer Malvern instruments. **(Đorđević et *al.*,**

2022).

2.7.2 Scanning Electron Microscopic (SEM)

The electron beam from a scanning electron microscope was used to attain the morphological features of the extract loaded nano emulsion were coated with a thin layer (2–20 nm) of metal(s) such as gold, palladium, or platinum using a sputter coater under vacuum. The pretreated specimen was then bombarded with an electron beam and the interaction resulted in the formation of secondary electrons called auger electrons. From this interaction between the electron beam and the specimen's atoms, only the electrons scattered at 90° were selected and further processed based on Rutherford and Kramer's Law for acquiring the images of surface topography **(Ahmed *et al.*, 2020).**

2.8 Formulation of nano emulsion loaded Gel

Initially carbopol-934 was immersed in 50 mL of warm water (A) for 2 hr and was homogeneously dispersed using magnetic stirrer at 600 rpm. In separate container carboxymethyl cellulose and methyl paraben was added into 50 ml warm water (B) and stirred continuously to make stiff gel. Both the mixtures A and B were mixed with the continuous stirring. Then tri-ethanol amine (Drop wise) was added to neutralize the pH and nano emulsion of optimized formulation was incorporated into the dispersion to obtained Gel. At this stage, permeation enhancer (Propylene glycol) was added. The final dispersion was agitated until smooth gel was formed without lumps (**Abbas *et al*., 2019, Silpa *et al*., 2021**).

Table 2: Composition of gel formulation

Excipients	Quantity
Carbopol 934	1.00 gm
Carboxymethyl cellulose	1.00 gm
Propylene glycol	0.5 ml
Methyl paraben	0.2 ml
Nano emulsion	10 ml
Tri-ethanolamine	q.s
Water	100 ml

2.9 Characterization of Nano emulsion loaded Gel

2.9.1 Physical appearance

The prepared Gel formulation was evaluated for appearance, Color, Odor, and homogeneity by visual observation **(Kumar and Eswaraiah 2020).**

2.9.2 pH

pH of the formulation was determined by using Digital pH meter (EI).

2.9.3 Viscosity

The viscosity of the gel formulations was determined using Brookfield viscometer with spindle no. 61 at 100 rpm at the temperature of 25^0C **(Monica and Gautami 2014).**

2.9.4 Spread ability

An ideal topical gel should possess a sufficient spreading coefficient when applied or rubbed on the skin surface. This was evaluated by placing about 1g of formulation on a glass slide. Another glass slide of the same length was placed above that, and a mass of 50 mg was put on the glass slide so that the gel gets sandwiched between the two glass slides and spreads at a certain distance. The time taken for the gel to travel the distance from the place of its position was noted down. Spread ability was determined by the following formula

S= M*L/T

Where, S-Spread ability, g.cm/s M-Weight put on the upper glass L-Length of glass slide T- Time for spreading gel in sec **(Sandeep, D. S. 2020).**

2.9.5 Skin irritation test

The intact skin of Wistar rats of either sex with average weight 150– 200 g was used. The hairs were removed from the rat 2-3 days before the experiment. The gel was applied on the properly shaven skin of rat. The animals were treated daily for 2-3 days, and undesirable skin changes, i.e., change in colour, change in skin morphology was checked for a period of 24 h and erythema and edema on the treated skin were examined **(Giri and Bhalke 2019, Murthy and Hiremath 2001).**

2.10 Invitro Antibacterial activity

2.10.1 Antibacterial Activity by Well Diffusion Assay

- **Preparation of Nutrient Media**

One litter of distilled water was used to dissolve twenty-eight grams of nutritional agar material. The media's pH was measured prior to sterilization. The media was autoclaved for 15 minutes at 121 degrees Celsius and 15 pounds of pressure. After sterilization, media was allowed to be cool but not solidify. Nutrient media was poured into plates and placed in the laminar air flow until the agar was get solidified.

- **Well Diffusion Assay**

Culture of bacterial strains was spread on the nutrient agar media (NAM). Then 1ml of test sample (emulgel of *S.asoca*) was taken directly from the stock. Then 250 µg of standard (Methanolic extract of *S.aso*ca leaves) was taken with 750µl solvent (Methanol) to make 10mg/10ml solution. Then, inoculum of one-gram positive bacteria (*S. aureus* (MTCC 736) and one gram negative (*E. coli* (MTCC 8076) was prepared; test organisms were inoculated in 10ml Nutrient broth. The bacterial suspension was standardized to 108 CFU/ml of bacteria and kept into the shaker. Then, 100µl of the inoculum from the broth (containing 10^8 CFU/ml) was taken with a micropipette and then transferred to fresh and sterile solidified Agar Media Plate **(Mohammadi-Sichani et al., 2012).** The agar plate was inoculated by spreading the inoculum with a sterile spreader, over the entire sterile agar surface. Three wells of 6 mm were bored in the inoculated media with the help of sterile cork-borer. Each well was filled with different component (methanolic extract of leaves, nanoemulsion &emulgel) of sample. It was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37^o C. After the incubation period, plates were checked to see if a clear zone formed around the well, indicating that the investigated substances had antibacterial action. A measurement was taken of the zone of inhibition (ZOI) in millimeters. A ruler was placed on the back of the inverted Petri plate and used to measure zones to the closest millimeter. A few inches above a non- reflective black background were where the Petri plate was placed. The measurements included the well's diameter as well as the diameter of the zone of total inhibition as determined by unassisted vision. **(Manandhar et al., 2019).**

3 RESULT AND DISCUSSION

3.1 Plant Extraction:

The plant material of *Saraca asoca* was extracted by Soxhlation extraction method and the percentage yield calculated by the following formula: -

$$\% \text{ yield} = \frac{\text{Actual yield}}{\text{Theoretical yield}} \times 100$$

Table 3 Percentage yield of *Saraca asoca* extract

Solvent	Colour of extract	Weight of plant material (gm)	Weight of extract (gm)	%yield
Petroleum ether	Green	40.32	0.957	2.35
Methanol	Green	38.20	8.211	21.49

3.2 Solubility Determination: -

Table 4 Solubility determination of extract

Solvent	Solubility of P.E. extract	Solubility of methanolic extract
Water	Insoluble	Insoluble
Ethanol	Soluble	Soluble
Petroleum ether	Soluble	Soluble
DMSO	Insoluble	Insoluble
Methanol	Slightly soluble	Soluble
Chloroform	Soluble	Soluble
Acetone	Soluble	Soluble
Ethyl acetate	Soluble	Soluble

3.3 Phytochemical investigation: -

3.3.1 Qualitative estimation: -

Table 5 Phytochemical testing of extract

S.NO.	Experiment	RESULTS	
		Petroleum ether extract	Methanolic extract
Test for carbohydrates			
1.	Molisch's Test	-	-
2.	Fehling's Test	-	-
3.	Benedict's Test	-	-
Test for Alkaloids			
1.	Mayers Test	-	+
2.	Hager's Test	-	+
3.	Wagner's Test	-	+
Test for Triterpenoids and Steroids			
1.	Salkowski's Test	-	+
Test for Flavonoids			
1.	Lead Acetate Test	-	+
2.	Alkaline Reagent Test	-	+
Test for Tannin and Phenolic compound			
1.	Ferric chloride Test	-	+
2.	Lead Acetate Test	-	+

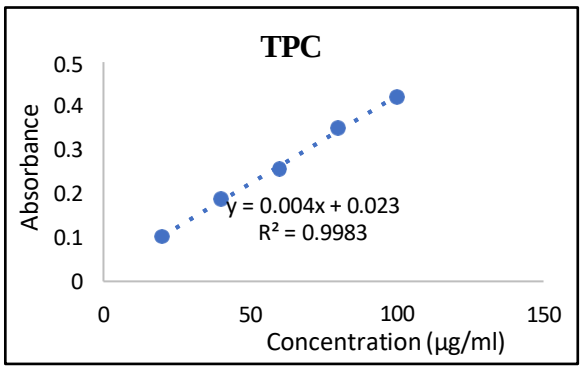
3.	Gelatine Test	-	+
Test for Saponins			
1.	Froth Test	-	+
Test for Fats and oils			
1.	Solubility Test	+	+
Test for Protein and Amino acids			
1.	Biurets Test	-	-
2.	Ninhydrin Test	-	-
Test for Glycosides			
1.	Bontrager's Test	-	-
2.	Keller-Killian Test	-	-

3.3.2 Quantitative estimation

3.3.2.1 Total phenolic content: -

Table 6 Total phenolic content

Concentration (µg/ml)	Absorbance
20	0.102
40	0.187
60	0.256
80	0.349
100	0.421
Extract	0.22



Graph 1 Graph represent standard curve of Gallic acid

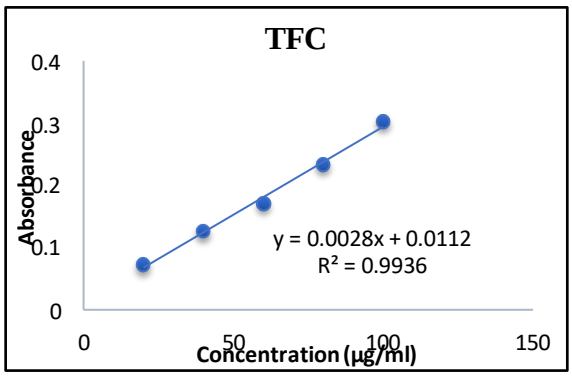
From the analysis it was observed that methanol extract of *S.asoca* leaves showed phenolic content (49.25mg/ml) (Table 6).

3.3.2.2 Total Flavonoid content: -

Table 7 Total flavonoid content present in methanolic extract of *S.asoca* leaves

Concentration(µg/ml)	Absorbance
20	0.072
40	0.127
60	0.171
80	0.233

100	0.302
Extract	0.128

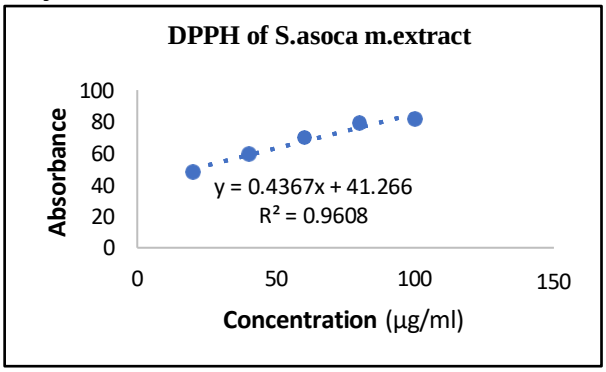


Graph 2 Graph represent standard curve of Rutin

The methanol extract of *S.asoc*a leaves showed flavonoid content of (41.71mg/ml).

3.4 Anti-oxidant activity- DPPH: -

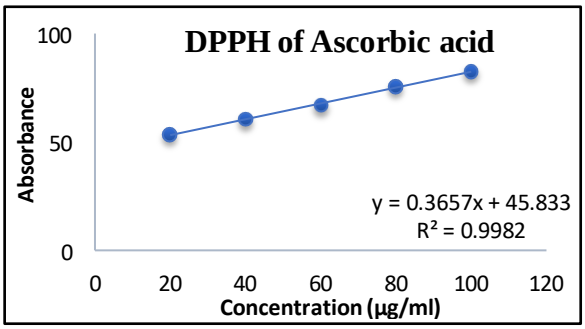
3.4.1 Anti-oxidant activity- DPPH of *S.asoca* methanolic extract:-



Graph 3 Graph representing DPPH activity of *S.asoca* methanolic extract

In the present study DPPH radical scavenging activity was highest in methanolic extract (IC50=20.032µg/ml) of *Saraca asoca* leaves.

3.4.2 DPPH activity of Ascorbic acid



Graph 4 Graph representing DPPH activity of Ascorbic acid

3.5 Evaluation parameter of Nano emulsion

3.5.1 Particle Size

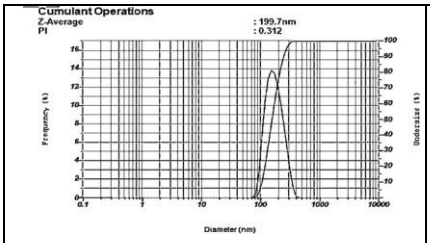


Figure 1: Particle size (F1)

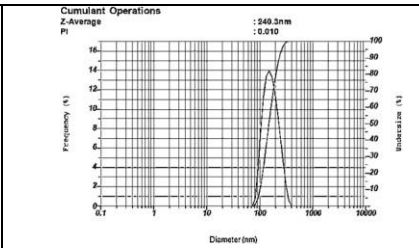


Figure 2: Particle size (F2)

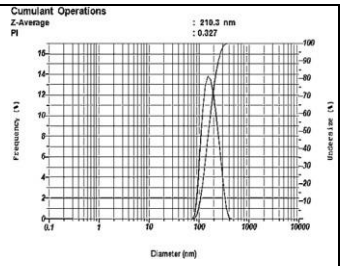


Figure 3:Particle size (F3)

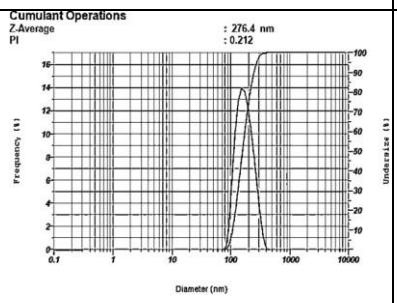


Figure 4: Particle size (F4)

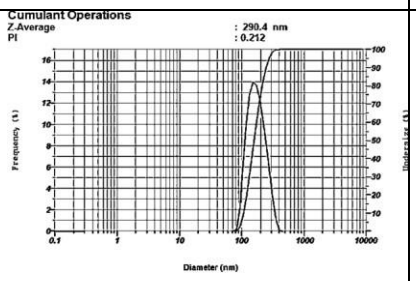


Figure 5: Particle size (F5)

Table 8 Particle size determination

Formulation code	Particle size (nm)	PI Value
NE 1	**199.7 nm**	**0.312**
NE2	240.3 nm	0.010
NE3	210.3 nm	0.327
NE 4	276.4 nm	0.212
NE 5	290.4 nm	0.213

Particle size analysis showed that the average particle size of extract loaded nano-emulsion was found to be range 199.7 nm to 290.4 nm.

3.5.2 Zeta potential

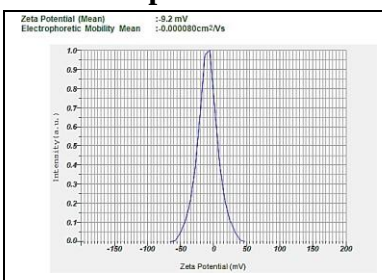


Figure 6 Zeta potential (F1)

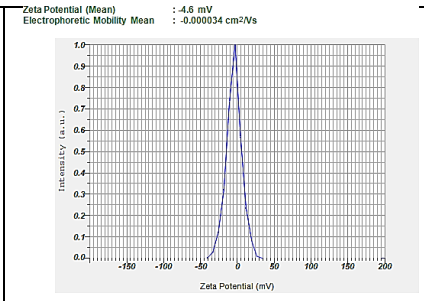


Figure 7 Zeta potential (F2)

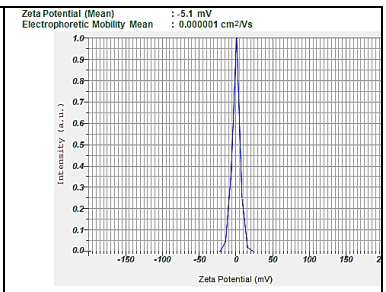


Figure 8 Zeta potential (F3)

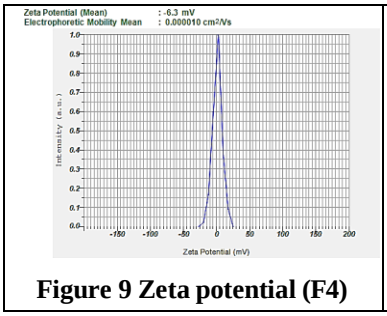


Figure 9 Zeta potential (F4)

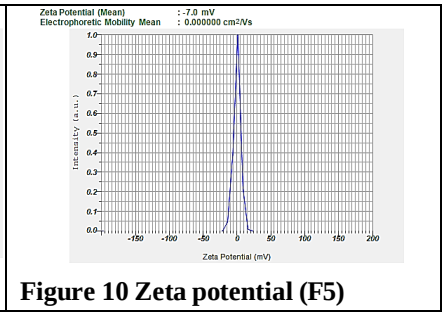


Figure 10 Zeta potential (F5)

Table 1 9 Zeta potential

Formulation Code	Zeta potential
Nano emulsion (F1)	-9.2 mV
Nano emulsion (F2)	-4.6 mV
Nano emulsion (F3)	-5.1 mV
Nano emulsion (F4)	-6.3 mV
Nano emulsion (F5)	-7.0 mV

The zeta potential range for all formulations was determined to be -4.6 mV to -9.2 mV. mV at the 100% intensity peak area.

3.5.3 Scanning electron microscope (SEM) of F1 Formulation (Optimized)

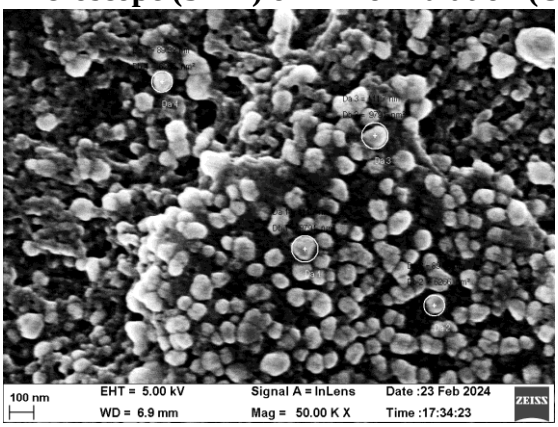


Figure 11 Scanning electron microscope

3.6 Characterization of Nano emulsion loaded gel Table

3.6.1 : Physical appearance

Parameter	Result
Colour	creames yellow
Odour	Odourless
Appearance	whitish colour
Homogeneity	Homogeneous

Table 11: Viscosity, pH, Skin irritation study and Spreadability test of gel formulation

Formulation	Results (pH)	Viscosity (cps)	Spreadability test (gm.cm/sec)	skin irritation study
Gel	6.23	6891±0.37	11.18	Not irritant observed

3..7. Antimicrobial testing

3.7.1 Zone of inhibition in *E. coli*-

Table 12 Antibacterial activity of nano emulsion, methanolic leaves extract of *S.asoca* &emulgel against *E.coli*

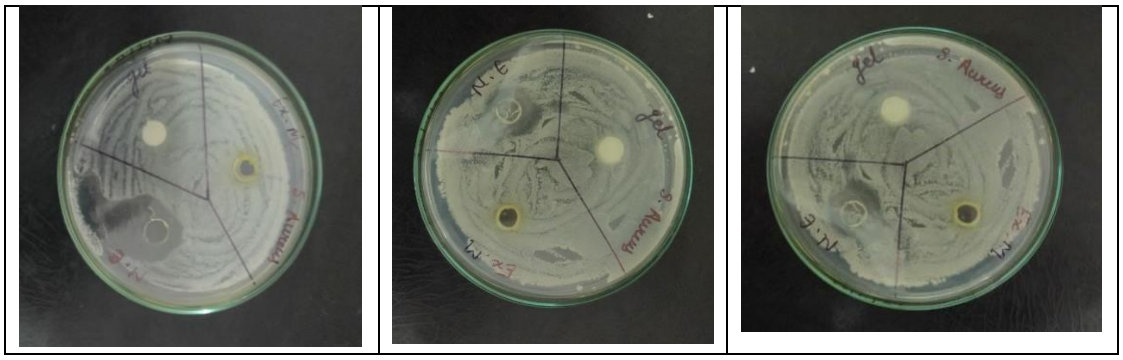
Name	Zone of Inhibition plate 1 (mm)	Zone of Inhibition plate 2 (mm)	Zone of Inhibition plate 3 (mm)	Mean ± SD (mm)
Emulgel	22	24	22	22.66 ± 0.94
Nano emulsion	25	20	20	22.66 ± 0.94
Extract	11	10	13	11.33 ± 1.24

Emulgel of *S.asoca* methanolic extract shows zone of inhibition of 22.66 ± 0.94, Nano- emulsion shows zone of inhibition of 22.66 ± 0.94 in *E.coli* as shown in table no.12.

3.7.2 zone of inhibition in S. aureus-

Table 13: Antibacterial activity of nano emulsion, methanolic leaves extract of *S.asoca* &emulgel against *S.aureus*-

Name	Zone of Inhibition plate 1 (mm)	Zone of Inhibition plate 2 (mm)	Zone of Inhibition plate 3 (mm)	Mean ± SD (mm)
Emulgel	8	8	8	8 ± 0
Nano-emulsion	22	19	20	20.33 ± 1.24
Extract	07	07	07	07 ± 0



Justification

Emulgel of *S.asoca* methanolic extract shows zone of inhibition of 8 ± 0, Nano-emulsion shows zone of inhibition of 20.33 ± 1.24 in *S. aureus*. The emulgel is enable to inhibit the growth of *S.aureus* may be due to the low concentration of nano-emulsion in emulgel. *S. aureus* is a gram -positive bacteria and have a thick cell wall which cannot be ruptured easily.\

4. CONCLUSION

The study demonstrates that *Saraca asoca* extracts possess potent antimicrobial and antioxidant activities, attributed to its rich phytochemical composition such as flavonoid 41.71 mg/ml. Flavonoids are known for their antioxidant, antibacterial, anti-inflammatory, and phenol 49.25mg/ml, Phenolic compounds play crucial roles in plant physiology and exhibit antioxidant properties. The nano emulsion was incorporated into a gel is creamy yellow in color, odorless and homogeneous in appearance. Its viscosity is 6891 cps, suitable for topical application.ph of 6.23, within the skin's normal pH range, ensuring compatibility and the spreadability of 11.18 g.cm/s, facilitating easy application on the skin.

These findings support the potential use of *Saraca asoca* in developing therapeutic agents against bacterial infections, warranting further investigation into its bioactive components and mechanisms of action. The emulgel successfully inhibited the growth of *E. coli* and shows highly antimicrobial and antioxidant properties. These research results in finding of a successful herbal medicine for the urinary health.

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