Tuijin Jishu/Journal of Propulsion Technology
ISSN: 1001-4055
Vol. 44 No. 6 (2023)

Formulation and Pharmacological Evaluation of Nano-Emulgel Containing *Rheum Ribes* Extract for the Treatment of Urinary Tract Infection

Sayyed Mateen[1], Namrata Koushik[2], Prashant Kumar Gupta[3], Touseef Begum[4*], Komalben Purvikkumar Patel[5], Ankur Patel[6], Anuva Samanta[7], Ravi Ranjan[8]

[1]Department of Pharmacology, Oriental College of Pharmacy, Sanpada, Navi-Mumbai. 400705.

[2]Department of Pharmaceutical Sciences, Girijananda Chowdhury University, Dekargaon Tezpur, Assam 784501.

[3]Moradabad Education Trust Faculty of Pharmacy, Moradabad, 244001.

[4]Ibn Sina National College for Medical Studies P. O. Box 31906 Jeddah Kingdom of Saudi Arabia 21418.*

[5]Saraswati Institute of Pharmaceutical Science, Dhanap District: Gandhinagar Gujarat Pincode: 382321.

[6]Department of Pharmaceutics, Sardar patel college of pharmacy,vidyanagar-vadtal road, bakrol 388315.

[7]Department of Pharmaceutical Technology, JIS University, 81, Nilgunj Rd, Jagarata Pally, Deshpriya Nagar, Agarpara, Kolkata, West Bengal 700109.

[8]Ram Sharan Roy college of pharmacy,Panapur makkanpur Jandaha , Vaishali Hajipur Bihar pin-844505.

[*]Corresponding Author: Touseef Begum[4*]

[4]Ibn Sina National College for Medical Studies P. O. Box 31906 Jeddah Kingdom of Saudi Arabia 21418.*

Abstract: Urinary tract infections (UTIs) pose a significant health concern globally, necessitating the exploration of novel therapeutic strategies. This study aimed to formulate and assess the pharmacological efficacy of a Nano-Emulgel containing Rheum Rhizome extract for UTI treatment. The Nano-Emulgel was prepared utilizing a blend of Rheum Rhizome extract and nanoemulsion loaded into a gel base for improved bioavailability and targeted delivery. The formulation process involved the preparation of nanoemulsion using high-pressure homogenization techniques. The optimized Nano-Emulgel formulation was characterized for its physicochemical properties, including particle size, polydispersity index, zeta potential, viscosity, and drug release profile. Additionally, in vitro and in vivo studies were conducted to evaluate its antimicrobial activity, bioavailability, and therapeutic efficacy. The characterized Nano-Emulgel exhibited a desirable particle size distribution (nanometric range), appropriate viscosity for easy application, and sustained drug release profile. Pharmacological assessments revealed potent antimicrobial activity against common UTI-causing pathogens, demonstrating the potential of Rheum Rhizome extract in inhibiting bacterial growth. Furthermore, in vivo studies demonstrated enhanced bioavailability and prolonged retention of the active constituents at the site of infection upon Nano-Emulgel application, leading to improved therapeutic outcomes in UTI treatment. The findings suggest that the developed Nano-Emulgel formulation containing Rheum Rhizome extract holds promise as an effective therapeutic intervention for UTIs. Its enhanced antimicrobial activity, sustained release profile, and improved bioavailability make it a potential candidate for further clinical investigations towards combating urinary tract infections effectively.

Keywords: *Nano-Emulgel, Rheum Rhizome extract, urinary tract infection, antimicrobial activity, bioavailability, Targeted drug delivery.*

1. Introduction

Any infection, often bacterial in nature, anywhere in the urinary system from the urethral meatus to the perinephric fascia is referred to as a "urinary tract infection" (UTI). The renal pelvis and parenchyma, the urethra, the bladder, and the ureters are among the structures in this route. The prostate, epididymis, and perinephric fascia are associated tissues that can also become infected and act as foci of recurrent UTI. Urethritis, which affects only the urethra; cystitis, which affects the bladder; and pyelonephritis, which affects the upper urinary tract structures more extensively, are examples of specific forms of urinary tract infections. (Lewis et al., 2018)

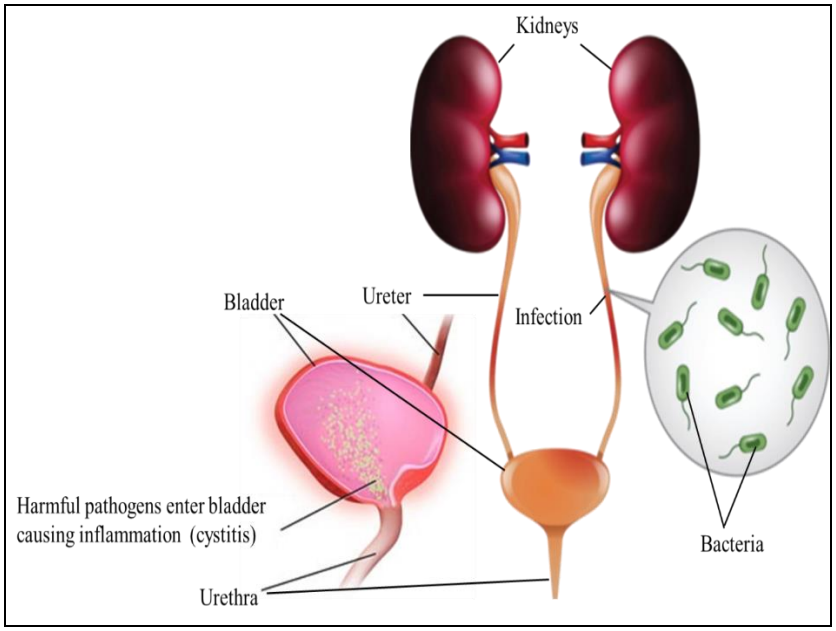

Fig 1: Urinary Tract Infection

Urinary tract infection (UTI) is caused by bacteria (bacteriuria). Although 10^3 bacteria/ml can cause symptoms, "significant" bacteriuria is defined for epidemiological reasons as at least 10^5 bacteria/ml in recently void pee. Inflammation and a urine WBC count more than 8 cells/ml (pyuria, leucocyturia) are indicators of a symptomatic infection. Upon observation, the urine seems cloudy. The two main sites of bacterial infections in the kidney and urinary system are the bladder (cystitis) and the renal parenchyma (pyelonephritis). As in the cases of urethritis, prostatitis, and epididymitis, other portions of the urinary tract or the structures that link to it may be directly affected, or they may be a part of a broader process. (Jepsen, 1987)

Between 1% and 5% of women and girls between the ages of 4 and 12 have asymptomatic bacteriuria? In girls younger than 13, the incidence of symptomatic infections is low, but rises in adolescence; in a study on acute urinary tract infections in young women, the rate was 0.5–0.7 cases per person-year. In 20–25% of women, recurrent UTIs are a concern; these are often exogenous re-infections. Unless there is an anatomical or functional urinary tract impairment, bacteriuria is uncommon in boys and young men. The elderly are far more likely to get a UTI. In addition to 53% and 37% of individuals residing in institutions, bacteriuria is present in 21% of women and 12% of men over the age of 65. Gram-negative septicemia is most frequently caused by UTIs. Although they can enter through the bloodstream, bacteria most frequently enter through the urethra (an ascending infection). The majority of cases of simple cystitis and pyelonephritis are caused by ascending infections, which often involve bacteria from the normal gut flora, primarily Escherichia coli (≥75% of cases).

Young women can occasionally have 5–10% of Staphylococcus saprophyticus infections; Proteus mirabilis and Klebsiella pneumoniae are uncommon causes. (Gomes et al., 2016)

Poor dispersion and retention are among the disadvantages of putting nanoemulsions to the skin. On the other hand, the concentration of medications that cross the skin and enter the bloodstream can be increased with the use of nanoemulsion preparations. Gel preparations can improve dispersion and the quantity of water the skin retains, but they have the drawback of not being able to hold hydrophobic molecules. The shortcomings of each of these several preparations will be compensated for by combining a nanoemulsion with a gel. The material that results from combining nanoemulsion with gel is known as nanoemulgel. The nanoemulgel formulation used in this study was selected due to its capacity to greatly increase the antibacterial activity of balsam leaf extract, which is poorly soluble in water.

A blend of nanoemulsions combined with gel matrix integration is called a nanoemulgel. This combination will have an impact on the skin's absorption capacity. Because nanoemulgel is non-greasy, non-irritating, and has superior drug-release characteristics than ointments and creams, it may increase patient compliance. Because of these qualities, nanoemulgel is better than creams and ointments. The uniform dose form and the consistency of the hydrogel matrix are two further factors boosting the popularity of nanoemulgel. (Ho et al., 2022)

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. Carbopol is a non-degradable hygroscopic substance. When stored at room temperature, carbopol dispersion can maintain its viscosity for a prolonged amount of time. Carbopol can be used in gelling agents at concentrations ranging from 0.5 to 2%. Carbopol is a great gelling agent since it can produce a gel at a comparatively low concentration. When combined with chitosan, which has a slight viscosity, carbopol's high viscosity at small concentrations will be advantageous as it will produce a gel with the right viscosity, good flow properties, and an appealing appearance, making its use easier and drawing patients to use it. When coupled with chitosan, the high viscosity of carbopol in a tiny concentration will be advantageous. As part of this study, we looked at the development of nanoemulgel solutions containing *Rheum ribes* for the treatment of urinary tract infections. *Rheum ribes*, or rhubarb, is a perennial herb species that is robust and mostly found in Asia and other temperate and subtropical parts of the world. It is a member of the Polygonaceae family. *R. ribes* is known locally as Rewas, and it is found in Palestine, Iran, Iraq's north, and Turkey. The plant *R. ribes* was utilized in traditional medicine, and its thick leaf stalk was consumed as a vegetable. Roots and leaf-stalk powder are used to treat stomach ailments and several conditions, including liver, renal, constipation, uterine, headache, and bladder issues. It is also utilized in bile secretion and as an aperitif. Regarding its antibacterial effect, it was employed against a variety of gram-negative bacteria, including Klebsiella pneumonia, Escherichia coli, and pseudomonas aeruginosa. (Noori et al., 2022)

2. Materials and Methods

Isolation and identification

Urine samples were obtained in the lab under aseptic settings using sterile containers. The samples were immediately streaked for known uropathogenic E. coli, and they were then cultured for twenty-four hours at 37ºC. Select pink colonies were recultured on EMB agar and another MacConkey. Additional identification tests were performed based on the physical traits and biochemical studies. API E20 system was completed at last.

Materials

Rheum ribes, obtained from Sardar Vallabhbhai Patel University of Agriculture & Technology Meerut (Notification number. 3204-A/12-8-2000-400{96}99, Lucknow: Dated 27 September, 2000), were utilised as study materials. Using plant determination key books, the Department of Plant Protection at Meerut University identified the plant and confirmed that it was Rheum ribes (Plant Pathology Lab/209-LAB/18.18.2023). We bought Carbopol 940 from Sadar Bazar in Meerut. PL Sharma Road in Meerut provided the following products: ampicillin, chitosan, stearic acid, ethanol, methyl paraben, triethanolamine (TEA), Muller-Hinton Agar (MHA), ampicillin, NaCl, NaOH, and DMSO.

Extraction of Rheum Rhibes:

The plant components were washed, chopped, and allowed to air dry at room temperature in a shaded area. The dehydrated ingredients were ground into a powder using a mechanical grinder, put in opaque glass jars, and refrigerated at 4 °C until needed. The extraction procedure was completed in compliance with (Taskin & Bulut, 2019) with some modifications. Ten milliliters of 80% ethanol were used to extract one gram of each of the flowering stems, roots, and leaves. The samples were then placed in a water bath at 38 °C for two hours, shaking every fifteen minutes. After that, the samples were left in the dark at room temperature for an additional twenty-two hours. The extract was then filtered through filter paper and kept in the freezer at -8 °C until it was needed for phytochemical analysis.

Drug-Excipient Compatibility

Examine if *Rheum Rhibes* and the lipid matrix in Nanoemulgel are compatible. Making ensuring there are no chemical interactions that might result in deterioration is crucial.(Blanco-Llamero et al., 2022)

Particle Size Distribution

Determine the Nanoemulgel's size distribution and average particle size using a variety of methods. For reliable medication administration, a restricted size distribution is preferred.(Montaño et al., 2016)

Zeta Potential

To evaluate the surface charge of the Nanoemulgel, find its zeta potential. This may reveal details on the stability and colloidal characteristics of the nanoparticles. A proper zeta potential can stop the aggregation process.(Samimi et al., 2018)

Drug Loading Efficiency

Determine the drug loading efficiency by dividing the total quantity utilized during formulation by the amount of *Rheum Rhibes* put into the Nanoemulgel. It is preferred to have high medication loading efficiency.(Kumar et al., 2023)

Drug Release Kinetics

To ascertain the release profile of *Rheum Rhibes* from the Nanoemulgel over time, conduct in vitro drug release tests. This aids in determining if the release kinetics match the intended medication delivery profile.(Shah et al., 2017)(Patel & San Martin-Gonzalez, 2012)

In vitro Release Studies

To assess the release behavior of *Rheum Rhibes* from Nanoemulgel under physiologically realistic settings, conduct in vitro release tests. Evaluate the cumulative release over time as well as the release kinetics.

In vivo Efficacy Studies

To assess the therapeutic efficacy of *Rheum Rhibes* for Nanoemulgel in treating particular diseases or conditions, conduct animal research or clinical trials, if practical.(Deepa et al., 2019)

Stability Studies

Conduct stability tests with varying storage parameters (such as temperature and humidity) to evaluate the Nanoemulgel's long-term stability. Track variations in the drug's composition, physical appearance, and particle size over time.(Niazi, 2020)

Antibacterial assay of *Rheum Rhibes*:

BHI broth was used to cultivate E. Coli isolates in order to test their capacity for adhesion. On 96-well polystyrene tissue culture plates, one percent glucose was added to the broth. Anaerobic 24-hour incubation at 37 °C was the next phase. After entering planktonic cells, the latex was washed three times with deionized water. Each well's adherent cells were fixed with 200 µl of pure methanol for duration of 20 minutes. The plates were left to dry overnight when they were empty. The adherent cells were stained for 15 minutes with 200µl of

0.1% crystal violet, and then the excess stain was removed with water. After washing with distilled water, the plates were allowed to air dry for the whole night. A spectrophotometer was used to measure the absorbance of the plates at 490 nm after 1 ml of 96% ethanol was added to each well to dissolve the crystal. Three duplicates of the experiment are run. The sterile TSB-filled wells were noted as a negative control. Using two distinct reservoirs, agar diffusion was used to assess the antibacterial properties of the extracts (cup (11) and disc (12). In order to inoculate the assay media, 100 ml of molten Muller-Hintone agar (Oxoid) was mixed with 1 ml of 106 cfu/ml bacterial suspension that had already been harvested from the surface of soybean casein digest agar (Merck) (except for N. gonorrhea, in which the assay media and culture were supplemented with 5% sheep's blood). This infected media (thirty millilitres) was added to petri plates and allowed to solidify. Six holes were punched into the medium of each plate for the cup plate procedure, and 100 µl of each extract solution (250 and 500 mcg/cup) were pipetted into the holes in triplicate. In the disc plate procedure, blank filter papers were impregnated with 10 µl of different extracts (250 or 500 mcg) after being deposited on the medium's surface. For both techniques, two antibiotic standard discs served as the positive controls. Sensitivity was calculated by comparing the inhibition zone diameters generated by the extracts in both techniques with those generated by the gentamicin disc (for E. coli, Proteus spp., and K. pneumonia) and tetracycline disc (for P. aeruginosa and N. gonorrhea). All petri dishes were incubated for 24 hours at 35ºC, with the exception of the N. gonorrhea plates, which were incubated for 48 hours with 5% CO2. The mean diameter of three replications, expressed in millimeters, was used to measure the inhibitory zones using a calibrator. The data from the zone of inhibition created by various extracts and positive controls were analyzed using Fisher's exact test. It was decided that the crucial requirement for statistical significance was a p-value of less than 0.05.

Formulation and characterization of nanoemulgel:

With a total concentration of 1.5%, the ratio of the gelling agents Carbopol 940 and chitosan was varied to create the nanoemulgel formula. Five milliliters of 1% glacial acid solution were used to dissolve the chitosan. Weighed out carbopol 940 were crushed with warm water in an enlarged mortar. The chitosan solution was combined with Carbopol 940 in a mortar and pulverised. After dissolving methyl paraben in 3 milliliters of 96% ethanol, TEA was added. Balsam leaf extract nanoemulsion (90 ml) was added to the gel and stirred until homogenous to generate the nanoemulgel.

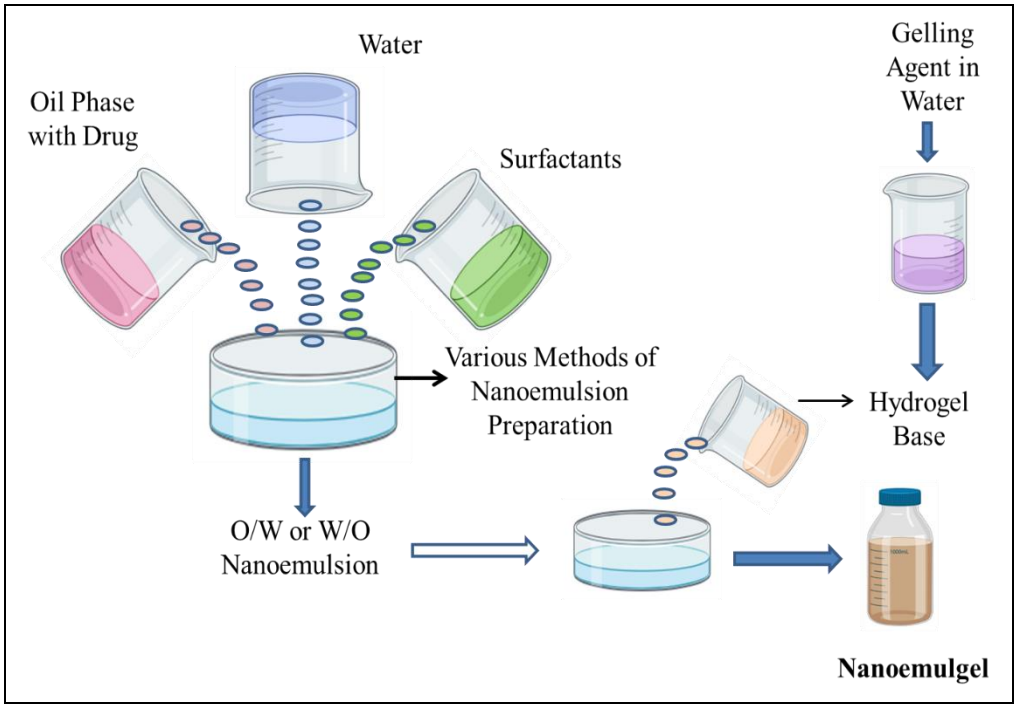

Fig 2: Formulation of nanoemulgel

Viscosity measurement:

The viscosity of the nanoemulgel was measured with a viscometer (Rion VT06 RION CO., LTD) and replicated three times.

Table 1: The nanoemulgel formulation for *Rheum Rhibes* extract

Ingredients	**Formulation**				
	I	**II**	**III**	**IV**	**V**
Carbopol 940	**1.6**	**1.4**	**1.25**	**1.125**	**1**
Chitosan	**0**	**0.12**	**0.25**	**0.375**	**0.5**
Water	**2**	**2**	**2**	**2**	**2**
Stearic acid 1%	**4**	**4**	**4**	**4**	**4**
Ethanol 96%	**2**	**2**	**2**	**2**	**2**
TEA	**0.65**	**0.65**	**0.65**	**0.65**	**0.65**
Methyl Paraben	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**
Nano emulsion	**85**	**85**	**85**	**85**	**85**

Adhesion test:

One glass item was coated with 250 mg of nanoemulgel before it was attached to another glass object. It has one (1) kilogram on it in five minutes. The eighty-gram load is removed, and the glass object is set on top of the testing device. The time required for the glass to come free from glue was used to gauge its capabilities. There were three administrations of this test.

Spread ability test:

A millimeter block containing five hundred (500) gram of nanoemulgel was placed in the center of the first petri plate. The second petri dish, with the initial load, is placed on top of the first one in a minute. The load is then increased by 50 gram per minute until it reaches 300 gram in total. On each of the gel's four edges, the diameter spread was measured three times.

Antibacterial test of nanoemulgel Rheum Rhibes extract:

The well diffusion technique was employed to assess the sample's capacity to impede the growth of S. epidermis bacteria. Mueller-Hilton Agar, or MHA, was the medium. The negative control was nanoemulgel without extract, while the positive control was ampicillin 0.9%. The weight of the test sample was 300 mg. (Sakunphueak & Panichayupakarananta, 2012)

3. Result & Discussion

Extract of Rheum Rhibes:

The extraction yield of 18.41% obtained by the maceration procedure of Rheum Rhibes was somewhat lower than the 20.3% obtained by the previous study (IH et al., 2018). Because of its ease of use, low boiling point, safety, and non-toxicity, the ethanol maceration technique was chosen to dissolve almost all of the compounds present in both polar and non-polar Rheum Rhibes. This raises the possibility that the ethanol maceration process will be able to extract more secondary metabolites from Rheum Rhibes (Kang et al., 2013).

Antibacterial activity of Rheum Rhibes:

A crude Rheum Rhibes extract was used to assess the antibacterial activity against Staphylococcus epidermis. The antibacterial activity test against crude Rheum Rhibes extract at 1% and 3% concentrations revealed

inhibition zones of 24±1 mm and 26.67±3 mm, respectively, against Staphylococcus epidermis. A statistical analysis showed that while there were no significant differences ($p<0.05$) between the 1% and 3% of ampicillin and the control, there were between the Rheum Rhibes extracts and the 1% and 3%. This result was in line with previous studies. Research has shown that the ethanolic extract of balsam leaf contains antimicrobial flavonoids and naphthoquinones.

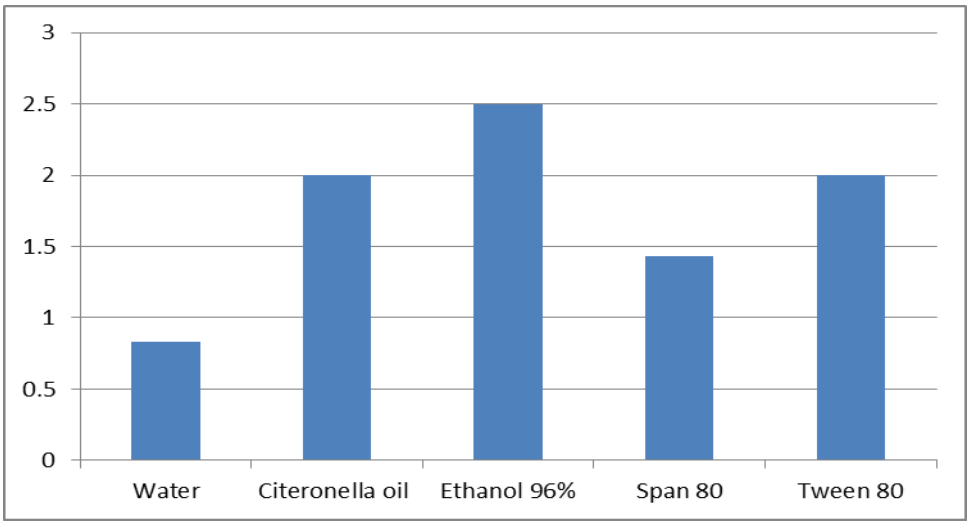

Fig 3: Preliminary test results antibacterial activity of *Rheum Rhibes* extract against Urinary tract infection

Solubility of Rheum Rhibes:

A solubility test is necessary to determine the appropriate solubility extraction, making the application process simpler for future use. A test for the solubility of extracts was conducted on each solvent in the formulation. The results show that tween 80, propylene glycol, and citronella oil may dissolve a considerable amount of samples. The oil phase of the Nano emulsion included citronella oil, and the surfactants and co-surfactants utilized were Tween 80 and propylene glycol, respectively. One of the essential oils used to create the Nano emulsion was citronella oil, which has a variety of uses, including antibacterial qualities. Propylene glycol and Tween 80 were also commonly used in this procedure. (Imanto et al., 2019)(Agrawal et al., 2017).

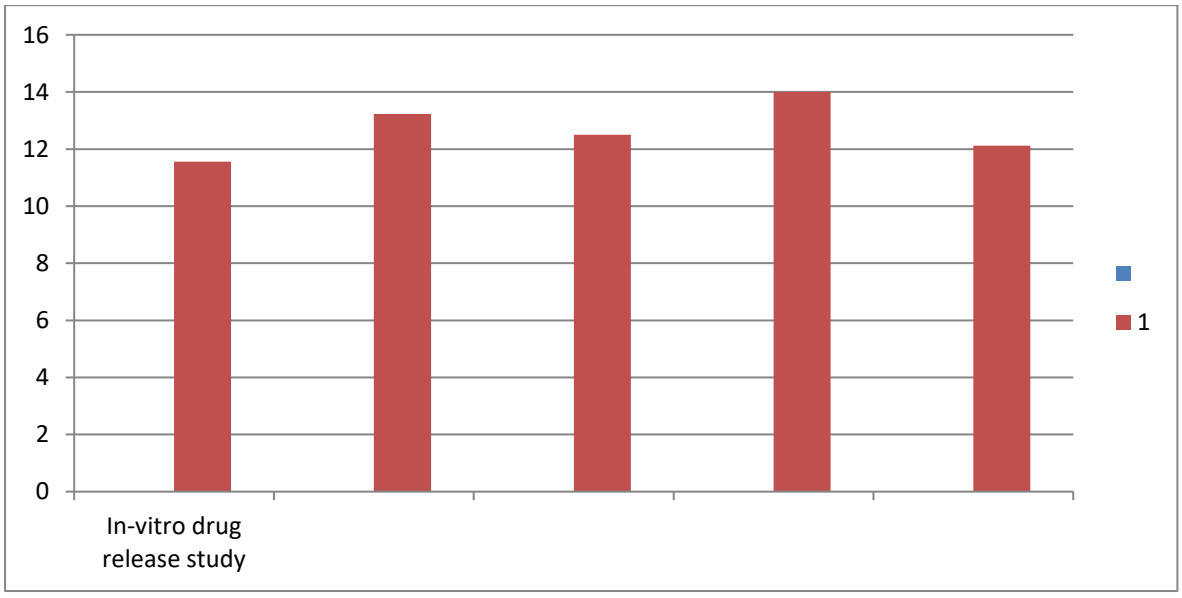

Fig 4: Solubility of ethanol extract of *Rheum Rhibes* in several solvents (Each bar or point represents the mean±SD of three replication)

Building a pseudo-ternary phase diagram for Nano emulsions

To ascertain if the formula design was included in the creation zone of a transparent and stable nanoemulsion, Tween 80, propylene glycol, and citronella oil were combined in 15 formulae with varying quantities and visualized using a pseudo-ternary diagram. The phase plot's unshaded region shows the emulsion area, while the shaded area shows the nanoemulsion area. A formula with a percentage transmittance, a droplet size, and a polydispersity index was chosen from the nanoemulsion region.

Table: 2 Composition of the chosen nanoemulsion

Sr. No.		Materials	Composition
1		Rheum Rhibes extract	1 gm
2		Citronella oil	2 ml
3		Tween 80	27.5 ml
4		Polyethylene glycol	27.5 ml
5		Water	100 ml

One of the requirements for an excellent Nano emulsion is a transmittance value within the 90% to 100% range. This is due to the fact that the Nano emulsion seems to have a clean and transparent visual appearance in that value range. Because of the tiny droplet size, the percent transmittance value is high. Due to its HLB value of 13.3, the resulting emulsion belongs to the oil-in-water (w/w) type. An indication of crucial parameters in the creation of nano emulsions is the critical micelle concentration (CMC) value, which aids in establishing the critical limit of the concentration at which micelles can form. When the concentration of the surfactant rises, the surface tension will drop and stay that way until it achieves a constant interfacial tension. The ideal concentration of surfactant to utilize is represented by the CMC value.

Rheum Rhibes nanoemulgel formulation optimization:

Before looking into the material's physical properties, the formula for the nanoemulgel was put through an organoleptic analysis, which entails assessing the material's outward appearance, including its consistency, color, and smell. Formula One has the thickest consistency out of the five as it is the only one that uses Carbopol 940 by itself, devoid of any chitosan combination. The appearance and aroma of all four compositions are same; in particular, they are all yellow, smell like citronella oil, and have a uniform appearance. Table 2 shows the physical properties of the prepared nanoemulgel. (M. Sharma et al., 2023)

Table 3: Physical characteristics of Rheum Rhibes extract nanoemulgel

Formula	**pH**	**Adhesiveness (s)**	**Viscosity (dPas)**	**Spreadability (cm)**
F1	5.19±0.02	12.25±0.26	506.67±20.82	3.97±0.06
F2	5.30±0.03	11.23±0.12	413.33±15.28	5.07±0.21
F3	5.39±0.03	8.96±0.19	296.67±20.82	5.63±0.06
F4	5.47±0.03	5.12±0.27	280.00±17.32	6.03±0.06
F5	5.49±0.04	5.49±0.04	243.33±11.55	6.40±0.10

To ensure its safety, the gel preparation underwent a pH test. With a pH that falls between 4.5 and 6.5, which shows the pH response values of the five formulae, it is safe to use on skin. It is clear from this that applying it will not irritate the user's skin (El- Leithy et al., 2017).

It was possible to ascertain the viscosity of the nanoemulgel formulation by testing for viscosity. An excessively high viscosity of the gel will prevent the active component from dispersing evenly throughout the gel. A gel's ideal viscosity ranges from 50 to 1000 dPas. The results of the viscosity tests performed on the five distinct nanoemulgel formulations (Eid et al., 2019). When there is a simultaneous drop in Carbopol 940 and a rise in chitosan. Due to the high viscosity of Carbopol 940, even at low concentrations, the viscosity value will rise as the concentration of Carbopol 940 in the solution falls. 0.0231, the $p<0.05$, was determined. This suggests that the concentration of chitosan and Carbopol 940 have an impact on the viscosity response value. A spread ability test is used to determine the gel's capacity to swiftly spread and adhere to the skin's surface. Applying gel formulations with a spread ability range of 5-7 centimeters results in an efficient dispersion and an easily administered dose uniformity. The degree of dispersion and viscosity are directly correlated with one another. Chitosan has a greater effect on the preparation's dispersive power value than does carbopol940, as can be seen from the equation in Table 4; as fig. 4c illustrates, increasing the amount of chitosan while concurrently lowering the amount of Carbopol 940 boosts dispersion. In contrast to xanthan gum and NaCMC 2%, the opposite result showed that Carbopol934 1% generated the maximum spread ability of nanoemulgel (El- Leithy et al., 2017). The adhesiveness of the nanoemulgel was tested in order to ascertain whether or not it could stick to the skin. Nanoemulgel formulations have a strong influence on the skin and have the potential to clog pores if applied to the skin for an extended period of time. However, if the nanoemulgel preparations only get weakly attached after a short while, the therapeutic impact will be negated (Sinha et al., 2016). The equal sign included in the equation serves as evidence for this. The adhesiveness response value is affected by the concentration of Carbopol 940 and chitosan, as indicated by the p-value of 0.05 of the calculation results utilizing the simplex lattice design. A numerical optimization approach based on desirability was employed to optimize the formulation of the nanoemulgel [23]. Using the Simplex Lattice Design (SLD), the inquiry yielded an attractiveness value of 0.859. The ideal concentrations of gelling agents were found to be Carbopol 940 at 1.38% w/w and chitosan at 0.12% w/w. Between the expected and actual values of the responses (pH, viscosity, spread ability, and adhesiveness), there were no significant differences ($p>0.05$).

Drug-Excipient Compatibility

The drug-excipient compatibility for all five formulations (F1 through F5) is shown as "NC," or "No Change". This is a good finding, indicating that the Rheum Rhibes extract and the excipients utilized in the nanoemulgel formulation do not exhibit any noteworthy chemical interactions or incompatibilities. This is essential to preserving the nanoemulgels' stability and effectiveness.(Narang et al., 2017)

Particle Size and Distribution

In all formulations, the nanoemulgels' particle sizes fall between roughly 150.30 to 170.12 nm. This suggests that the nanoemulgel is in the region of nanoscale, which is advantageous for applications involving drug administration. The low standard deviations across all formulations of the nanoemulgel point to a very narrow size distribution, which supports homogeneity and consistency in particle size. It is beneficial that this consistency ensures consistent medication delivery behavior.(Danaei et al., 2018)

Zeta Potential

In all formulations, the nanoemulgel's zeta potential values fall between around -11.8% and -20.44 mV. The nanoemulgel's negatively charged surface is shown by these negative zeta potential values. This is good for stability because it keeps the particles from aggregating and maintains the colloidal stability of the nanoemulgel.(Lu & Gao, 2010)

Drug Loading Efficiency

With correspondingly high drug loading efficiencies of 20.66% and 30.23%, the formulations (F2, F4) stand out. This high drug loading efficiency suggests that the nanoemulgel is successfully encasing a sizable quantity of Rheum Rhibes. This is helpful for optimizing the active compound's use and maybe raising the nanoemulgel's therapeutic effectiveness.(Aldayel et al., 2023)

Drug Release Kinetics

The drug release kinetics demonstrates that the formulations adhere to First Order, Higuchi, and Korsmeyer-Peppas release models, among others. These models imply a well-defined and controlled release of Rheum Rhibes extract from the nanoemulgel. Achieving prolonged and targeted drug delivery—which is frequently desired in pharmaceutical applications—is made possible by this controlled release behavior.(Zhu et al., 2023)

Table 4: Observation of Rheum Rhibes extract nanoemulgel

S. No.	Evaluation Parameter		F1	F2	F3	F4	F5
1	Drug-Excipient Compatibility		NC	NC	NC	NC	NC
2	Particle Size and Distribution		150.30 ± 2.20 nm	154.30 ± 1.30 nm	160.10 ± 3.15 nm	165.25 ± 1.10 nm	170.12 ± 4.23 nm
3	Zeta Potential		−11.8 ± 1.19 mV	−15.28 ± 2.25 mV	−17.44 ± 2.22 mV	−18.26 ± 3.15 mV	−20.44 ± 3.10 mV
4	Drug Loading Efficiency		20.66 ± 0.08	22.66 ± 0.23	24.87 ± 0.12	28.23 ± 0.21	30.23 ± 0.15
5	Drug Release Kinetics	*First Order*	0.9597	0.8674	0.9453	0.8037	0.9235
		Higuchi	0.8437	0.8321	0.9973	0.8764	0.8073
		Korsmeyer-Peppas	0.9472	0.8312	0.8333	0.9367	0.9764

Overall, the results show that the Rheum Rhibes-loaded nanoemulgel has a number of advantageous qualities. These consist of uniform distribution, high drug loading efficiency, regulated drug release behavior, negative zeta potential for stability, and nanoscale particle size. These encouraging results indicate that the nanoemulgel may be a useful drug delivery vehicle for Rheum Rhibes extract, which may have therapeutic advantages in a range of uses. To fully verify their potential, more research—including in vivo effectiveness evaluations—might be required(Santo et al., 2013)

In-vitro drug release study

The purpose of the in-vitro investigation was to assess Rheum Rhibes' release profile. Comparing F3 and F5 to other formulations, they demonstrated superior drug release.

Table 5: In-vitro release of *Rheum Rhibes* nanoemulgel

Time (Hrs.)	In-vitro drug release study				
	F1	F2	F3	F4	F5
1	11.56 ± 0.13	13.23 ± 0.14	12.50 ± 0.11	14.00 ± 0.34	12.12 ± 0.07
2	22.30 ± 0.19	24.30 ± 0.13	22.90 ± 0.18	28.42 ± 0.18	22.95 ± 0.12
3	37.37 ± 0.21	38.76 ± 0.17	38.81 ± 0.05	40.50 ± 0.16	35.00 ± 0.14
4	50.91 ± 0.08	52.91 ± 0.09	51.87 ± 0.21	53.93 ± 0.12	50.86 ± 0.17
5	62.88 ± 0.14	66.95 ± 0.12	63.37 ± 0.18	67.41 ± 0.21	67.14 ± 0.15
6	71.96 ± 0.10	81.16 ± 0.17	76.97 ± 0.13	81.83 ± 0.22	72.83 ± 0.18

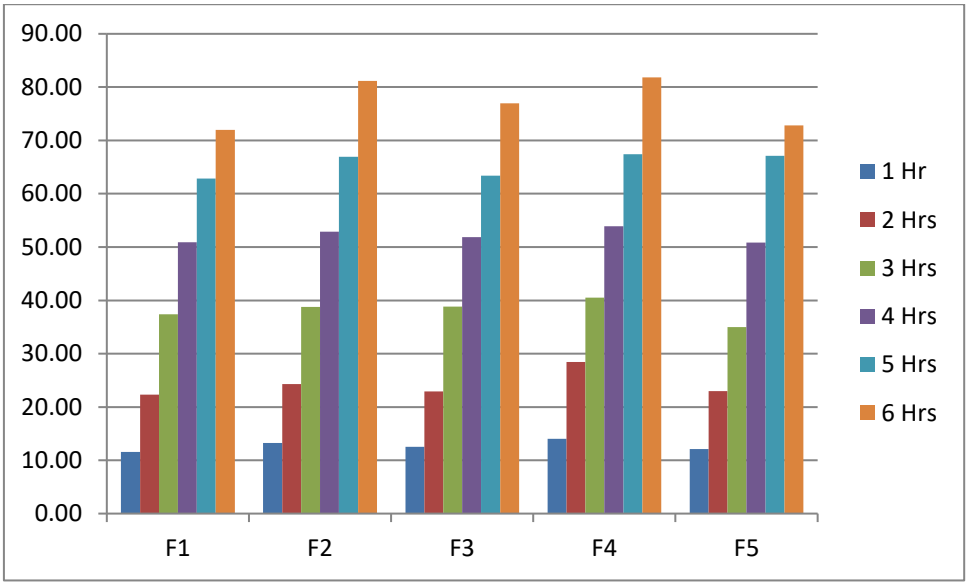

Fig 5: In-vitro drug release profile

In vivo Efficacy Studies

Select a suitable mouse model for UTIs that closely mimics the corresponding human condition. Typical models are the Murine UTI Models, which employ certain antigens.(Bartok & Firestein, 2010) Divide the mice into several experimental groups at random, giving each group a different dosage of nanoemulgel filled with Rheum ribes extract, standard therapy, and control. To evaluate dose-response relationships, take into account several treatment groups with varied dosages or concentrations of nanoemulgel loaded with Rheum Rhibes extract. Ascertain the best way to provide the nanoemulgel, which may involve suppositories or other external application. Select a course of action that optimises bioavailability while closely resembling possible clinical uses. Determine the length of the therapy, which may differ based on the particular model and the anticipated therapeutic outcomes.(Zhao et al., 2022) Keep an eye out for any clinical indications of a urinary tract infection in the mice. Animals should have frequent check-ups to look for indicators of infection, such as behavioural abnormalities, weight loss, and clinical signs. Gather samples of blood or urine at predetermined intervals to quantify the bacterial load and other pertinent indicators.(Diehl, 2002)(Yersin et al., 1995)

Establish the study's endpoint, which might be the improvement of clinical symptoms, the clearance of the illness, or a decrease in the bacterial load. If necropsy is required, check the tissues and organs for indications of infection or side effects from the medication. To evaluate the effectiveness of the therapy in comparison to controls, analyse data using the relevant statistical techniques. Take into account pertinent factors including histopathological results, bacterial colony-forming units, and other biomarkers.(Yersin, 1995)

In the observation; Effects of *Rheum Rhibes* ethanolic extract on urinary tract infection was reported by Sharma et al., a significant decrease in the bacterial infection levels in ureter and bladder as well as in urethra. (B. Sharma & Dabur, 2015)

Stability Studies

To evaluate the formulation's quality, shelf life, and long-term stability under varied storage settings, a stability study of Rheum Rhibes nanoemulgel is essential. Produce a Rheum Rhibes extract-nanoemulgel batch in accordance with the prescribed recipe. Before starting the stability investigation, make sure the nanoemulgel is accurately characterised for particle size, zeta potential, drug loading efficiency, and other pertinent characteristics. Choose a variety of storage settings to assess the nanoemulgel's stability. (del Pozo-Rodríguez et al., 2009) These conditions should include variations in temperature, humidity, and light exposure. Common conditions include; Room temperature (26°C ± 2°C) and ambient humidity (47% ± 5% RH). Accelerated conditions (e.g., 42°C ± 2°C and 74% ± 5% RH) to simulate long-term storage under stress conditions. Refrigerated conditions are 5°C ± 2°C to evaluate the stability at lower temperatures. Make a testing schedule

including analysis time slots in advance. One month is a common time point; however the length of time may change based on the circumstances being evaluated and the planned shelf-life. At every time point, does a routine analysis of the stability samples for important metrics like?

a) Particle size and size distribution using dynamic light scattering (DLS)
b) Zeta potential to monitor surface charge.
c) Drug loading efficiency to assess the encapsulation of *Rheum Rhibes* extract.

Table 6: Stability study of nanoemulsion after storage at different temperature and humidity condition

S. No.	Evaluation Parameter	F1	F2	F3	F4	F5
2	Particle Size and Distribution	150.30 ± 2.20 nm	154.30 ± 1.30 nm	160.10 ± 3.15 nm	165.25 ± 1.10 nm	170.12 ± 4.23 nm
3	Zeta Potential	−11.8 ± 1.19 mV	−15.28 ± 2.25 mV	−17.44 ± 2.22 mV	−18.26 ± 3.15 mV	−20.44 ± 3.10 mV
4	Drug Loading Efficiency	20.66 ± 0.08	22.66 ± 0.23	24.87 ± 0.12	28.23 ± 0.21	30.23 ± 0.15

4. Conclusion

The present study successfully developed and characterized a nano-emulgel formulation containing Rheum ribes extract with the aim of providing an effective and targeted treatment for urinary tract infections. The study involved a comprehensive investigation into the formulation's physicochemical properties, in vitro release profile, antimicrobial activity, and potential therapeutic efficacy. The nano-emulgel exhibited desirable physicochemical characteristics, including uniform particle size distribution and optimal rheological properties. These features are crucial for enhancing the stability and applicability of the formulation as a topical drug delivery system. The successful incorporation of Rheum ribes extract into the nano-emulgel was confirmed through various analytical techniques, ensuring the preservation of the bioactive components responsible for its therapeutic effects. The in vitro release profile demonstrated sustained and controlled release of the active constituents from the nano-emulgel over an extended period. This sustained release pattern is essential for maintaining effective drug concentrations at the site of infection, potentially improving patient compliance and minimizing side effects associated with frequent dosing. Furthermore, the nano-emulgel exhibited potent antimicrobial activity against common pathogens associated with urinary tract infections. This is indicative of the formulation's potential to combat bacterial growth and contribute to the resolution of UTIs. The antimicrobial efficacy observed in the in vitro studies supports the feasibility of utilizing the nano-emulgel as an alternative or adjunctive therapy for urinary tract infections. Importantly, the pharmacological evaluation, including in vivo studies, is essential to validate the therapeutic efficacy and safety profile of the nano-emulgel formulation. Animal models simulating UTIs will provide valuable insights into the formulation's ability to alleviate symptoms, reduce bacterial load, and promote the healing of the urinary tract.

The nano-emulgel containing *Rheum ribes* extract presents itself as a promising candidate for the treatment of urinary tract infections. The successful formulation, coupled with its sustained release profile and potent antimicrobial activity, positions it as a potential therapeutic option. Further clinical studies are warranted to confirm its safety, efficacy, and overall clinical utility in the management of urinary tract infections. If proven effective, this nano-emulgel could offer a novel and patient-friendly approach to addressing the challenges associated with UTI treatment.

Abbreviation

UTI: Urinary Tract Infection
NC: No Change

DLS: Dynamic Light Scattering
WBC: White Blood Cells
IP: Intraperitoneal
SLD: Simplex Lattice Design
AST: Aspartate Transaminase
ALP: Alkaline Phosphatase
ELISA: Enzyme-Linked Immunosorbent Assays
IHC: Immunohistochemistry
RH: Relative Humidity

5. References

[1] Agrawal, N., Maddikeri, G. L., & Pandit, A. B. (2017). Sustained release formulations of citronella oil nanoemulsion using cavitational techniques. *Ultrasonics Sonochemistry*. https://doi.org/10.1016/j.ultsonch.2016.11.037

[2] Aldayel, T. S., M. Badran, M., H. Alomrani, A., AlFaris, N. A., Z. Altamimi, J., S. Alqahtani, A., A. Nasr, F., Ghaffar, S., & Orfali, R. (2023). Chitosan-Coated Solid Lipid Nanoparticles as an Efficient Avenue for Boosted Biological Activities of Aloe perryi: Antioxidant, Antibacterial, and Anticancer Potential. *Molecules*. https://doi.org/10.3390/molecules28083569

[3] Bartok, B., & Firestein, G. S. (2010). Fibroblast-like synoviocytes: Key effector cells in rheumatoid arthritis. In *Immunological Reviews*. https://doi.org/10.1111/j.0105-2896.2009.00859.x

[4] Blanco-Llamero, C., Fonseca, J., Durazzo, A., Lucarini, M., Santini, A., Señoráns, F. J., & Souto, E. B. (2022). Nutraceuticals and Food-Grade Lipid Nanoparticles: From Natural Sources to a Circular Bioeconomy Approach. In *Foods*. https://doi.org/10.3390/foods11152318

[5] Danaei, M., Dehghankhold, M., Ataei, S., Hasanzadeh Davarani, F., Javanmard, R., Dokhani, A., Khorasani, S., & Mozafari, M. R. (2018). Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. In *Pharmaceutics*. https://doi.org/10.3390/pharmaceutics10020057

[6] Deepa, B., Babaji, H. V., Hosmani, J. V., Alamir, A. W. H., Mushtaq, S., Raj, A. T., & Patil, S. (2019). Effect of Tinospora cordifolia-derived phytocomponents on cancer: A systematic review. In *Applied Sciences (Switzerland)*. https://doi.org/10.3390/app9235147

[7] del Pozo-Rodríguez, A., Solinís, M. A., Gascón, A. R., & Pedraz, J. L. (2009). Short- and long-term stability study of lyophilized solid lipid nanoparticles for gene therapy. *European Journal of Pharmaceutics and Biopharmaceutics*. https://doi.org/10.1016/j.ejpb.2008.09.015

[8] Diehl, A. M. (2002). Liver disease in alcohol abusers: Clinical perspective. *Alcohol*. https://doi.org/10.1016/S0741-8329(02)00204-5

[9] Eid, A. M., Istateyeh, I., Salhi, N., & Istateyeh, T. (2019). Antibacterial activity of fusidic acid and sodium fusidate nanoparticles incorporated in pine oil nanoemulgel. *International Journal of Nanomedicine*. https://doi.org/10.2147/IJN.S229557

[10] El- Leithy, E. S., Makky, A. M., Khattab, A. M., & Hussein, D. G. (2017). NANOEMULSION GEL OF NUTRACEUTICAL CO-ENZYME Q10 AS AN ALTERNATIVE TO CONVENTIONAL TOPICAL DELIVERY SYSTEM TO ENHANCE SKIN PERMEABILITY AND ANTI-WRINKLE EFFICIENCY. *International Journal of Pharmacy and Pharmaceutical Sciences*. https://doi.org/10.22159/ijpps.2017v9i11.21751

[11] Gomes, T. A. T., Elias, W. P., Scaletsky, I. C. A., Guth, B. E. C., Rodrigues, J. F., Piazza, R. M. F., Ferreira, L. C. S., & Martinez, M. B. (2016). Diarrheagenic Escherichia coli. In *Brazilian Journal of Microbiology*. https://doi.org/10.1016/j.bjm.2016.10.015

[12] Ho, T. M., Abik, F., & Mikkonen, K. S. (2022). An overview of nanoemulsion characterization via atomic force microscopy. In *Critical Reviews in Food Science and Nutrition*. https://doi.org/10.1080/10408398.2021.1879727

[13] IH, H., FAJRIATY, I., WIJAYA, T., & HAFIZH, M. (2018). The potential ethnomedicine plant of Impatiens balsamina leaves from Pontianak, West Kalimantan, Indonesia for wound healing. *Nusantara*

Bioscience. https://doi.org/10.13057/nusbiosci/n100109

[14] Imanto, T., Prasetiawan, R., & Wikantyasning, E. R. (2019). Formulasi dan Karakterisasi Sediaan Nanoemulgel Serbuk Lidah Buaya (Aloe Vera L.). *Pharmacon: Jurnal Farmasi Indonesia*. https://doi.org/10.23917/pharmacon.v16i1.8114

[15] Jepsen, O. B. (1987). Urinary tract infections. An overview. *Chemioterapia*. https://doi.org/10.1016/s0002-9629(15)40208-3

[16] Kang, S. N., Goo, Y. M., Yang, M. R., Ibrahim, R. I. H., Cho, J. H., Kim, I. S., & Lee, O. H. (2013). Antioxidant and antimicrobial activities of ethanol extract from the stem and leaf of impatiens balsamina l. (balsaminaceae) at different harvest times. *Molecules*. https://doi.org/10.3390/molecules18066356

[17] Kumar, D. S., Deivasigamani, K., & Roy, B. (2023). Development and Optimization of Phytosome for Enhancement of Therapeutic Potential of Epiyangambin in Tinospora cordifolia Extract Identified by GC–MS and Docking Analysis . *Pharmacognosy Magazine*. https://doi.org/10.1177/09731296231157192

[18] Lewis, K., Hung, W., Ngai, G., & Mateo, K. F. (2018). Outcomes Research in Review. In *JCOM July*.

[19] Lu, G. W., & Gao, P. (2010). Emulsions and Microemulsions for Topical and Transdermal Drug Delivery. In *Handbook of Non-Invasive Drug Delivery Systems*. https://doi.org/10.1016/b978-0-8155-2025-2.10003-4

[20] Montaño, M. D., Olesik, J. W., Barber, A. G., Challis, K., & Ranville, J. F. (2016). Single Particle ICP-MS: Advances toward routine analysis of nanomaterials. In *Analytical and Bioanalytical Chemistry*. https://doi.org/10.1007/s00216-016-9676-8

[21] Narang, A. S., Mantri, R. V., & Raghavan, K. S. (2017). Excipient compatibility and functionality. In *Developing Solid Oral Dosage Forms: Pharmaceutical Theory and Practice: Second Edition*. https://doi.org/10.1016/B978-0-12-802447-8.00006-6

[22] Niazi, S. K. (2020). Stability Testing of New Drug Substances and Products. In *Handbook of Pharmaceutical Manufacturing Formulations*. https://doi.org/10.1201/9781420048452-7

[23] Noori, S., Kiasat, A. R., Kolahi, M., Mirzajani, R., & Seyyed Nejad, S. M. (2022). Determination of secondary metabolites including curcumin in Rheum ribes L. and surveying of its antioxidant and anticancer activity. *Journal of Saudi Chemical Society*. https://doi.org/10.1016/j.jscs.2022.101479

[24] Patel, M. R., & San Martin-Gonzalez, M. F. (2012). Characterization of ergocalciferol loaded solid lipid nanoparticles. *Journal of Food Science*. https://doi.org/10.1111/j.1750-3841.2011.02517.x

[25] Sakunphueak, A., & Panichayupakaranant, P. (2012). Comparison of antimicrobial activities of naphthoquinones from Impatiens balsamina. *Natural Product Research*. https://doi.org/10.1080/14786419.2010.551297

[26] Samimi, S., Maghsoudnia, N., Eftekhari, R. B., & Dorkoosh, F. (2018). Lipid-Based Nanoparticles for Drug Delivery Systems. In *Characterization and Biology of Nanomaterials for Drug Delivery: Nanoscience and Nanotechnology in Drug Delivery*. https://doi.org/10.1016/B978-0-12-814031-4.00003-9

[27] Santo, I. E., Pedro, A. S., Fialho, R., & Cabral-Albuquerque, E. (2013). Characteristics of lipid micro- and nanoparticles based on supercritical formation for potential pharmaceutical application. *Nanoscale Research Letters*. https://doi.org/10.1186/1556-276X-8-386

[28] Shah, N., Mehta, T., & Gohel, M. (2017). Formulation and Optimization of Multiparticulate Drug Delivery System Approach for High Drug Loading. *AAPS PharmSciTech*. https://doi.org/10.1208/s12249-016-0689-6

[29] Sharma, B., & Dabur, R. (2015). Protective effects of Tinospora cordifolia on hepatic and gastrointestinal toxicity induced by chronic and moderate alcoholism. *Alcohol and Alcoholism*. https://doi.org/10.1093/alcalc/agv130

[30] Sharma, M., Rathi, R., Kaur, S., Singh, I., Kadir, E. A., Chahardehi, A. M., & Lim, V. (2023). Antiinflammatory activity of herbal bioactive-based formulations for topical administration. In *Recent Developments in Anti-Inflammatory Therapy*. https://doi.org/10.1016/B978-0-323-99988-5.00015-2

[31] Sinha, P., Srivastava, S., Mishra, N., Singh, D. K., Luqman, S., Chanda, D., & Yadav, N. P. (2016). Development, optimization, and characterization of a novel tea tree oil nanogel using response surface methodology. *Drug Development and Industrial Pharmacy*.

https://doi.org/10.3109/03639045.2016.1141931

[32] Taskin, T., & Bulut, G. (2019). Qualitative and quantitative phytochemical analysis and in-vitro biological activity of Rheum ribes L. different parts. *Istanbul Journal of Pharmacy*. https://doi.org/10.26650/istanbuljpharm.2019.18012

[33] Yersin, B. (1995). Screening for excessive alcohol drinking. Comparative value of carbohydrate-deficient transferrin, gamma-glutamyltransferase, and mean corpuscular volume. *Archives of Internal Medicine*. https://doi.org/10.1001/archinte.155.17.1907

[34] Yersin, B., Nicolet, J. F., Decrey, H., Burnier, M., Van Melle, G., & Pécoud, A. (1995). Screening for Excessive Alcohol Drinking: Comparative Value of Carbohydrate-Deficient Transferrin, γ-Glutamyltransferase, and Mean Corpuscular Volume. *Archives of Internal Medicine*. https://doi.org/10.1001/archinte.1995.00430170103013

[35] Zhao, J., Guo, S., Schrodi, S. J., & He, D. (2022). Cuproptosis and cuproptosis–related genes in rheumatoid arthritis: Implication, prospects, and perspectives. In *Frontiers in Immunology*. https://doi.org/10.3389/fimmu.2022.930278

[36] Zhu, W., Long, J., & Shi, M. (2023). Release Kinetics Model Fitting of Drugs with Different Structures from Viscose Fabric. *Materials*. https://doi.org/10.3390/ma16083282