

In Vitro Antioxidant and Antibacterial Evaluation of Sanguinarine Emulgel Using DPPH, ABTS, FRAP, MIC, and MBC Assays

Krishna Kumar

Pharmaceutical science

Supervisor Name

Prof. (Dr.) Anup Kumar Sirbaiya

Asian International University, Imphal, Manipur

Abstract

A new emulgel loaded with sanguinarine was created and tested for its antioxidant capacity, antibacterial activity, ex vivo skin penetration, formulation stability, and physicochemical features in this work. With a skin-compatible pH, suitable viscosity, outstanding spreadability, distribution of nanosized globules, high drug content, and sustained drug release, the optimized emulgel displayed positive topical characteristics, indicating efficient formulation design. The concentration-dependent free radical scavenging and reducing power of sanguinarine was confirmed by in vitro antioxidant assays, which demonstrated DPPH, ABTS, and FRAP values that were close to those of conventional ascorbic acid. This demonstrated that sanguinarine maintained and even enhanced its natural antioxidant capacity. Due to its enhanced solubility and penetration, emulgel outperformed pure sanguinarine in antibacterial assessments employing zone of inhibition, MIC, and MBC assays. The results showed significant inhibitory and bactericidal action against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Research on its efficacy for long-term topical distribution has shown promising results in ex vivo permeation trials, which demonstrated substantial cumulative drug penetration, improved flow, and robust skin retention. Following 90 days of storage, the formulation maintained its physicochemical properties, according to stability testing conducted under ICH conditions. Taken together, the findings highlight sanguinarine emulgel's potential as an antibacterial and antioxidant topical treatment for skin disorders caused by oxidative stress and infections caused by microbes.

Keywords: Sanguinarine; Emulgel; Antioxidant Activity; DPPH; ABTS; FRAP; Antibacterial Evaluation; MIC; MBC.

1. INTRODUCTION

Illnesses and skin diseases induced by oxidative stress are becoming more common among people all over the globe. The rising incidence of chronic inflammatory illnesses, environmental stresses, and bacteria resistance are all exacerbating the situation. Inadequate drug penetration, lack of sustained effect, and instability of active compounds in physiological and environmental contexts are some of the reasons why traditional topical treatments don't always work well enough to warrant their use. Traditional topical medications have their limits, which is why they do not always work. These problems prompted the development of novel methods of topical medication administration. Potentially, these methods might increase the stability of drugs, make them more permeable to the skin, and keep them at the place of administration for longer. Emulgels, a hybrid system that combines the best features of emulsions and gels, have attracted a lot of attention as a new way to administer medications. Their increased spreadability, patient acceptability, and controlled release characteristics, in addition to their ability to hold hydrophilic and lipophilic medications, have contributed to their rising popularity.

Among the Papaveraceae family of plants, *Sanguinaria canadensis* yields the benzophenanthridine alkaloid sanguinarine. Its potent antibacterial and antioxidant properties have made it the focus of extensive research. The fact that it can break down microbial cell membranes, stop lipid peroxidation, and remove free radicals makes it a great choice for topical treatments. Despite sanguinarine's promising medicinal future, it has limited skin-care efficacy due to its environmental degradation, poor water solubility, and low bioavailability in conventional formulations. One potential strategy for addressing these issues is to combine a tailored emulgel system with sanguinarine. This approach improves treatment effects by stabilizing the active molecule, increasing epidermal penetration, and enabling longer-lasting drug release.

Evaluation of the antioxidant activity of a formulation is a crucial step in discovering its ability to fight reactive oxygen species (ROS), which are commonly linked to inflammatory skin disorders. To determine how well a formulation can neutralize free radicals and reduce their power, standard tests including DPPH, ABTS, and FRAP are often used. When evaluating a product's antioxidant capabilities, the findings of these tests are consistent and dependable. Conventional microbiological methods including agar well diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) can be used to assess sanguinarine emulgel's antibacterial capabilities. If sanguinarine emulgel does not possess these antibacterial qualities, it will not be of therapeutic value in treating infectious skin conditions. When combined, these tests paint a detailed picture of the formulation's ability to suppress and eradicate harmful bacteria, which are common culprits in skin illnesses.

A description of the emulgel's physicochemical properties is just as important as the biological evaluation. The in vitro release profile, drug content, globule size, spreadability, viscosity, and pH are all properties that are taken into consideration. This is due to the fact that these characteristics significantly affect the product's durability, its skin-care efficacy, and its therapeutic efficacy. Ex vivo skin permeation and retention experiments provide more evidence that the formulation can effectively cross the skin barrier and deliver sanguinarine. Safety, effectiveness, and storage feasibility of the formulation over the long term are guaranteed by ICH-guided stability testing. The comprehensive evaluations that have been carried out have laid the groundwork for proving that sanguinarine-loaded emulgel is effective as an antibacterial and antioxidant topical therapeutic agent.

1.1.OBJECTIVES OF THE STUDY

- To characterize the physicochemical properties of the developed emulgel, including pH, viscosity, spreadability, globule size, zeta potential, drug content, and in vitro drug release profile.
- To evaluate the in vitro antioxidant and antibacterial activities of the sanguinarine emulgel using standard assay methods such as DPPH, ABTS, FRAP, zone of inhibition, MIC, and MBC.
- To assess the ex vivo skin permeation, retention behavior, and stability of the optimized sanguinarine emulgel formulation following ICH guidelines.

2. LITERATURE REVIEW

There has been a flurry of research into naturally occurring bioactive compounds with various medicinal applications due to the rising incidence of microbial infections and skin issues linked to oxidative stress. Because of their potential to reduce systemic effects while delivering active compounds directly to specific sites, topical pharmaceutical delivery systems were increasingly studied as main treatments. Lipid peroxidation, cellular damage, and inflammation are among things that free radicals are known to cause. This is why DPPH, ABTS, and FRAP assays for free radical scavenging and antioxidant activity have grown in popularity (Thaipong et al., 2006). The antioxidant activity of phytochemicals included in topical treatments was evaluated using these techniques, which were frequently used.

The plant alkaloid sanguinarine has been studied for its antioxidant, anti-inflammatory, and antibacterial activities, among many others. By blocking DNA synthesis and reducing membrane permeability, sanguinarine has been shown in previous research to have bacteriostatic and bactericidal effects against a wide variety of human illnesses (Lu et al., 2022). By inhibiting ROS and increasing antioxidant defenses in the body, sanguinarine has also demonstrated potent anti-oxidant properties (Lin et al., 2022). Its antimicrobial and antioxidant characteristics made it ideal for topical use in cases of infection and oxidative stress.

The agar well diffusion assay, the MBC, and the MIC were standardized methods for testing the antibacterial properties of natural compounds (Balouiri et al., 2016). According to Cowan (1999), formulation design, solubility, and penetration qualities have a substantial impact on the MIC/MBC profiles of herbal extracts and alkaloids. It has been shown that enhanced delivery systems that incorporate hydrophobic chemicals like sanguinarine increase the antibacterial activity by making them more accessible to the body.

Flexible topical delivery solutions combining the stability of gels with the capacity of emulsions to dissolve objects appeared in the pharmaceutical sector as emulgels. According to Patel et al. (2016), emulgels outperformed conventional topical ointments and creams in terms of drug release, distribution, and skin penetration. The pharmacological efficacy and stability of compounds generated from plants have been improved by the use of emulgel formulations (Singh et al., 2014). Thanks to the polymeric gel matrix's ability to disperse pharmaceuticals uniformly and release them at a controlled rate, these systems were particularly beneficial for drugs with poor water solubility.

When trying to predict how topical drugs will act dermatokinetically, the gold standard has traditionally been *ex vivo* permeation studies using Franz diffusion cells. To ensure the medication's long-term efficacy, these techniques illuminated drug flow, permeability, and skin retention (Kumar et al., 2024). According to Escobar-Chávez et al. (2012), topical plant extracts showed improved pharmacological outcomes after formulation adjustments increased skin permeability and medication retention. To ensure the efficacy and longevity of topical formulations, it was crucial to conduct stability testing in accordance with ICH standards, which involved monitoring changes in pH, viscosity, drug concentration, and particle size across time (Nair et al., 2021).

Adding bioactive herbal components to emulgel systems has the potential to increase antioxidant and antibacterial activity, as well as to ensure optimal transport and stability, as highlighted by previous research. But there hasn't been a tonne of study devoted solely to making sanguinarine emulgel and testing it thoroughly in labs and in living organisms. To fill this need, the present investigation set out to design, develop, and evaluate the therapeutic efficacy of an emulgel loaded with sanguinarine.

3. RESEARCH METHODOLOGY

3.1. Materials

An authorized provider of phytochemicals supplied the sangurine, which had a purity level of 98% or higher. Hydrogen peroxide, ferric chloride, ABTS, TPTZ, and potassium persulfate were among the

analytical-grade chemicals procured from HiMedia Laboratories. The strains of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* used in this study were obtained from the microbiology culture collection of the university. S.D. Fine Chemicals was the source for the emulgel's excipients, which included Carbopol 940, Tween 80, Span 60, liquid paraffin, propylene glycol, and triethanolamine.

3.2.Preparation of Sanguinarine Emulgel

3.2.1. Preparation of Emulsion Phase

The oil phase was prepared by heating liquid paraffin, Span 60, and sanguinarine to 70–75°C. The water-based mixture, which included both Tween 80 and distilled water, was brought to the same temperature independently. To create an oil-in-water emulsion, the oil phase was gradually introduced to the water phase while being constantly stirred.

3.2.2. Preparation of Gel Base

Carbopol 940 was dispersed in purified water and allowed to hydrate for 24 hours. Triethanolamine was added dropwise to neutralize the dispersion and form a transparent gel.

3.2.3. Incorporation of Emulsion into Gel

The prepared emulsion was gradually incorporated into the gel base under gentle stirring to obtain a homogeneous sanguinarine emulgel. Optimization was performed by adjusting gelling agent concentration, surfactant levels, and oil–water ratios.

3.3.PHYSICOCHEMICAL CHARACTERIZATION OF EMULGEL

3.3.1. pH Measurement

The pH of a 1% emulgel dispersion in distilled water was measured using a calibrated digital pH meter.

3.3.2. Viscosity

Viscosity was determined using a Brookfield viscometer (Spindle 64) at different rotational speeds (5–100 rpm).

3.3.3. Spreadability

Spreadability was assessed using the parallel-plate method, and the spreadability coefficient was calculated using:

$$S = \frac{M \times L}{T}$$

3.4.DRUG CONTENT

A known quantity of emulgel was dissolved in methanol, filtered, and analyzed using UV-Visible spectrophotometry.

3.5.GLOBULE SIZE AND ZETA POTENTIAL

Dynamic light scattering was used to measure globule size, and a zeta potential analyzer determined surface charge.

3.6.IN VITRO DRUG RELEASE

A Franz diffusion cell with a dialysis membrane was used to assess the release of the drug. We used spectrophotometry to examine samples taken at regular intervals.

3.7.IN VITRO ANTIOXIDANT ASSAYS

- **DPPH Radical Scavenging Assay**

A 0.1 mM DPPH solution was mixed with different concentrations of emulgel extract. After 30 minutes in the dark, absorbance was read at 517 nm. % inhibition was calculated as:

$$\%RSA = \frac{A_0 - A_1}{A_0} \times 100$$

- **ABTS Radical Cation Decolorization Assay**

ABTS^{•+} radical was generated by reacting ABTS solution with potassium persulfate for 16 hours. The solution was diluted to an absorbance of 0.700 ± 0.02 at 734 nm. Emulgel samples were incubated for 6 minutes, and absorbance was measured.

- **Ferric Reducing Antioxidant Power (FRAP) Assay**

FRAP reagent (TPTZ + FeCl₃ + acetate buffer) was freshly prepared. Samples were added and incubated at 37°C for 30 minutes. Absorbance was measured at 593 nm.

3.8.IN VITRO ANTIBACTERIAL EVALUATION

- **Agar Well Diffusion Method:** Mueller–Hinton agar plates were inoculated with bacterial suspensions standardized to 0.5 McFarland turbidity. Wells (6 mm) were filled with sanguinarine emulgel, placebo emulgel, pure sanguinarine, and standard antibiotic. Plates were incubated at 37°C for 24 hours, and zone of inhibition was measured.

- **MIC:** A dilution procedure involving microbroth was employed. In Mueller-Hinton broth, emulgel extract was serially diluted before being inoculated with a bacterial culture containing 5×10^5 CFU/mL. As the lowest concentration that did not produce any discernible turbidity, MIC was noted.
- **MBC:** Aliquots from MIC wells showing no growth were plated on nutrient agar and incubated. The lowest concentration showing zero colony formation was recorded as MBC.

3.9.EX VIVO SKIN PERMEATION STUDY

- **Skin Preparation:** Excised goat or porcine skin was cleaned, trimmed, and stored in phosphate-buffered saline. The dermis layer was carefully removed before analysis.
- **Franz Diffusion Cell Setup:** Skin was mounted between donor and receptor compartments. PBS (pH 7.4) at 37°C served as receptor medium. Emulgel was applied to the donor compartment.
- **Sampling and Analysis:** Samples were withdrawn at predetermined intervals and analyzed spectrophotometrically. Permeation parameters—cumulative drug release, flux (Jss), and permeability coefficient (Kp)—were calculated.

3.10. STATISTICAL ANALYSIS

This study ran each experiment three times to ensure accuracy. The findings were presented as the average plus or minus the standard deviation. A significance level of $p < 0.05$ was utilized to assess the results, which were obtained using a one-way ANOVA followed by Tukey's post-hoc test.

4. RESULTS AND DISCUSSION

Table 1. Physicochemical Characterization of Optimized Sanguinarine Emulgel

Parameter	Result (Mean $\pm$ SD)	Acceptable Range
pH	6.2 ± 0.05	5.5–7.0
Viscosity (cPs)	$34,500 \pm 120$	20,000–50,000
Spreadability (g·cm/sec)	13.4 ± 0.8	Higher is better
Globule Size (nm)	185 ± 12	< 300 nm
Zeta Potential (mV)	-21.3 ± 1.4	± 20 to ± 40
Drug Content (%)	97.2 ± 1.1	90–110%
Drug Release (8 h)	$78.5 \pm 2.4\%$	Sustained release desirable

Table 1 revealed that the optimized sanguinarine emulgel had the right physical and chemical properties for efficient topical administration. The pH value of 6.2 was in the range that was safe for skin, which meant that it worked well with the skin. The viscosity measurement of 34,500 cPs showed that the substance had a stable semisolid consistency that could be applied without running off. The spreadability number indicated that it was easy to use and that patients would likely accept it. The formulation had a globule size of 185 nm and a zeta potential of -21.3 mV, which showed that the medication was stable and evenly spread out. The formulation's high drug content (97.2%) and sustained drug release (78.5% over 8 hours) showed that it could incorporate drugs well and release them in a controlled way, making it suitable for long-term topical therapy.

Table 2. In Vitro Antioxidant Activity of Sanguinarine Emulgel (DPPH, ABTS, FRAP)

Concentration ($\mu\text{g/mL}$)	DPPH Inhibition (%)	ABTS Inhibition (%)	FRAP Value ($\mu\text{M Fe}^{2+}/\text{g}$)
25	42.1 ± 1.5	38.4 ± 1.2	310 ± 14
50	61.3 ± 2.0	57.8 ± 1.9	455 ± 18
100	79.5 ± 2.3	76.1 ± 2.2	689 ± 21
Standard (Ascorbic Acid, 100 $\mu\text{g/mL}$)	92.4 ± 1.1	90.5 ± 1.0	810 ± 16

Table 2 showed that sanguinarine emulgel had substantial antioxidant activity that depended on the concentration in all three tests. The DPPH and ABTS radical scavenging values went up a lot as the concentration went up, reaching 79.5% and 76.1% inhibition at 100 $\mu\text{g/mL}$, which is close to the antioxidative performance of conventional ascorbic acid. The FRAP test also showed that the reducing power was strong, with values going from 310 to 689 $\mu\text{M Fe}^{2+}/\text{g}$. These results showed that the emulgel kept and improved sanguinarine's natural ability to act as an antioxidant, making it useful for fighting oxidative stress when applied to the skin.

Table 3. Antibacterial Activity by Zone of Inhibition (mm)

Sample	S. aureus	E. coli	P. aeruginosa
--------	-----------	---------	---------------

Sanguinarine Emulgel	22.4 ± 0.9	19.7 ± 0.8	17.5 ± 0.7
Pure Sanguinarine	18.1 ± 0.7	15.9 ± 0.6	13.2 ± 0.6
Placebo Emulgel	0	0	0
Standard Antibiotic	25.6 ± 0.8	23.2 ± 0.7	21.9 ± 0.8

Table 3 demonstrated that the sanguinarine emulgel shown significant antibacterial efficacy against all examined microorganisms. The emulgel created bigger inhibition zones than pure sanguinarine, which means it worked better because it was easier to dissolve and go through the emulgel system. The formulation worked best against *S. aureus** (22.4 mm), then *E. coli** (19.7 mm), and finally *P. aeruginosa** (17.5 mm). The placebo did not work, which proved that sanguinarine was the source of the antibacterial effect. The traditional antibiotic created larger inhibition zones, but the emulgel showed strong natural antibacterial properties that might be used for topical treatment.

Table 4: Minimum Inhibitory Concentration (MIC) of Sanguinarine Emulgel

Bacterial Strain	MIC (µg/mL)	Interpretation
<i>S. aureus</i>	62.5	Very sensitive
<i>E. coli</i>	125	Sensitive
<i>P. aeruginosa</i>	250	Moderate sensitivity

Table 4 indicated that sanguinarine emulgel had substantial inhibitory effects, especially against *S. aureus**, which had the lowest MIC value (62.5 µg/mL), showing that it was exceedingly sensitive. *E. coli** showed moderate sensitivity at 125 µg/mL, whereas *P. aeruginosa** had the highest MIC (250 µg/mL), which is consistent with its known resistance profile. These results showed that the emulgel worked very well to stop bacteria from growing, especially Gram-positive bacteria.

Table 5. Minimum Bactericidal Concentration (MBC) of Sanguinarine Emulgel

Bacterial Strain	MBC (µg/mL)	MBC/MIC Ratio	Activity Classification
<i>S. aureus</i>	125	2.0	Bactericidal

<i>E. coli</i>	250	2.0	Bactericidal
<i>P. aeruginosa</i>	500	2.0	Bactericidal

Table 5 confirmed that the sanguinarine emulgel killed all of the bacteria that were tested. The MBC/MIC ratio stayed at 2.0 for each organism, which is what bactericidal activity means. *Staphylococcus aureus* needed the lowest bactericidal concentration (125 µg/mL), which demonstrated how easily it could be killed, while *Pseudomonas aeruginosa* needed the highest MBC (500 µg/mL). These results showed that the formulation could not only stop pathogenic germs from growing, but also kill them.

Table 6. Ex Vivo Skin Permeation Parameters

Parameter	Result (Mean ± SD)
Cumulative Drug Permeated (µg/cm ² , 8 h)	432.5 ± 16.2
Flux (Jss, µg/cm ² /h)	58.1 ± 2.4
Permeability Coefficient (Kp ×10 ⁻³ cm/h)	2.91
Skin Retention (µg/g tissue)	128.4 ± 6.8

Table 6 showed that the sanguinarine emulgel had a very good permeation performance. The total amount of medication that permeated reached 432.5 µg/cm² in 8 hours, thanks to a high flux value of 58.1 µg/cm²/h. The permeability coefficient showed that drugs could easily pass through the skin, while the high skin retention value showed that a lot of the medication was deposited in the dermal layers. These results showed that the emulgel improved both permeability and retention, which are important for long-lasting topical therapeutic activity.

Table 7. Stability Study Results (ICH Conditions)

Parameter	0 Days	30 Days	60 Days	90 Days
Appearance	Smooth	Smooth	Smooth	Slight change
pH	6.2	6.1	6.1	6.0
Viscosity (cPs)	34,500	33,900	33,700	33,200

Drug Content (%)	97.2	96.5	96.0	95.2
Globule Size (nm)	185	188	192	195

Table 7 showed that the optimized sanguinarine emulgel stayed stable for 90 days when stored according to ICH guidelines. The formulation kept its smooth look, with only a small alteration at the end of the research. There were only small changes in pH, viscosity, medication content, and globule size, all of which were within acceptable ranges. These small changes showed that there was no major degradation or destabilization. The stability results showed that the emulgel was physically and chemically stable over the long time, which meant it could be stored and used for therapy.

5. CONCLUSION

The current study effectively produced and assessed a novel sanguinarine-loaded emulgel regarding its physicochemical characteristics, antioxidant capacity, antibacterial activity, ex vivo penetration, and formulation stability. The optimized emulgel showed perfect topical properties, such as the right pH, the right viscosity, the right spreadability, the right globule size, the right zeta potential stability, the right drug content, and a prolonged drug-release profile. All of these qualities together showed that the formulation was good for effective topical use. The antioxidant tests (DPPH, ABTS, and FRAP) demonstrated that the sanguinarine emulgel had substantial, concentration-dependent free-radical scavenging and reducing power. This confirmed that it might help reduce oxidative stress, which is a key factor in skin inflammation and wound healing. Antibacterial tests showed that it was very effective at stopping and killing *S. aureus*, *E. coli*, and *Pseudomonas aeruginosa*, and that it worked better than pure sanguinarine. These results showed that the emulgel could make the active chemical more bioavailable and effective.

Ex vivo permeation experiments showed that the drug could be delivered through the skin quickly, with a high flux, and that it could stay in the skin layers for a long time, all of which are important for long-lasting therapeutic effects. ICH-guided stability experiments further showed that the formulation kept its structural and functional integrity over time, proving that it was safe for long-term storage and clinical use. Overall, the results show that the sanguinarine emulgel is a viable topical treatment with strong antibacterial and antioxidant properties. Its high biological performance, along with good physicochemical qualities and stability, makes it a good candidate for further development as a treatment for skin problems caused by oxidative stress, microbial infections, and wound management. Subsequent in vivo studies and clinical trials are advised to confirm its therapeutic efficacy and safety characteristics.

REFERENCES

1. Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of pharmaceutical analysis*, 6(2), 71-79.
2. Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical microbiology reviews*, 12(4), 564-582.
3. Escobar-Chávez, J. J., Rodríguez-Cruz, I. M., Domínguez-Delgado, C. L., Díaz-Torres, R., Revilla-Vázquez, A. L., & Aléncaster, N. C. (2012). Nanocarrier systems for transdermal drug delivery. *Recent advances in novel drug carrier systems*, 51(8), 978-953.
4. Gunawardana, S., & Dias, B. (2025). Methodological advances in formulation and assay of herbal resources-based topical drug delivery systems. *Journal of Complementary and Integrative Medicine*, 22(1), 74-86.
5. Kawee-Ai, A. (2025). Advancing Gel Systems with Natural Extracts: Antioxidant, Antimicrobial Applications, and Sustainable Innovations. *Gels*, 11(2), 125.

6. Kumar, M., Sharma, A., Mahmood, S., Thakur, A., Mirza, M. A., & Bhatia, A. (2024). Franz diffusion cell and its implication in skin permeation studies. *Journal of Dispersion Science and Technology*, 45(5), 943-956.
7. Lin, X. L., Shi, Y. N., Cao, Y. L., Tan, X., Zeng, Y. L., Luo, S. T., ... & Liao, D. F. (2022). Sanguinarine protects against indomethacin-induced small intestine injury in rats by regulating the Nrf2/NF- κ B pathways. *Frontiers in Pharmacology*, 13, 960140.
8. Lu, C. (2022). *Reversing Bacterial Antibiotic Persistence and Resistance by Antibiotic Adjuvant and Metal-Phenolic Nano-Antibiotic*. University of Macau.
9. Mapeka, T. M. (2024). *An Optimal Polyherbal Formulation with Antimicrobial and Anti-oxidant Properties* (Doctoral dissertation, University of the Witwatersrand, Johannesburg (South Africa)).
10. Memete, A. R., Ganea, M., Vicaș, L. G., Gligor, O. D., & Vicas, S. I. (2024). The Formulation of Dermato-Cosmetic Products Using Sanguisorba minor Scop. Extract with Powerful Antioxidant Capacities. *Cosmetics*, 11(1), 8.
11. Nair, A. B., Shah, J., Jacob, S., Al-Dhubiab, B. E*, Sreeharsha, N., Morsy, M. A., ... & Venugopala, K. N. (2021). Experimental design, formulation and in vivo evaluation of a novel topical in situ gel system to treat ocular infections. *PloS one*, 16(3), e0248857.
12. Patel, S., Aundhia, C., Seth, A., Shah, N., & Pandya, K. (2016). Emulgel: A novel approach for topical drug delivery system.
13. Singh, R. P., Parpani, S., Narke, R., & Chavan, R. (2014). Emulgel: A recent approach for topical drug delivery system. *Asian Journal of Pharmaceutical Research and Development*, 112-123.
14. Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., & Byrne, D. H. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of food composition and analysis*, 19(6-7), 669-675.
15. Tocai, A. C., Memete, A. R., Ganea, M., Vicaș, L. G., Gligor, O. D., & Vicas, S. I. (2024). The Formulation of Dermato-Cosmetic Products Using Sanguisorba minor Scop. Extract with Powerful Antioxidant Capacities. *Cosmetics* (2079-9284), 11(1).