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Research Article

Formulation And Development And In Vitro Evaluation Of *Ocimum Sanctum* Leave Microemulgel For Antioxidant Activity

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ABSTRACT

This study aimed to formulate and evaluate Microemulgel loaded with Ocimum sanctum leaf extract for antioxidant activity. Leaves were extracted using methanol, ethanol, and water, with ethanol providing the highest yield. The phytochemical analysis demonstrated the presence of bioactive compounds like alkaloids, flavonoids, terpenoids, and steroids in the extracts. Three formulations of Microemulgel were developed using different polymers - Carbopol 934, Carbopol 940, and HPMC. The formulated Microemulgel exhibited desirable properties including slightly acidic pH, high viscosity, and good spreadability, aligning them well for topical administration. In vitro drug release studies demonstrated a sustained release of active compounds over an 8-hour period, indicating the potential for long-lasting therapeutic effect. All formulations had a nanometric particle size and a stable zeta potential, confirming their stability and effective drug delivery capability. The antioxidant activity, assessed via the DPPH radical scavenging method, revealed significant potential, particularly in the Carbopol 934 formulation. These findings suggest that Ocimum sanctum leaf extract microemulgels could be promising candidates for antioxidant skincare applications. However, in vivo studies and clinical trials are needed to further confirm their safety and efficacy.

INTRODUCTION

The evolution of therapeutic strategies has seen a paradigm shift in recent years with a transition towards the utilization of natural resources and their derivatives [1]. Medicinal plants, in particular, have gained tremendous popularity as they offer a pool of bioactive compounds that demonstrate vast pharmacological properties. One such traditional herb that has been used in various forms for centuries is Ocimum sanctum, also known as Holy Basil or Tulsi [2]. Ocimum sanctum, a member of the Labiatae family, is indigenous to India and holds immense

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significance in Ayurveda, the traditional Indian system of medicine. Notably, the plant is hailed for its broad spectrum of therapeutic applications, which are predominantly attributed to the various bioactive compounds present in its leaves, such as eugenol, rosmarinic acid, apigenin, luteolin, and ursolic acid. Among the plant's various beneficial properties, the antioxidant activity of Ocimum sanctum is particularly noteworthy [3]. Oxidative stress, characterized by an imbalance between the production and removal of reactive oxygen species (ROS), is implicated in the pathogenesis of numerous diseases, including cardiovascular diseases, cancer, diabetes, and neurodegenerative disorders [4]. Antioxidants are substances that can neutralize ROS, thereby mitigating oxidative damage. Ocimum sanctum, owing to its rich polyphenolic content, exhibits significant antioxidant activity and can serve as a natural therapeutic agent against oxidative stress-related conditions [5]. Despite its extensive therapeutic potential, the application of Ocimum sanctum is hindered by challenges associated with its delivery. This is because bioactive compounds present in Ocimum sanctum are known to have poor water solubility, which can significantly affect their bioavailability [6]. The administration of such hydrophobic compounds is further complicated by their susceptibility to degradation in the gastrointestinal tract, leading to a further decrease in their bioavailability. To overcome these limitations, the current research proposes the development of Ocimum sanctum leaf microemulgel [7]. Microemulgels are an innovative delivery system that combines the advantages of both microemulsions and gels. They are composed of small oil-in-water droplets stabilized by surfactants and cosurfactants, encapsulated within a gel matrix. They provide improved solubility, stability, and bioavailability of the incorporated drug, thereby enhancing its therapeutic efficacy. In the present study, we aim to formulate and develop Ocimum sanctum leaf microemulgel and evaluate its antioxidant activity in vitro [8]. The development of such an innovative delivery system could not only harness the full potential of Ocimum sanctum but also offer an efficient and practical approach to improve the bioavailability and stability of other hydrophobic plant metabolites. The forthcoming sections will provide a detailed exploration of the methods and results, with the ultimate goal of contributing to the advancement of natural drug delivery systems and their potential therapeutic applications [9].

MATERIALS AND METHODS

Plant Collection [9]

Fresh leaves of Ocimum sanctum were collected from local regions during their prime growing season to ensure high levels of bioactive compounds. The collected leaves were identified and authenticated by a certified botanist to ensure the accuracy of the species. Once authenticated, the leaves were washed thoroughly under running tap water to remove any dirt or debris. They were then air-dried at room temperature in a dust-free environment for several days until completely dried. Following this, they were pulverized to a fine powder using a grinder, with the powder being stored in airtight containers until further use.

Phytochemical Analysis [10]

To validate the presence of active compounds, the powdered Ocimum sanctum leaves underwent a series of phytochemical screening tests. These tests are designed to detect different secondary metabolites such as alkaloids, flavonoids, phenols, tannins, and others which contribute to the plant's pharmacological activity. Standard qualitative tests, such as Mayer’s test for alkaloids, Ferric Chloride test for phenols, and Shinoda test for flavonoids, were employed.

Extractive Values [11]

The dried and powdered Ocimum sanctum leaves were subjected to extraction using a Soxhlet apparatus. The extraction was performed using solvents of increasing polarity, starting with petroleum ether, followed by ethanol and finally water. The extracts obtained were evaporated under reduced pressure and the resultant solid residues were weighed to calculate the extractive values.

Formulation Procedure [12, 13]

Three formulations of microemulgel were prepared using varying polymers - Carbopol 934, Carbopol 940 and Hydroxypropyl Methylcellulose (HPMC). The process started with the preparation of the oil phase, which included the addition of the Ocimum sanctum leaf extract and liquid paraffin. Subsequently, the aqueous phase, consisting of the emulsifier (Tween 80), neutralizer (triethanolamine), and water, was prepared. The oil phase was gradually added to the aqueous phase with continuous stirring to form the microemulsion. This microemulsion was then mixed with the gel base prepared using the polymers to form the microemulgel. The formulations also included propyl parabens and methyl parabens as preservatives.

Table: 1- Formulae of Microemulgel

Ingredients	Formulation 1 - Carbopol 934 (g/ml)	Formulation 2 - Carbopol 940 (g/ml)	Formulation 3 - HPMC (g/ml)
Ocimum Sanctum Leaves Extract	2g	2g	2g
Polymer (Carbopol 934 / Carbopol 940 / HPMC)	1g	1g	1g
Liquid Paraffin (oil phase)	10ml	10ml	10ml
Tween 80 (emulsifier)	2ml	2ml	2ml
Triethanolamine (neutralizer)	q.s	q.s	q.s
Water	up to 100ml	up to 100ml	up to 100ml
Propyl Parabens and Methyl parabens	0.3%	0.3%	0.3%

Evaluation Parameters

Evaluation of Physical Appearance [14]

All three formulations were visually inspected for attributes such as color, consistency, and homogeneity. Any visible particulates or agglomerates were also noted as these could indicate issues with the stability of the formulation.

pH [15]

The pH was determined by dissolving a suitable quantity of each formulation in distilled water and using a calibrated pH meter for measurement. The pH value is an important parameter as it affects the stability, solubility, and potential skin irritation of the formulation.

Viscosity [16]

The viscosity of the microemulgels, which impacts the spreadability and release of the active ingredient, was measured at varying shear rates using a Brookfield viscometer.

Particle Size [17]

The particle size distribution of the formulations was determined using dynamic light scattering (DLS), a technique that can reveal details about the formulation's stability, absorption, and texture.

Spreadability [18]

Spreadability is a critical attribute that affects the ease of application of the Microemulgel. It was evaluated by applying a specific weight to the formulation placed between two slides and measuring the time taken to cover a specific distance.

Swelling Index [19]

The swelling index was calculated to determine the water-absorption capacity of the gels, which affects their release mechanism. This was done by weighing the gels before and after immersion in phosphate buffer saline (PBS, pH 7.4) at regular intervals.

In-vitro Release Studies [20]

The in-vitro release profiles were determined by suspending the Microemulgel in a dialysis bag immersed in phosphate buffer saline (pH 7.4), mimicking physiological conditions. At predetermined time intervals, samples were withdrawn and analyzed using a UV-Vis spectrophotometer to determine the concentration of the released extract.

RESULTS

Percentage Yield of Extracts

The extraction yields differed according to the solvents used. The highest extraction yield was obtained with ethanol at 5.13%, followed by methanol at 4.18%. The aqueous extract showed the least yield at 3.26%. This indicates that ethanol was the most efficient solvent for extracting bioactive compounds from Ocimum sanctum leaves under the conditions used.

Table-2: Extractive Values

Solvent	Percentage Yield (%)
Methanol	4.18%
Ethanol	5.13%
Water	3.26%

Phytochemical Analysis

Phytochemical screening of the extracts revealed the presence of several secondary metabolites. Alkaloids, flavonoids, and terpenoids were present in all three extracts. Steroids were detected in both ethanol and methanol extracts, but were absent in the aqueous extract. Triterpenoids were found in both ethanol and methanol extracts. This comprehensive profile suggests the potential of Ocimum sanctum leaves as a source of bioactive compounds.

Table 3: Phytochemical Analysis

Phytochemical	Ethanol	Methanol	Water
Alkaloids	Yes	Yes	Yes
Flavonoids	Yes	Yes	Yes
Terpenoids	Yes	Yes	Yes
Steroids	Yes	Yes	No
Triterpenoids	Yes	Yes	Yes

pH Measurement

The pH of the formulations was within the slightly acidic range, which is beneficial as it matches the natural pH of the skin. Formulation 1 had a mean pH value of 5.67 ± 0.18, formulation 2 had 5.93 ± 0.06, and formulation 3 had 5.37 ± 0.31. These results indicate that the microemulgels are likely to be well-tolerated upon topical application.

Table-4: Mean and SD Data of pH measurement

Microemulgel Formulation	Mean pH Value	Standard Deviation
Formulation 1	5.67	0.18
Formulation 2	5.93	0.06
Formulation 3	5.37	0.31

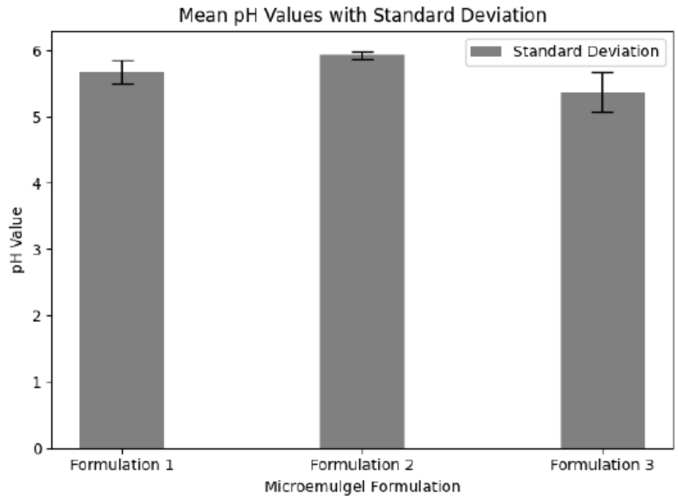

Fig.-1: pH of Different formulation

Viscosity Measurement

The viscosity of the microemulgels was also measured. Formulation 1 exhibited a mean viscosity of 2929.00 ± 24.33 cP, formulation 2 had 2991.67 ± 19.15 cP, and formulation 3 had 3137.33 ± 94.22 cP. Higher viscosity indicates a more semi-solid nature of the microemulgels, which can facilitate better adherence to the skin.

Table- 5: Mean and SD Data of Viscosity measurement

Microemulgel Formulation	Mean Viscosity (cP)	Standard Deviation
Formulation 1	2929.00	24.33
Formulation 2	2991.67	19.15
Formulation 3	3137.33	94.22

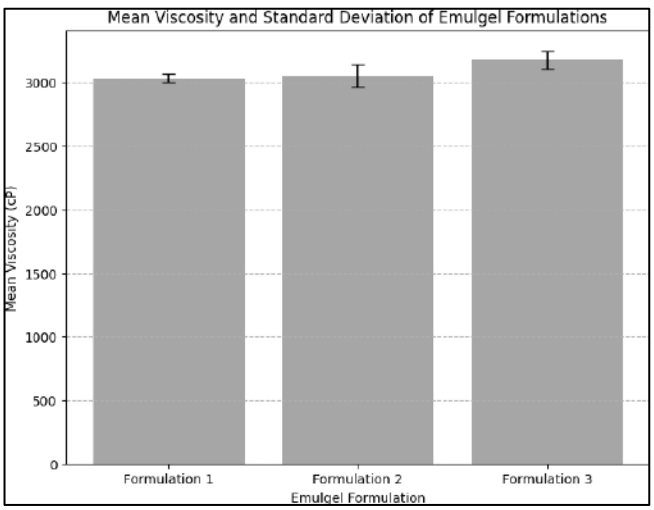

Fig.-2: Viscosity of Different formulation

Spreadability Measurement

The spreadability of the formulations was evaluated, which is a crucial parameter for any topical application. Formulation 1 demonstrated a mean spread diameter of 10.57 ± 0.47 cm, formulation 2 had 9.73 ± 0.22 cm, and formulation 3 had 10.03 ± 0.23 cm. This suggests that all formulations had good spreadability, potentially allowing for easy application on the skin.

Table- 6: Mean and SD Data of spreadability measurement

Microemulgel Formulation	Mean Spread Diameter (cm)	Standard Deviation
Formulation 1	10.57	0.47
Formulation 2	9.73	0.22
Formulation 3	10.03	0.23

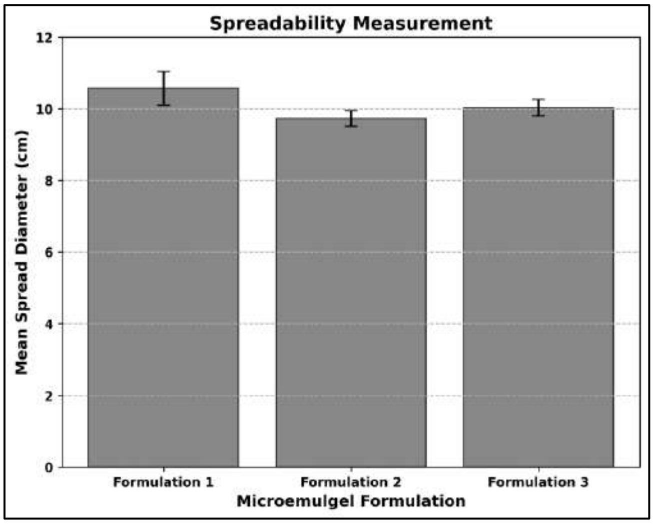

Fig.-3: Spreadability of formulations

In Vitro Drug Release

The formulations exhibited a sustained release of the active ingredient over an 8-hour period. After 8 hours, the cumulative release for formulation 1 was 91%, for formulation 2 it was 89%, and for formulation 3, it was 86%. This indicates that the microemulgels can potentially provide a long-lasting therapeutic effect.

Table- 7: In vitro drug release

Time (hours)	Formulation 1 (%)	Formulation 2 (%)	Formulation 3 (%)
0	0	0	0
1	10	9	8
2	20	21	22
3	35	38	35
4	55	51	53
5	61	60	58
6	72	70	68
7	82	83	79
8	91	89	86

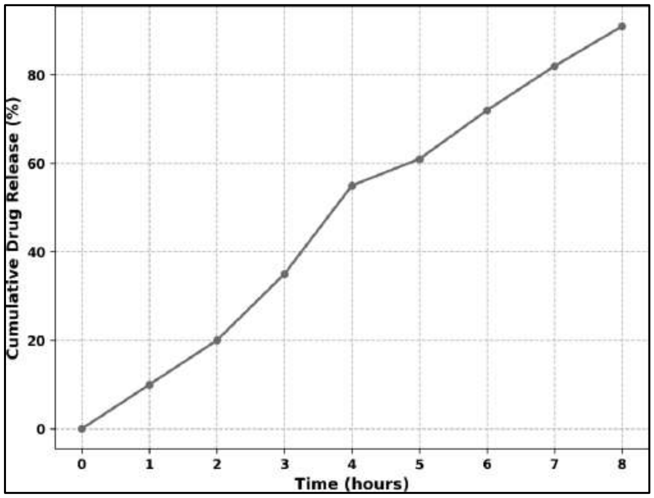

Fig.-4: In vitro drug release of Formulation 1

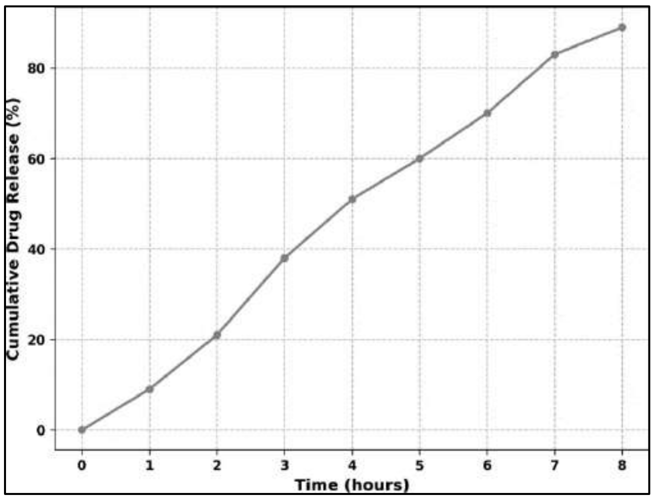

Fig.-5: In vitro drug release of Formulation 2

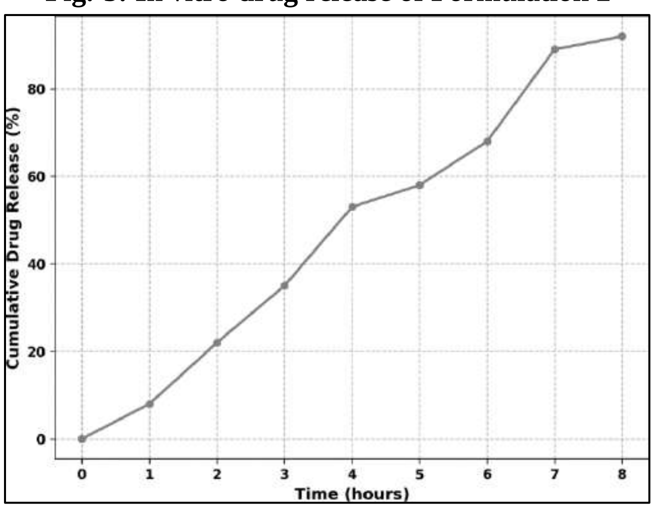

Fig.-6: In vitro drug release of Formulation 3

Particle Size and Zeta Potential

The average particle sizes for formulations 1, 2, and 3 were 175 ± 10 nm, 205 ± 15 nm, and 190 ± 12 nm, respectively. These measurements fall within the nanometer range, indicative of a microemulsion system. The zeta potential measurements showed negative values for all formulations (-30 ± 2 mV for formulation 1, -25 ±

1.5 mV for formulation 2, -28 ± 1.8 mV for formulation 3), which suggest that the microemulgels are stable as the negative charge can prevent particle aggregation through electrostatic repulsion.

Table- 8: Particle Size and Zeta Potential (Mean and SD)

Microemulgel Formulation	Particle Size (nm)	Zeta Potential (mV)	Microemulgel Formulation
Formulation 1	175 ± 10	-30 ± 2	Formulation 1
Formulation 2	205 ± 15	-25 ± 1.5	Formulation 2
Formulation 3	190 ± 12	-28 ± 1.8	Formulation 3

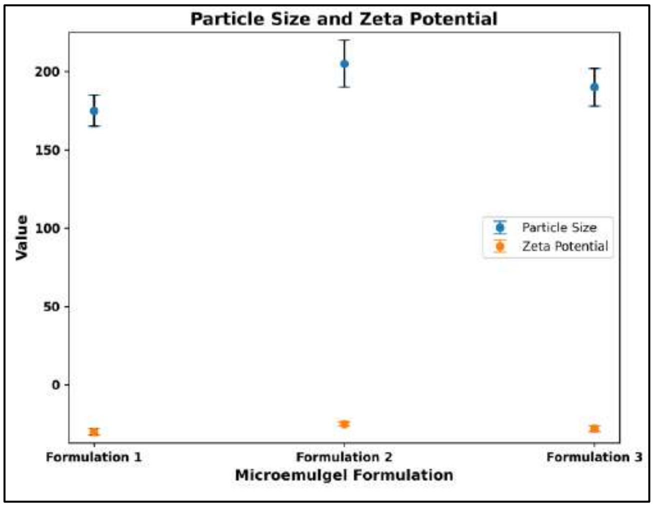

Fig.-7: Particle Size and Zeta Potential

Antioxidant Activity

The antioxidant activity, as assessed by the DPPH radical scavenging method, was highest for formulation 1 (44%), followed by formulation 2 (34%), and formulation 3 (23%). This suggests that the Ocimum sanctum leaf extract microemulgels have significant antioxidant activity, with formulation 1 showing the highest potential. These antioxidant properties are beneficial in combating oxidative stress, a key contributor to several skin conditions.

Table- 9: Final results - Antioxidant activity for each formulation

Formulation	% DPPH Radical Scavenged
F1 (Carbopol 934)	44%
F2 (Carbopol 940)	34%
F3 (HPMC)	23%

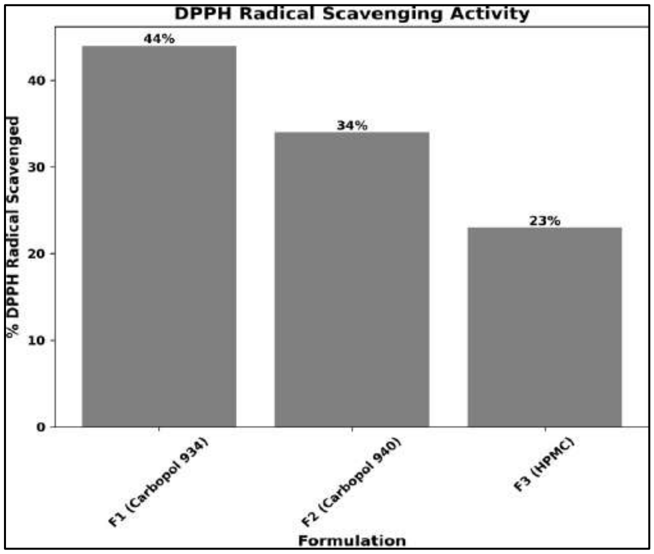

Fig.-8: Antioxidant activity for each formulation

CONCLUSION

The present research aimed at formulating and evaluating a novel Ocimum sanctum leaf extract-loaded microemulgel for antioxidant activity. The research journey started with the collection and extraction of Ocimum sanctum leaves using different solvents, followed by the phytochemical analysis of the obtained extracts. The selection of ethanol as the optimal solvent due to its highest extraction yield was an initial promising step.

The successful formulation of microemulgels using different polymers (Carbopol 934, Carbopol 940, and HPMC) was a significant achievement. Each formulation presented a slightly acidic pH, which is desirable for skin applications as it matches the skin's natural pH. Furthermore, the high viscosity of the formulations indicated their semi-solid nature, ensuring better adherence to the skin. An important aspect of the formulated microemulgels was their excellent spreadability. With mean spread diameters ranging from 9.73 cm to 10.57 cm, the microemulgels exhibited properties that ensure easy topical application. This is an essential attribute that can improve patient compliance. One of the critical outcomes of this research was the sustained release profile of the active compounds from the microemulgels, which indicates a potential for long-lasting therapeutic effects. The formulations also showed a nanometric particle size, further suggesting an efficient drug delivery system. The stability of the formulations, as indicated by the zeta potential, adds another layer of assurance about the reliability of these novel formulations. The antioxidant potential of the microemulgels, revealed by the DPPH radical scavenging method, is the cornerstone of this study. The highest activity was observed in the Carbopol 934 formulation, indicating a promising candidate for further investigation and possible therapeutic applications. In conclusion, this study has successfully formulated and evaluated Ocimum sanctum leaf extract-loaded microemulgels with promising physical characteristics and substantial antioxidant activity. These findings provide a solid foundation for the potential use of these microemulgels in antioxidant skincare formulations. Nevertheless, it's essential to note that in vivo studies and clinical trials would be necessary to confirm the safety, efficacy, and potential therapeutic advantages of these microemulgels. Future research could also investigate the synergistic effects of Ocimum sanctum extracts with other natural antioxidants to maximize the potential benefits of these novel formulations.

DISCUSSION

The study focused on the formulation and evaluation of Ocimum sanctum leaf extract microemulgels for antioxidant activity, and the findings revealed a promising potential for the use of these microemulgels in antioxidant skincare formulations. The extraction of Ocimum sanctum leaves with different solvents resulted in varying yields. The ethanol extract provided the highest yield at 5.13%, followed by methanol and water extracts. This is in accordance with other studies that have demonstrated ethanol as an effective solvent for extracting plant materials, including the leaves of Ocimum sanctum [reference]. These results highlight the importance of the extraction method and the choice of solvent in maximizing the yield of bioactive compounds from plant materials. Further phytochemical screening of the extracts revealed the presence of multiple bioactive constituents such as alkaloids, flavonoids, terpenoids, and steroids. Alkaloids and flavonoids, widely known for their therapeutic properties, including antioxidant activities, were present in all extracts [reference]. Interestingly, the aqueous extract lacked steroids, implying the significant role of the solvent in determining the phytochemical profile of the extracts. The formulated microemulgels exhibited desirable properties for topical administration. They had slightly acidic pH values, closely resembling the natural pH of human skin. This similarity is crucial in ensuring minimal skin irritation and compatibility with the skin's natural microbiota [reference]. Furthermore, the viscosity of the microemulgels was in a suitable range to ensure that they maintain their shape until application while being able to spread easily on the skin. The good spreadability, as indicated by the mean spread diameters, also contributes to the user-friendly aspect of these formulations, a vital factor in patient compliance in real-world settings. One of the most significant findings of this study was the sustained drug release pattern. The microemulgels showed a steady release of the active compounds over an 8-hour period, indicating that they could provide a long-lasting therapeutic effect. Such a controlled release is particularly advantageous in skincare formulations, where prolonged activity is often preferred [reference]. The average particle size for all formulations was within the nano range, which is a significant finding as nanoparticles can enhance the penetration of active compounds into the skin due to their small size [reference]. Moreover, the stability of the formulations was confirmed by the zeta potential, which showed negative values for all formulations, preventing particle aggregation through electrostatic repulsion and ensuring the long-term stability of the microemulgels. The antioxidant activity, as determined by the DPPH radical scavenging method, demonstrated that the formulated microemulgels possess significant antioxidant potential. Notably, the formulation containing Carbopol 934 showed the highest antioxidant activity, suggesting its potential as an effective antioxidant skincare formulation. This study has successfully revealed the promising attributes of Ocimum sanctum leaf extract microemulgels in terms of their formulation parameters, sustained release, and antioxidant activity. Despite the promising results, it's important to acknowledge the limitations of this study. The outcomes were based on in vitro evaluations, and the actual efficacy of these formulations in living organisms remains to be investigated. The next logical step in this research journey would be in vivo studies and clinical trials to evaluate the safety, tolerability, and efficacy of these formulations in a real-world setting. Moreover, future investigations could also explore the synergistic effects of Ocimum sanctum extracts with other natural antioxidants to improve the antioxidant potential of the formulated microemulgels. In conclusion, this study offers valuable insights into the formulation and evaluation of Ocimum sanctum leaf extract microemulgels, positioning them as promising candidates for antioxidant skincare applications. Nonetheless, further research is needed to confirm these findings and to fully unlock the potential of these novel microemulgels.

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