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Bioavailability-Enhanced Nanoemulsion of Andrographolide for Oral Delivery: A Novel Antiviral Drug Delivery Strategy – A Research Study

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ABSTRACT

Background: *Andrographis paniculata* is a medicinal herb widely recognized for its broad-spectrum antiviral activity, primarily due to the presence of andrographolide. However, its therapeutic potential is significantly limited by poor water solubility, low oral bioavailability, and rapid metabolism.

Objective: This study aimed to formulate and evaluate a stable nanoemulsion-based oral syrup containing andrographolide to enhance its solubility, bioavailability, and antiviral efficacy.

Methods: Andrographolide was extracted from A. paniculata using microwave-assisted extraction (MAE). A nanoemulsion was prepared using castor oil (oil phase), Tween 80 (surfactant), and propylene glycol (co-surfactant), followed by high-speed homogenization and ultrasonication. The formulation was evaluated for particle size, zeta potential, size distribution (polydispersity index), pH, viscosity, preliminary phytochemical evaluation, terpenoid content in extract and appearance.

Results: The developed nanoemulsion had an average droplet size of 191.1 nm and a zeta potential of –31.5 mV, indicating good stability. The pH between 5.5 and 7.0 and viscosity (75.7 cP) were within the acceptable range for oral administration. Phytochemical tests confirmed the presence of terpenoids. The nanoemulsion showed enhanced physicochemical stability and uniformity compared to conventional suspensions.

Conclusion: The formulated nanoemulsion-based syrup significantly improves the solubility and bioavailability of andrographolide. It holds promise as a stable, effective, and patient-friendly antiviral delivery system..

Key Words: *Andrographolide, Nanoemulsion, Oral syrup, zeta potential, Antiviral activity, Bioavailability, Phytoconstituents, Drug delivery system*

1. INTRODUCTION

Viral infections remain a major global health concern, with illnesses such as influenza, dengue, hepatitis, and COVID-19 contributing to high rates of illness and death. In the ongoing search for effective antiviral therapies, medicinal plants have attracted growing interest due to their abundance of biologically active compounds. One such plant is Andrographis paniculata, also known as Kalmegh, which has been traditionally used in Ayurvedic and Chinese medicine. Its primary bioactive compound, andrographolide a labdane-type diterpenoid has been extensively studied for its immune-enhancing, anti-inflammatory, and especially antiviral activities1.

Although andrographolide shows promising therapeutic effects, its clinical use is limited by several drawbacks, including poor water solubility (approximately ~3.3 mg/mL), an intensely bitter taste, and low oral bioavailability. It is rapidly metabolized during the first-pass effect and is categorized as a Biopharmaceutics Classification System (BCS) Class II compound, indicating that its absorption is restricted by its low dissolution rate2. To address these limitations, advanced drug delivery systems such as nanoemulsions have been investigated. Nanoemulsions consist of nanoscale droplets stabilized by surfactants and co-surfactants, and they offer a significant enhancement in the solubility and gastrointestinal uptake of lipophilic agents like andrographolide3.

Oral liquid formulations, particularly syrups, offer several benefits, including ease of administration, improved patient

compliance, and a quicker onset of action. Incorporating nanoemulsion technology into such dosage forms can further enhance the delivery and controlled release of poorly soluble plant-derived compounds. This study aims to develop and evaluate a nanoemulsion-based oral syrup loaded with andrographolide, targeting improvements in physicochemical stability, bioavailability, and antiviral potential2,3

1.1 Plant Profile: Andrographis paniculata

Andrographis paniculata (Burm.f.) Wall. ex Nees, widely known as Kalmegh, is a prominent medicinal herb belonging to the Acanthaceae family. Commonly called the "King of Bitters" because of its intensely bitter flavor, this plant is native to South and Southeast Asia, including regions such as India, Sri Lanka, and Thailand. It thrives in tropical and subtropical environments1,4.

Fig. 1. *Andrographis paniculata* plant and flower.

1.2 Botanical Description

Feature	Description
Botanical Name	Andrographis paniculata (Burm.f.) Wall. ex Nees
Family	Acanthaceae
Common Names	Kalmegh, King of Bitters, Creat, Green Chiretta
Habitat	Native to India and Sri Lanka; found in tropical and subtropical Asia
Habit	Erect annual herb
Height	30–110 cm
Stem	Slender, green, quadrangular, hairless or sparsely hairy
Leaves	Simple, opposite, lanceolate, glabrous; 2–10 cm long, 1–3 cm wide
Flowers	Small, tubular, pale violet with purple markings; arranged in racemes
Flowering Season	July to October (varies with region)
Fruits	Linear capsules, ~2–3 cm long, with acute apex
Seeds	Numerous, yellow-brown, sub-quadrate, glabrous

Feature	Description
Part Used	Mainly leaves and aerial parts
Phytochemicals	Andrographolide, neoandrographolide, deoxyandrographolide, flavonoids
Medicinal Properties	Antiviral, anti-inflammatory, hepatoprotective, immunostimulant

Table 1: Botanical description of *Andrographis paniculata*[5]

1.3 Traditional and Medicinal Uses

Kalmegh (Andrographis paniculata) is a traditional medicinal herb widely used in Ayurveda, Traditional Chinese Medicine, and other Asian practices for treating infections, fevers, inflammation, and digestive problems. Known as a bitter febrifuge and immune booster, it is commonly used for respiratory, gastrointestinal, and liver disorders[1]. The herb's main active compound, andrographolide, has antiviral, antimicrobial, anti-inflammatory, and liver-protective effects. Traditionally, Kalmegh has been used for illnesses like flu, malaria, tuberculosis, and HIV symptoms[6]. Modern research supports its effectiveness against viruses such as influenza, dengue, Zika, hepatitis, and coronaviruses by blocking viral replication and boosting the immune response.

1.4 Nanoemulsion

Nanoemulsions are colloidal systems where tiny droplets (20–200 nm) of one liquid, like oil, are dispersed in another immiscible liquid, like water. They appear clear or slightly cloudy and remain stable over time without droplets merging or settling. Surfactants and co-surfactants stabilize these droplets by reducing surface tension and maintaining uniformity. Each droplet has an oil core, which can encapsulate hydrophobic drugs for protection and delivery, surrounded by a surfactant shell that stabilizes and disperses the droplets in the continuous phase[7,8].

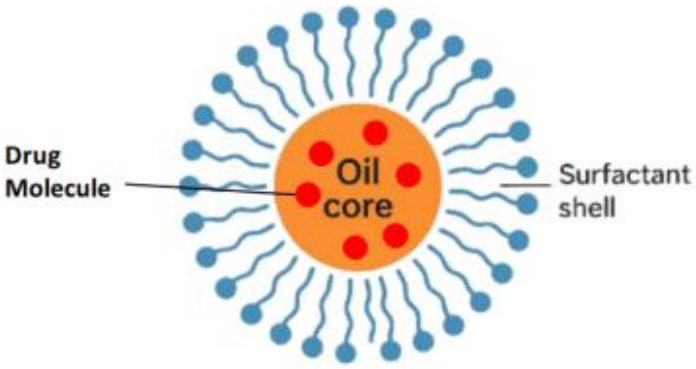


Fig. 2. A nanoemulsion droplet structure

1.5 Components of Nanoemulsion

A nanoemulsion system generally consists of four main components: an oil phase, a surfactant, a co-surfactant, and an aqueous phase[3,9].

Components	Role/Function	Examples
Oil phase	Dissolves hydrophobic drug in droplet core	Vegetable oils, MCT (medium-chain triglycerides), α-tocopherol
Surfactant	Stabilizes droplets by lowering interfacial tension; forms droplet shell	Tween 80, Cremophor EL, lecithin

Co- surfactant	Further reduces interfacial tension, enhances flexibility at interface	Ethanol, propylene glycol
Aqueous phase	Continuous water phase	Water, buffer

Table 2: Components of Nanoemulsion and their role with examples

2. MATERIALS AND METHODS

2.1 Materials

The materials used in the formulation included andrographolide (2.5 g), Tween 80 (5 mL), propylene glycol (5 mL), castor oil (30 mL), sodium benzoate (0.25 g), sorbitol (25 mL), citric acid (q.s.), honey/peppermint oil (2 mL), and distilled water (to make up 250 mL)[3,10,11]. The complete composition is shown in Table 3.

S.No.	Ingredient	Purpose	Quantity
1	Andrographolide	Active pharmaceutical ingredient	2.5 g
2	Tween 80	Surfactant	5 mL
3	Propylene Glycol	Co-surfactant	5 mL
4	Castor Oil	Oil phase	30 mL
5	Sodium Benzoate	Preservative	0.25 g
6	Sorbitol	Sweetening agent	25 mL
7	Citric Acid	pH adjuster	q.s. (as required)
8	Honey/Peppermint oil	Flavoring Agent	2ml
9	Distilled Water	Vehicle/Base	184 mL (q.s.)

Table 3: Composition of Nanoemulsion Oral Syrup

2.2 Extraction Method

In this study, Microwave-Assisted Extraction (MAE) was used to extract andrographolide from *Andrographis paniculata* as a faster and more efficient alternative to traditional methods like Soxhlet extraction. MAE offers benefits such as reduced time, lower solvent use, and better preservation of heat-sensitive compounds. A hydroalcoholic solvent (ethanol:water, 70:30) was chosen for its ability to dissolve bioactive compounds and absorb microwave energy effectively[12,13].

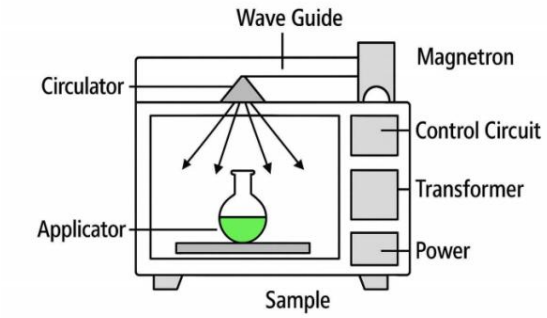


Fig. 3. Microwave-Assisted Extraction (MAE)

Dried plant material was coarsely powdered, sieved through a #60 mesh, and mixed with the solvent in a 1:10 or 1:15 w/v ratio. The mixture was stirred for 10–15 minutes, then subjected to microwave bursts (400–600W) in a domestic microwave for a total of 5–10 minutes, with intermittent stirring. After heating, the extract was cooled, filtered, and concentrated using a water bath at 40–50°C to evaporate the solvent gently. The final semi-solid extract was collected and stored for further use[14].

Fig. 4. Hydroalcoholic extract of Andrographis paniculata after solvent evaporation following Microwave-Assisted Extraction (MAE)[15].

2.3 Instrumentation

The formulation process employed the following equipment: a magnetic stirrer with a hot plate (ISKO®) for initial phase mixing, a mechanical stirrer (Elecopto, Model No. HV-LS-157) for emulsification, and an ultrasonic probe sonicator (OSC-3L) for particle size reduction[10].

2.4 Formulation of Nanoemulsion Oral Syrup

The preparation of the nanoemulsion was conducted using the oil-in-water (O/W) emulsification technique, wherein andrographolide was incorporated into the oil phase and subsequently dispersed within the aqueous phase to form a stable nanoemulsion (Figure 5,6)[4,10]. Table 3 contains the composition and functional role of Ingredients used in the preparation of andrographolide-loaded nanoemulsion oral syrup[16,17].

2.5 Formulation Procedure

Oil Phase Preparation: Castor oil (30 mL) was transferred into a clean beaker, and andrographolide (2.5 g) was added. The mixture was gently tirred and heated to ~40–45 °C using a magnetic stirrer to facilitate drug solubilization. Tween 80 (5 mL) and propylene glycol (5 mL) were then added sequentially with continuous stirring until a clear, homogenous oil phase was obtained.

Aqueous Phase Preparation: Separately, distilled water (184 mL) was measured and combined with sorbitol (25 mL) and sodium benzoate (0.25 g) under magnetic stirring until fully dissolved. Citric acid was added dropwise to adjust the pH to ~6.0, and 2 mL of honey or peppermint oil was added for flavor enhancement[3,18].

Pre-emulsion Formation: The oil phase was slowly added to the aqueous phase under continuous stirring at 500–1000 rpm to form a coarse emulsion. This initial mixing ensured uniform distribution of the oil droplets within the aqueous matrix[20,21].

Homogenization: The pre-emulsion was subjected to high-speed mechanical homogenization at 10,000–15,000 rpm for several minutes. This reduced the droplet size, enhancing the physical stability and uniformity of the emulsion[20,21].

Ultrasonication: The homogenized emulsion was further processed using ultrasonication at 20–25 kHz for 5–10 minutes (in pulse mode) to achieve nanometric droplet size. Ultrasonication generated intense cavitation, further reducing droplet size and enhancing kinetic stability[23,24].

Final Adjustment and Storage: The final volume was adjusted to 250 mL with distilled water. The formulation was thoroughly mixed and evaluated for clarity, phase separation, and pH. The finished nanoemulsion was then stored in airtight glass containers at room temperature, away from light and moisture, for subsequent characterization[20].

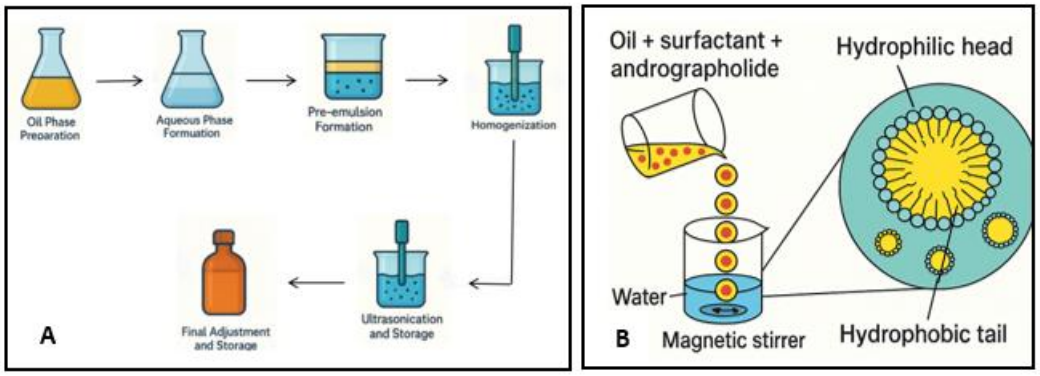


Fig. 5. (A) Schematic representation of the formulation process; (B) Technique applied for the development of andrographolide-loaded nanoemulsion.

2.6 Evaluation Parameters

The prepared nanoemulsion was assessed for **physical characteristics**: particle size, zeta potential, size distribution (polydispersity index), pH, viscosity, preliminary phytochemical evaluation, terpenoid content in extract, appearance and antiviral performance.

2.6.1 Particle Size

Particle size reflects the average droplet diameter, which significantly influences the drug's solubility, rate of absorption, and overall bioavailability. Smaller droplet sizes (typically under 200 nm) contribute to improved formulation stability and quicker absorption[25].

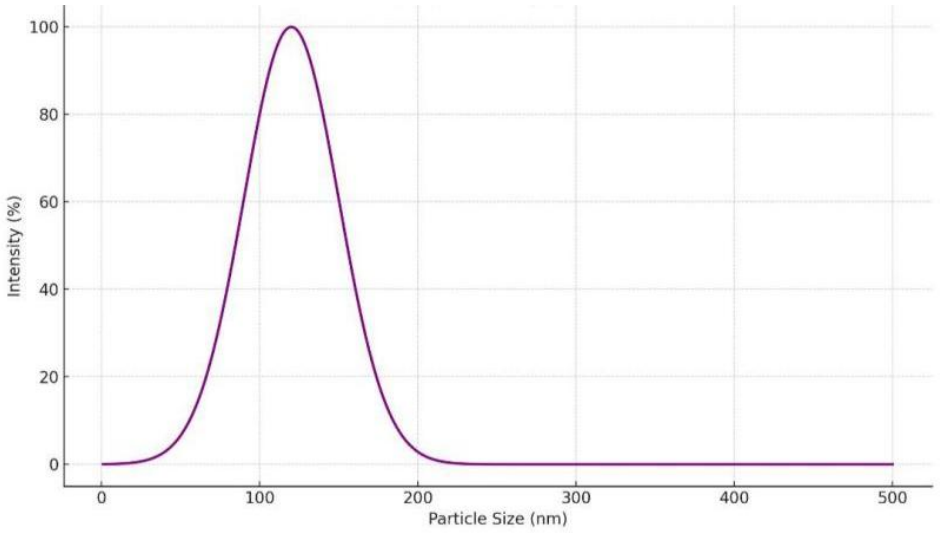


Fig. 6. This DLS graph suggests that the nanoemulsion is stable, well-dispersed, and within the ideal nanometric range (1–200 nm).

2.6.2 Zeta Potential

Zeta potential measures the electrical charge on the surface of the droplets and is a key indicator of the system's physical stability. Higher positive or negative zeta potential values create electrostatic repulsion between particles, preventing them from sticking together. This minimizes the risk of aggregation. Zeta potential values beyond +30 mV or below –30 mV are generally considered a sign of good stability. Together, particle size and zeta potential provide valuable insights into the performance, consistency, and shelf-life of the nanoemulsion[26].

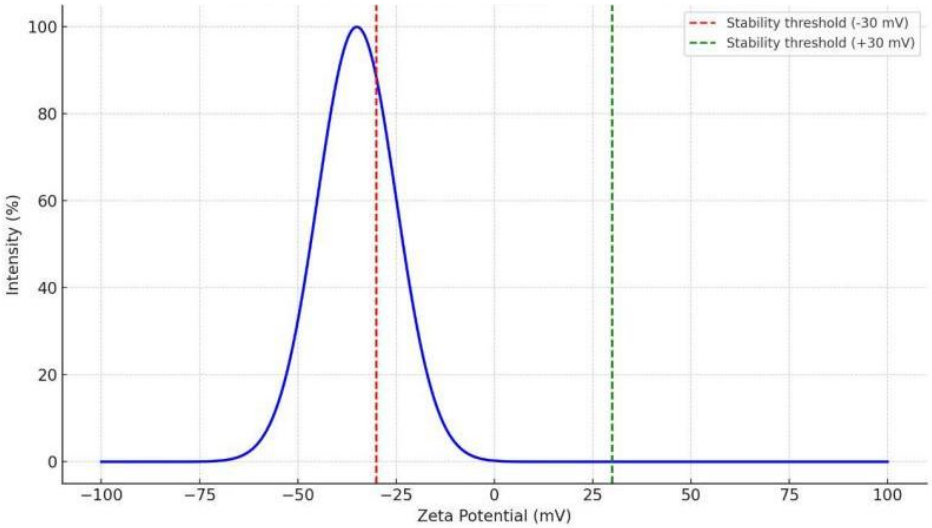


Fig. 7. This zeta potential graph suggests that the nanoemulsion is physically stable, as the zeta potential value (around –35 mV) exceeds the ±30 mV threshold.

2.6.3 PDI (Polydispersity index)

The Polydispersity Index (PDI) is a number (without any units) that tells us how evenly the particle sizes are distributed in systems like nanoemulsions, nanoparticles, or liposomes. It helps to check the quality, uniformity, and stability of nanocarrier-based

drug delivery systems. A lower PDI means the particles are almost the same size, which makes the formulation mo re stable and reliable for drug delivery. In pharmaceutical nanoformulations, a PDI below 0.3 is usually considered good. However, in some emulsions or more complex systems, a value up to 0.5 can also be acceptable[27].

2.6.4 pH determination

The pH of a nanoemulsion plays an important role in its overall stability, effectiveness, and how well it suits the human body. Since this nanoemulsion is meant for oral use, it is necessary to keep the pH in a range that will not irritate the stomach or intestines and will keep the drug (andrographolide) stable[28].

The pH of the final formulation is expected to be between 5.5 and 7.0, which is slightly acidic to neutral. This range is ideal for oral formulations and helps in better compatibility with the digestive system. Andrographolide, the main active compound, is sensitive to very acidic or basic environments, which can break it down or reduce its effectiveness. So, maintaining the pH around neutral helps in keeping the drug stable. It also supports the proper functioning of surfactants and co-surfactants in the nanoemulsion[19].

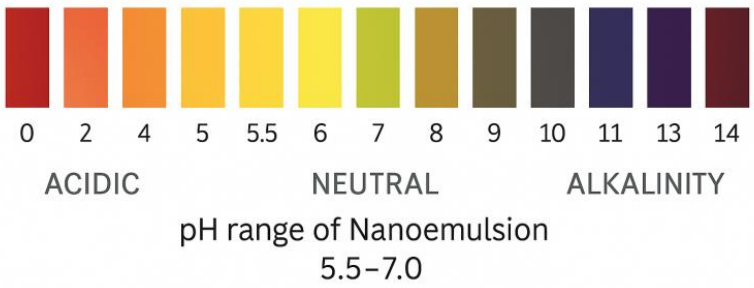


Fig. 8. ph range of nanoemulsion.

2.6.5 Viscosity Measurement

Viscosity is a critical physicochemical parameter in the evaluation of nanoemulsion based formulations, particularly for oral administration.It affects how easily the liquid can be poured, how good it tastes, how stable it stays over time, and how much people will accept it. Viscosity shows how the liquid flows, which is very important for nanoemulsions because it not only makes it easier to take but also controls how the drug spreads and how stable the tiny droplets remain in the liquid.

The expected viscosity of the formulated nanoemulsion is anticipated to lie in the lower to moderate range (e.g., 50–100 cP), based on the typical composition of oil, surfactant, co-surfactant, and aqueous phase used in nanoemulsion systems[29].

2.6.6 Preliminary Phytochemical Evaluation

Preliminary phytochemical evaluation is an important step in the assessment of nanoemulsion formulations, especially those derived from plant-based extracts. This evaluation helps to confirm the presence of key phytoconstituents such as alkaloids, flavonoids, terpenoids, glycosides, saponins, and tannins, which contribute to the therapeutic effects of the formulation[31,39] .

In the case of nanoemulsions containing andrographolide, a major terpenoid compound, specific tests like the Salkowski test are employed to detect the presence of terpenoids. In this test, a reddish-brown coloration at the interface upon the addition of concentrated sulfuric acid indicates a positive result for terpenoids[30].

2.6.7 Terpenoid Content in Extract

Estimation of terpenoid content is an important step in the evaluation of herbal extracts used for nanoemulsion formulation. Since andrographolide, the primary active compound in Andrographis paniculata, belongs to the terpenoid class, assessing its presence ensures effective drug loading in the nanoemulsion[31].

Additionally, Fourier Transform Infrared Spectroscopy (FTIR) is commonly used to identify the characteristic functional groups of the bioactive constituents. FTIR analysis provides valuable information about the chemical structure, functional integrity, and stability of the extract[25-31].

2.6.8 Appearance (visual observation)

The physical appearance of a nanoemulsion provides initial insight into its stability and uniformity. When prepared using herbal extracts, the formulation may exhibit a characteristic color and opacity depending on the nature of the active constituents. A greenish opaque appearance is often observed when plant-based actives like andrographolide are used. Consistency in texture without any visible phase separation, creaming, or sedimentation indicates effective emulsification and physical stability of the formulation[10].

2.6.9 Antiviral performance

The antiviral effect was considered the main goal of the study. While it was not directly evaluated, existing literature suggests that improved bioavailability of andrographolide is likely to enhance its antiviral effectiveness[32,33].

3. RESULTS

The formulated andrographolide-loaded nanoemulsion oral syrup was subjected to various evaluation parameters to ensure its physicochemical stability, uniformity, and potential suitability for antiviral application.

3.1 Particle Size

The particle size analysis of the formulated nanoemulsion was performed using a **Malvern Zetasizer Nano ZS90**. The average particle size (Z-Average, d.nm) was found to be **191.1 nm**, indicating the successful formation of a nano-sized emulsion system. The small droplet size is beneficial for improving the **bioavailability of poorly water-soluble compounds such as andrographolide**, as it increases the surface area available for absorption[34].

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	191.1	Peak 1:	272.7	84.6	121.0
PdI:	0.302	Peak 2:	64.04	12.6	16.18
Intercept:	0.924	Peak 3:	4997	2.8	609.9
Result quality	Good				

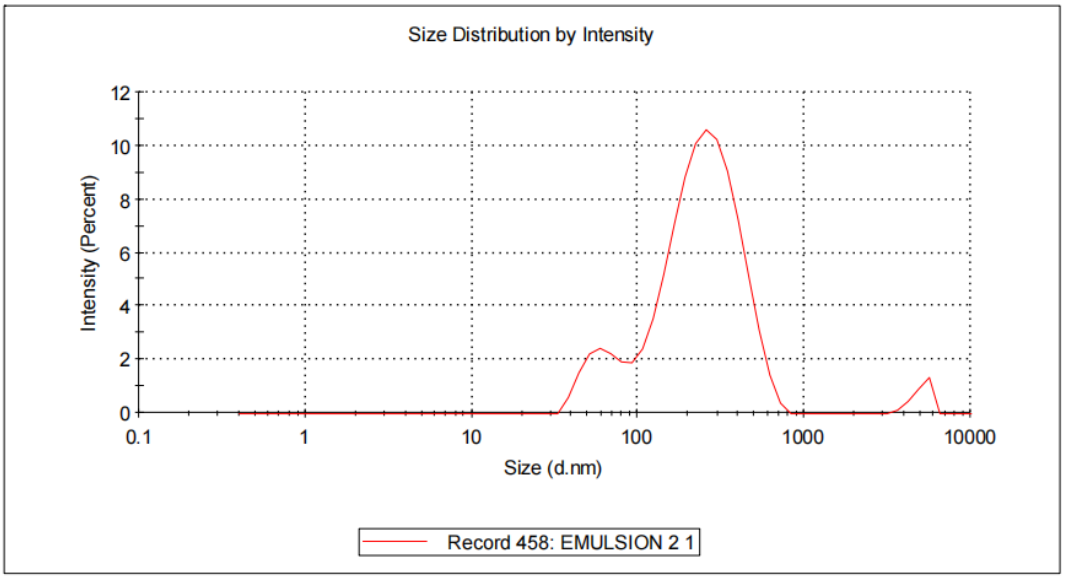
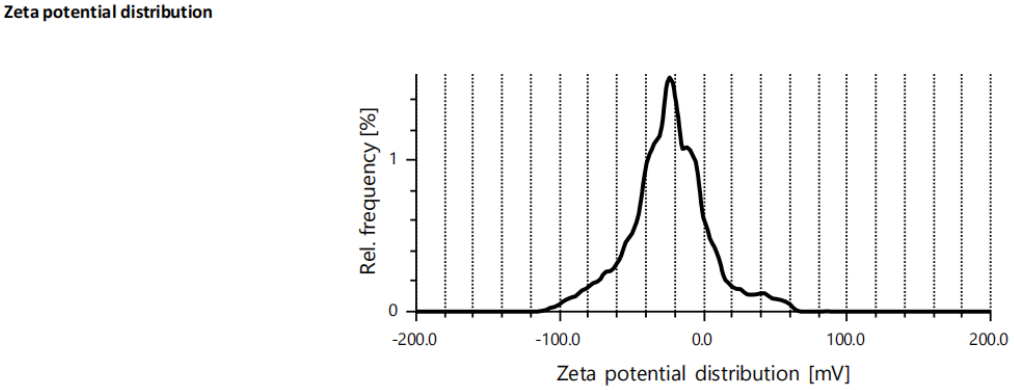


Fig. 9. Intensity-Based Size4 Distribution Curve.

3.2 Zeta Potential

The zeta potential of the prepared andrographolide-loaded nanoemulsion was measured using the Litesizer™ 500 (Anton Paar). The recorded zeta potential was -31.5 mV, indicating that the formulation possesses moderate electrostatic stability.



Result

Mean zeta potential	-31.5 mV	**Mean intensity**	710.1 kcounts/s
Standard deviation	1.1 mV	**Filter opical density**	2.7126
Distribution peak	-27.2 mV	**Conductivity**	0.059 mS/cm
Electrophoretic Mobility	-2.1621 µm*cm/Vs	**Transmittance**	46.7 %

Fig. 10. Zeta potential distribution.

3.3 PDI (Polydispersity index)

The PDI (Polydispersity index) analysis of the formulated nanoemulsion was performed using a **Malvern Zetasizer Nano ZS90**. The obtained PDI value was 0.301, indicating a moderately polydisperse system. Although slightly above the ideal monodisperse range (<0.3), the value suggests a reasonably acceptable particle size distribution suitable for nanoemulsion-based drug delivery systems.

Results

Z-Average (d.nm): 191.1

PdI: 0.302

Intercept: 0.924

Result quality Good

3.4 pH determination

The pH of the formulated nanoemulsion was found to be in the range of 6 to 7, as determined using pH indicator paper. This slightly acidic to neutral pH falls within the acceptable range for oral administration. Such pH conditions are considered suitable for maintaining the chemical stability of andrographolide and ensuring patient compliance, while minimizing the risk of gastrointestinal discomfort[37].

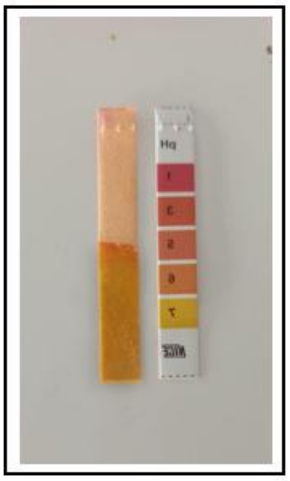


Fig. 11. ph result of the nanoemulsion.

3.5 Terpenoid Content in Extract

The terpenoid content in the plant extract was quantitatively estimated using Fourier Transform Infrared (FTIR) spectroscopy. The analysis was conducted using a PerkinElmer FTIR spectrometer. Characteristic absorption peaks corresponding to terpenoid functional groups were observed, confirming the presence of andrographolide and related compounds. The total terpenoid content was found to be 4.85%, indicating a sufficient concentration of bioactive constituents suitable for nanoemulsion formulation[38].

TEST RESULTS

S.No.	Test Parameters	Results	Units	Specifications	Method Reference
Chemical					
1	FTIR Scanning	Complies	-	-	IHS
2	Terpenoids	4.85	%	-	IHS

Remarks : Note :- Party asked for the above tests only.

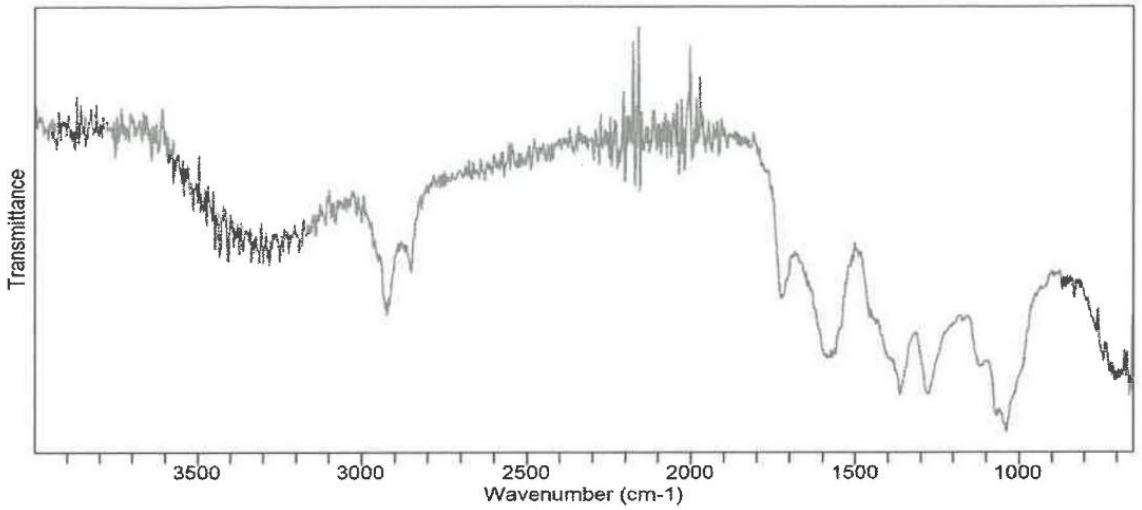


Fig. 12. FT-IR spectra of Terpenoid

3.6 Viscosity

The viscosity of the formulated nanoemulsion was measured using a Brookfield Viscometer (Model: LVDVE, Serial Number: 8503379, Made in U.S.A.). The measurement was conducted using Spindle 61 at a speed of 50 rpm, and the viscosity was found to be 75.7 centipoise (cP). This moderate viscosity is suitable for oral liquid formulations, ensuring ease of pouring and swallowing while also enhancing physical stability by reducing the risk of creaming or sedimentation during storage[29].

Fig. 13. Viscosity Measurement

3.7 Preliminary Phytochemical Evaluation

The presence of terpenoids in the herbal extract was confirmed using the Salkowski test, a standard qualitative method for detecting terpenoid compounds. In this test, the extract was treated with chloroform followed by concentrated sulfuric acid, resulting in the formation of a reddish-brown interface, which is indicative of terpenoid presence. This positive result supports the presence of andrographolide, a major diterpenoid compound in *Andrographis paniculata*, and justifies further formulation into a nanoemulsion for enhanced delivery[30,31].

Fig. 14. Preliminary Phytochemical Evaluation

3.8 Appearance (visual observation)

The physical appearance of the nanoemulsion was assessed visually. The formulation exhibited a light yellowish-brown (beige) opaque color, which can be attributed to the presence of the plant extract and oil components. The emulsion displayed a smooth and uniform consistency without any signs of phase separation, creaming, or sedimentation, indicating good physical stability and effective emulsification of the oil and aqueous phases[10].

Fig. 15. physical appearance of the nanoemulsion.

3.9 Antiviral performance

Although direct antiviral testing was not performed, the nanoemulsion formulation of andrographolide (AG) showed promising potential by significantly enhancing its oral bioavailability. This could lead to higher drug levels in the body and improved antiviral effects, especially against viruses like influenza, dengue, Zika, and SARS-CoV-2. The nano-syrup effectively solubilizes, protects, and controls the release of AG, making it a promising oral delivery system. Future studies using cell or animal models are needed to confirm its antiviral efficacy[32,33,40].

4. DISCUSSION

This study developed a nanoemulsion-based oral syrup of *Andrographis paniculata* to enhance the solubility and bioavailability of andrographolide, its key antiviral compound. Microwave-assisted extraction ensured efficient andrographolide recovery[1-5]. Castor oil, Tween 80, and propylene glycol were effectively used as oil, surfactant, and co-surfactant, contributing to a stable formulation[3,10,11]. The nanoemulsion showed favorable properties—191.1 nm particle size, –31.5 mV zeta potential, pH 6–7, PDI 0.301, and viscosity of 75.7 cP—ensuring stability and suitability for oral use. Phytochemical analysis confirmed terpenoid content (4.85%), and the stable physical appearance indicated successful emulsification. Although antiviral activity wasn't directly tested, the formulation shows strong potential for enhanced delivery of plant-based antiviral agents[25-38].

5. CONCLUSIONS

The present study successfully formulated and evaluated a nanoemulsion-based oral syrup of andrographolide derived from Andrographis paniculata. The formulation was developed using microwave-assisted extraction and optimized using Tween 80, propylene glycol, and castor oil as core excipients. Evaluation revealed an ideal average droplet size of 191.1 nm, a zeta potential of

-31.5 mV ensuring electrostatic stability, a near-neutral pH (6.4), and moderate viscosity (75.7 cP), all of which are suitable for oral administration.

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