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Optimization And Pharmacognostic Characterization Of Herbal Drug

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ABSTRACT:
This study investigates the organoleptic, physicochemical, and pharmaceutical properties of formulations FE-1 through FE-8, along with the phytochemical profiles of Zingiber officinale (ginger), Allium cepa (onion), and Citrus limon (lemon). Organoleptic evaluation revealed distinct characteristics, while physicochemical standardization and phytochemical profiling ensured formulation consistency. The particle size, viscosity, zeta potential, and polydispersity index (PDI) analyses highlighted the advantages of smaller particles (e.g., FE-7) for improved bioavailability, while higher viscosity formulations were identified for sustained release applications. Osmolality testing showed that FE-2, FE-7, and FE-6 are isotonic, while others require tonicity adjustment. The pH values of the formulations were suitable for a wide range of applications. Drug content analysis revealed variations, with FE-1 containing the highest drug concentration. SEM imaging confirmed the presence of smooth, oval nanoparticles, supporting the successful formulation process. These findings provide critical insights into optimizing formulations for pharmaceutical and cosmetic applications.

INTRODUCTION:
Wound The rising demand for plant-based therapeutics has propelled research into the use of phytoconstituents for targeted drug delivery systems, including ophthalmic formulations. Nanoemulsion-based drug delivery offers enhanced solubility, stability, and bioavailability of bioactive plant compounds, particularly for ocular applications where precision and sustained release are crucial. This study investigates the potential of three widely used medicinal plants—*Zingiber officinale* (ginger), *Allium cepa* (onion), and *Citrus limon* (lemon)—for the development of an optimized nanoemulsion formulation for ophthalmic delivery.

Zingiber officinale (Ginger)
Zingiber officinale is a well-known rhizomatous plant used extensively in both culinary and traditional medicine. Its bioactive constituents include gingerols, shogaols, paradols, and zingerone, which are known to possess anti-inflammatory, antioxidant, and antimicrobial activities (Ali et al., 2008; Butt & Sultan, 2011). Ginger extract has shown significant scavenging activity against reactive oxygen species (ROS), which are implicated in the pathogenesis of several ocular diseases including cataracts and age-related macular degeneration (Fajardo et al.,

2020). Studies also suggest that ginger can inhibit cyclooxygenase (COX) and lipoxygenase (LOX) pathways, reducing ocular inflammation (Pan et al., 2008).

Allium cepa (Onion)
Allium cepa is rich in phenolic acids and flavonoids, primarily quercetin, kaempferol, and sulfur-containing compounds like allicin. These components are known for their free radical scavenging and antimicrobial actions (Griffiths et al., 2002; Lanzotti, 2006). In ocular pharmacology, quercetin is of particular interest due to its ability to stabilize mast cells and inhibit histamine release, making it beneficial in allergic conjunctivitis and other inflammatory eye conditions (Nagai & Ito, 2011). Moreover, onion extract has shown protective effects against oxidative stress-induced retinal damage in animal models (Kang et al., 2012).

Citrus limon (Lemon)
Citrus limon, a member of the Rutaceae family, contains a wide range of bioactive compounds such as ascorbic acid (vitamin C), hesperidin, eriocitrin, and limonene (Rafiq et al., 2016; Gorinstein et al., 2001). These constituents contribute to lemon's strong antioxidant and antimicrobial properties. Vitamin C, in particular, plays a vital role in maintaining ocular health by preventing lens opacification and supporting corneal repair (Taylor et al., 2002). Furthermore, citrus essential oils have demonstrated inhibitory effects against various ocular pathogens, which could be beneficial in the management of bacterial conjunctivitis.

Rationale for Nanoemulsion Formulation
Nanoemulsions are thermodynamically stable dispersions with droplet sizes typically in the range of 20–200 nm. They are ideal carriers for poorly water-soluble phytochemicals, improving ocular penetration and retention time while minimizing systemic side effects (Mishra et al., 2008). The use of plant extracts in nanoemulsion formulations may enhance therapeutic outcomes by combining the pharmacological effects of multiple bioactives with improved delivery to ocular tissues.

Thus, the integration of *Zingiber officinale*, *Allium cepa*, and *Citrus limon* into a nanoemulsion delivery system presents a promising approach for the development of an effective and biocompatible ophthalmic formulation aimed at treating inflammation, oxidative stress, and microbial infections of the eye.

Procurement of Sample
Zingiber officinale, Allium cepa, and Citrus limon were procured from the local market and cleaned with distilled water. Ginger rhizomes were peeled and sliced; onions were peeled and chopped; lemon peels were separated, cut, and the juice stored if needed. All materials were air- or oven-dried to prevent microbial growth and concentrate actives. Dried samples were ground into fine powder and stored in airtight containers. Any liquid extracts were refrigerated in sterile, sealed containers to maintain quality.

Collection of the pant material
After the Zingiber officinale (ginger), Allium cepa (onion), and Citrus limon (lemon) plant materials were collected from the nearby market, the following steps were taken to process and store them for further use.

Processing and Storage of Sample:, Preparation of Powder, Storage
After collection, the plant materials were shade-dried by spreading them in a well-ventilated area away from direct sunlight. They were allowed to dry naturally until they became brittle and completely moisture-free, helping to preserve their active phytochemicals. Once dried, the materials were ground into a coarse powder using a clean mortar and pestle or suitable grinding equipment, ensuring they were in a manageable form for extraction. The coarse powders were then transferred into clean, airtight containers such as glass jars or food-grade plastic containers with tight-fitting lids. These containers were filled properly to reduce air exposure, and each was clearly labeled with the name of the plant material and the date of collection to ensure proper identification and traceability.

Pharmacognostic evaluation
Pharmacognostic evaluation refers to the systematic study of medicinal plants to determine their identity, purity, quality, and biological properties. .

Ash values
Ash values help assess the quality and purity of crude drugs, especially in powdered form. Ash represents the inorganic residue left after incineration, including natural mineral content and possible adulterants. Total ash indicates the drug's cleanliness, while acid-insoluble ash reflects the presence of silica or dirt. Sulphated ash, obtained by treating with sulphuric acid, is less fusible and gives more accurate results in some cases:

Total Ash Value = (Weight of Ash / Weight of Plant Material) × 100

Determination of total ash value
To determine the total ash value of a plant material, first, clean and dry a porcelain or silica crucible and weigh it accurately, recording the weight as "W1." Next, weigh a known amount of plant material (typically 2-5 grams) and place it in the crucible, noting the weight as "W2." The crucible with the plant material is then placed in a muffle furnace or high-temperature furnace (around 550-600°C) for several hours to incinerate the sample, leaving a white or grayish ash residue. After the incineration, the crucible is allowed to cool in a desiccator to prevent moisture absorption. Once cooled, the crucible with the ash is weighed, and the weight is recorded as "W3." Finally, the total ash value is calculated using the formula:

Total Ash Value = [(W3 - W1) / (W2 - W1)] × 100

In this formula, (W3 - W1) represents the weight of the ash residue, and (W2 - W1) represents the weight of the plant material.

Determination of acid insoluble ash value
To determine the acid-insoluble ash value, first, clean and weigh a porcelain or silica crucible (W1), then add 2-5 grams of the plant material (W2). Incinerate the sample in a furnace at 550-600°C until only ash remains, then cool and weigh the crucible with total ash (W3). Add 25 mL of dilute hydrochloric acid to the crucible and heat for 5-10 minutes to dissolve soluble ash. Filter and wash the residue with hot water, then dry the acid-insoluble ash in an oven at 105°C until it reaches a constant weight. Finally, weigh the residue (W4). The acid-insoluble ash value is calculated using the formula:

Acid-Insoluble Ash Value = [(W4 - W1) / (W2 - W1)] × 100
In this formula, (W4 - W1) represents the weight of the acid-insoluble ash residue, and (W2 - W1) represents the weight of the plant material.

Determination of water soluble ash value
To determine the water-soluble ash value, first, clean and weigh a porcelain or silica crucible (W1), then add 2-5 grams of the plant material (W2). Incinerate the sample in a furnace at 550-600°C until only ash remains, then cool and weigh the crucible with total ash (W3). Add 25 mL of distilled water to the crucible and stir to dissolve the water-soluble components. Filter the mixture and wash the residue with hot water. Dry the water-soluble ash on filter paper at 105°C until a constant weight is achieved, then weigh the residue (W4). The water-soluble ash value is calculated using the formula:

Water-Soluble Ash Value = [(W4 - W1) / (W2 - W1)] × 100

In this formula, (W4 - W1) represents the weight of the water-soluble ash residue, and (W2 - W1) represents the weight of the plant material.

Loss on drying
To determine the loss on drying (LOD), first, clean and dry a weighing dish or crucible and record its weight as "W1". Then, add 1-5 grams of the sample to the crucible and record its weight as "W2". Transfer the crucible with the sample to an oven set at a specified temperature (105°C to 110°C for organic substances, 60°C to 80°C for inorganic substances). Dry the sample until it reaches a constant weight, checking at regular intervals. After drying, cool the crucible in a desiccator and weigh the crucible with the dried sample (W3). The loss on drying is calculated using the formula:

Loss on Drying (LOD) = [(W2 - W3) / (W2 - W1)] × 100

In this formula, (W2 - W3) represents the weight loss after drying, and (W2 - W1) represents the initial weight of the sample.

Extractive values
To determine the water-soluble extractive value, weigh 5 grams of plant material (W1). Add enough water to cover the material and allow it to stand for 1-4 hours, shaking intermittently. Filter the mixture and evaporate the filtrate to dryness in a weighed evaporating dish. Dry the dish and residue to a constant weight in an oven. Weigh the dish with the residue (W2) and calculate the water-soluble extractive value using the formula:

Water-Soluble Extractive Value = [(W2 - W1) / W1] × 100

In this formula, (W2 - W1) represents the weight of the water-soluble extract, and W1 represents the weight of the plant material.

Determination of alcohol soluble extractive value
To determine the ethanol-soluble extractive value, 5 grams of air-dried plant powder was macerated with 100 ml of 90% ethanol for 24 hours. After filtration, 25 ml of the filtrate was evaporated to dryness, dried at 105°C, and weighed. The extractive value is calculated as a percentage based on the air-dried plant material, helping assess the solubility and extractability of active compounds for quality control in herbal medicines.

Determination of Total Crude Fibre Content
The crude fiber content of Zingiber officinale (ginger), Allium cepa (onion), and Citrus limon (lemon) was determined using the Kűrschner-Hanak method. First, 1,000 g of finely chopped sample was mixed with 25 mL of 80% acetic acid and 2.5 mL of concentrated nitric acid, and refluxed for 30 minutes. The hot mixture was filtered through a pre-weighed 1-G-3 filter crucible under weak evacuation, then washed with acetic-nitric acid, hot water, ethanol, and ether. The precipitate was dried at 105°C for 30 minutes, cooled, and weighed. The crude fiber content was calculated using the formula:

Crude fiber content (%) = a*100 /Ok
Where are they
- a - measured amount of crude fiber (g)
- Ok - measured quantity of sample taken for analysis (g)

Determination of Total Volatile matter
Petri dish was dried and weighed. 2 gm of sample were taken ad accurately weighted and dried at 105± 2^0C in an oven for 4 hours or till constant mass. Cooled in dessiccator and weighed.

Volatile content (%) = (loss in weight/weight of the sample in g) x 100

Determination of heavy metal and mineral analysis
To determine the major heavy metals and minerals content, 500 mg of air-dried stem powder was used. Ash was prepared from 2 g of Zingiber officinale (ginger), Allium cepa (onion), and Citrus limon (lemon) samples by heating them in a muffle furnace at 150°C until a constant weight was achieved. The major inorganic constituents of the powders and ash were quantified using an atomic absorption spectrometer (ASS) (Perkin Elmer-400), with argon as the carrier gas and a flow rate of 1 mL/2 min.

Behaviour of Powdered sample
Behaviors of stem powder of Zingiber officinale, Allium cepa, and Citrus limon plants parts with different chemical reagent were performed to detect the occurrence of phytoconstituents along with color changes under ordinary daylight by standard method.

Preparation of the extracts
Extraction was carried out in Soxhlet apparatus not exceeding 600 c and the extract thus obtained was concentrated below 600 c.

Soxhlet Extraction
The Soxhlet extraction method involves extracting compounds from plant materials using solvents of varying polarities. First, assemble the Soxhlet apparatus and weigh 1 kg of powdered plant material. Select solvents based on the target compounds. Place the plant material in the extraction chamber and fill the round-bottom flask with the solvent. Heat the flask, allowing the solvent to vaporize, condense, and extract compounds from the plant. The cycle repeats until extraction is complete. Afterward, collect and filter the extract, then concentrate and dry it using rotary evaporation and freeze-drying or vacuum drying.

Preliminary Phytochemical Screening of extract
Phytochemicals are plant-derived chemicals that play a protective role in plants and are researched for their potential health benefits. To establish the chemical profile of an extract, 1.5 grams of the extract is accurately measured and dissolved separately to prepare a test solution. Each extract is tested for various phytochemicals, including alkaloids, carbohydrates, steroids, glycosides, phenols, flavonoids, saponins, triterpenoids, phlobatannins, cardiac glucosides, anthraquinones, and proteins, using standard qualitative and quantitative methods. The prepared solution is labeled and stored in a refrigerator when not in use.

Detection of alkaloids:
These are nitrogenous organic compounds present in the plant. They have marked ans strong physiological action on humans.

Mayer's test
Mayer's Reagent Preparation:
Dissolve 1.35 g of mercuric chloride in 60 mL distilled water and 5 g of potassium iodide in 10 mL distilled water. Mix both solutions, then dilute to 100 mL with distilled water.

Procedure:
Add 3 mL of Mayer's reagent to 0.5 mL of the test solution (Unprocessed Turmeric or OT). A milky white precipitate indicates the presence of alkaloids.

Wagner's Test
Wagner's Reagent Preparation:
Dissolve 1.27 g of iodine and 2 g of potassium iodide in 20 mL distilled water. Dilute the solution to 100 mL with distilled water.
Procedure:
Add 3 drops of Wagner's reagent to 0.5 mL of the test solution (Unprocessed Turmeric or PT). A reddish-brown precipitate indicates the presence of alkaloids.

Hager's Test
Hager's Reagent Preparation:
Prepare a saturated aqueous solution of picric acid.
Procedure:
Add 1 mL of Hager's reagent to 0.5 mL of the test solution. A prominent reddish-yellow color indicates the presence of alkaloids.

Detection of Glycosides
Glycosides are also derived from plant and generally formed by the replacement of (OH) hydroxyl group in the sugar molecule.
Molish's Test
Dissolve 1 mg of Alpha-Naphtol in 2 mL of alcohol.
Procedure:
Add Molish's reagent to a few mL of the test solution. Mix, then carefully add a few drops of concentrated sulfuric acid along the side of the test tube. A blue-violet ring at the junction of the two liquids indicates the presence of glycosides.

Benedict's Test
Benedict's Reagent Preparation:
Dissolve 173 g sodium citrate, 100 g sodium carbonate in 800 mL water. Add 17.3 g copper sulfate in 100 mL water, then make the volume to 1000 mL.
Procedure:
Add 0.5 mL of Benedict's reagent to 1 mL test solution, mix, and heat for 20 minutes. Yellowish-brown color indicates glycosides/reducing sugars.

Fehling Test
Preparation of the reagent: Fehling's solution
Dissolve 34.66 g copper sulfate in 100 mL water, then make the volume to 500 mL with distilled water. Dissolve 173 g potassium sodium tartrate and 50 g sodium hydroxide in 200 mL water, then make the volume to 500 mL.
Procedure:

Add 0.2 mL of Fehling's A and B to 1 mL test solution, mix, and heat for 20 minutes. Yellowish-brown color indicates glycosides/reducing sugars.

Detection of Cardiac glycosides
Considerable number of plants scattered throughout the plant kingdom contain C21or C-24 steoidal glycosides which exert a slowing and strengthing effects on the heart failing (Wc, Evan16 th edition 2004).

Keller-Killani Test for Cardiac Glycosides:
50 mg of extracts were hydrolyzed with 10 mL hydrochloric acid for 2 hours, filtered, and the filtrate used. Ferric chloride (5g in 100 mL water) was added with glacial acetic acid and concentrated sulfuric acid. A reddish-brown color at the junction, turning blue, indicates deoxy sugars.

Borntrager's Test for Anthraquinone Glycosides:
2 mL test solution filtrate was mixed with 2 mL chloroform, shaken, and the chloroform layer separated. Adding ammonium solution led to a pink color, indicating the presence of Anthraquinone glycosides.

Foam Test for Saponin Glycosides:
50 mg of extracts were dissolved in 25 mL water, shaken for 15 minutes, and a foam layer formed, indicating saponins.

Alkaline Reagent Test for Flavonoids:
To 1 mL of extract, 0.5 mL 10% ammonium hydroxide was added. A yellow color appeared, indicating flavonoids.

Shinoda Test for Flavonoids:
50 mg of extracts were dissolved in methanol, and 1 mL test solution was treated with hydrochloric acid and magnesium turnings. A pink-red color appeared, indicating flavonoids.

Biuret Test for Proteins:
To 1 mL extract, 0.5 mL Biuret reagent was added, incubated for 5 minutes at 37°C. A violet color indicates the presence of proteins.

Lowry Test for Proteins:
A reagent (1 mL $CuSO_4$, 1% sodium citrate, Na_2CO_3, NaOH) was mixed with extract, followed by Folin-Ciocalteu reagent. A dark purple color indicates proteins.

Ninhydrin Test for Proteins:
3 mL extract solution was treated with 2 mL Ninhydrin reagent and heated in a water bath for 20 minutes. A purple-blue color indicates proteins.

Determination of Total phenol content
The total phenolic content of the hydroalcoholic leaf extract was determined using the Folin-Ciocalteu method. 1

mL of the extract (1.0 mg/mL) and 1 mL of Folin-Ciocalteu reagent were mixed, allowed to stand for 15 minutes, followed by the addition of 2 mL of 20% sodium carbonate solution. The mixture was incubated for 60 minutes at room temperature, and the phenolic content was measured spectrophotometrically at 650 nm. The total phenolic content was calculated from a calibration curve prepared using Gallic acid standards (100, 200, 300, 400, and 500 µg/mL). Results were expressed as gallic acid equivalents per gram of sample.

Determination of Total flavonoid content.

The total flavonoid content of the hydroalcoholic extract was determined using the aluminium chloride colorimetric method. 0.5 mL of the extract (1 mg/mL) was mixed with 0.3 mL of 10% aluminium chloride, 0.3 mL of 5% NaNO2 solution, and 4.0 mL of distilled water. After resting for 30 minutes at room temperature, the absorbance was measured at 510 nm against a blank. Quercetin was used as a standard, and the total flavonoid content was calculated from the calibration curve, expressed as mg/g of quercetin equivalents.

Preparation of Formulation Nanoemulsion

Formulations FE-1 to FE-8 were prepared by first mixing the oil phase ingredients (ginger extract, onion extract, lemon extract, and castor oil) for homogeneity. The aqueous phase was prepared separately by combining PEG 200, isopropyl myristate, Cremophor EL (except for FE-4 and FE-7), glycerin, and water while stirring. The oil phase was then slowly added to the aqueous phase, and the mixture was homogenized until smooth. The final nanoemulsion formulations were transferred to containers, labeled with their respective codes, and stored in a cool, dry place away from sunlight and moisture.

Table : Preparation of Formulation Nanoemulsion

Formulation Code	**FE-1**	FE-2	FE-3	FE-4	FE-5	FE-6	**FE-7**	**FE-8**
Ginger extract	**1.5**	-	-	**1.5**	-	**1.5**	**1.5**	**1.5**
Onion extract	-	**1.5**	-	**1.5**	**1.5**	**1.5**	-	**1.5**
Lemon extract	-	-	**1.5**	**1.5**	**1.5**	-	**1.5**	**1.5**
Castor oil	**1%**	**2%**	**3%**	**2%**	**1%**	**2%**	**1%**	**2%**
PEG 200 (ml)	**1.25**	**1.5**	**1.25**	**1.25**	**1.50**	**1.25**	**1.25**	**1.50**
Isopropyl myristate (ml)	**2.5**	**2.5**	**2.5**	**2.5**	**2.5**	**2.5**	**2.5**	**2.5**
Cremophor EL(ml)	**0.25**	**0.50**	**0.25**	**----**	**0.50**	**0.25**	**---**	**-----**
Glycerin	**2.25%**	**2.25%**	**2.25%**	**2.25%**	**2.25%**	**2.25%**	**2.25%**	**2.25%**
Water	**upto5ml**	**upto5ml**	**upto5ml**	**upto5ml**	**upto5ml**	**upto5ml**	**upto5ml**	**Upto5ml**

Physical evaluation

To perform the physical evaluation of the nanoemulsion formulation, several parameters need to be measured, including particle size, zeta potential, and polydispersity index. Here's a paragraph-form procedure for measuring these parameters:

Particle Size Measurement

Particle Size Measurement: Use a particle size analyzer (e.g., DLS) to determine the particle size of the nanoemulsion. Dilute the formulation with an appropriate solvent to the correct concentration, ensuring it is well-dispersed and free from aggregates. Follow the instrument's instructions to obtain data on mean particle size and size distribution.

Zeta Potential Measurement

Measure the zeta potential using a zeta potential analyzer to assess the stability of the nanoemulsion. Dilute the formulation in a suitable electrolyte or dispersant, ensuring proper dispersion. The instrument will provide the zeta potential value, indicating surface charge and stability. A higher magnitude of zeta potential generally suggests better stability.

Polydispersity Index (PDI) Measurement

Use the same particle size analyzer to determine the PDI, which quantifies the uniformity of particle sizes. The instrument calculates PDI from the size distribution data, and a lower PDI value indicates better uniformity and narrower size distribution.

Isotonicity or Tonicity adjustment

Osmolality is determined by four colligative properties of tears or eye formulations: freezing point, boiling point,

vapor pressure, and osmotic pressure. It measures the concentration of solute particles in a solution, either in moles per liter (osmolarity) or moles per kilogram (osmolality). Osmotic pressure is the pressure needed to prevent the movement of solvent molecules via osmosis and is influenced by the number of solute particles, regardless of their identity. Osmotic pressure can be calculated using the equation:

$\pi = i * n * R * T * C$

Where:

- π represents osmotic pressure,
- i is the van't Hoff factor (the number of particles formed per molecule or formula unit of solute when dissolved),
- n is the number of moles of solute,
- R is the ideal gas constant,
- T is the temperature in Kelvin, and
- C is the molar concentration of the solute.

Droplet size and Polydispersity

The key advantage of nanoemulsions over conventional emulsions is their small size and low polydispersity. These properties are influenced by both composition and preparation variables, such as emulsifying path, agitation, and emulsification time. While composition variables play a larger role in low-energy emulsification methods, preparation variables are more critical in shear emulsification. The droplet size is a crucial characteristic of nanoemulsion structure and stability. Photon correlation spectroscopy (PCS), or dynamic light scattering (DLS), is commonly used to measure droplet size by analyzing light scattering fluctuations caused by Brownian motion. This method, typically performed at 25°C and a 90° angle, is widely employed using instruments like the Zetasizer (Malvern Instruments, UK). Droplet size is considered the most representative parameter for evaluating emulsion stability.

Zeta Potential

Emulsifiers or surfactants stabilize emulsion droplets by creating both mechanical and electrostatic barriers. The electrical surface charge on the droplets results from the ionization of interfacial film-forming components. The surface and Zeta potential depend on the ionization extent of the emulsifying agents. The Zeta potential, which reflects the droplet's surface charge, is typically measured using instruments like the Zetasizer (Malvern Instruments, UK) or the moving boundary electrophoresis technique, providing accurate electrophoretic mobility data. Along with droplet size, Zeta potential is a key parameter for assessing nanoemulsion stability, and these factors are monitored during stability studies.

Morphological analysis by SEM

The formulation was lyophilized for SEM analysis with slight modifications to the standard method. 1 mL of the sample was combined with 0.2g each of sucrose and lactose. The samples were prepared in duplicates, frozen at -80°C for 12 hours, and then subjected to lyophilization in a freeze dryer (Virtis) for 72 hours at a condenser temperature of -53°C. After lyophilization, the samples were stored at -80°C until analysis. On the analysis day, the samples were vacuum-dried in a desiccator with anhydrous calcium chloride for 2 hours to remove moisture. The dried samples were analyzed using a FEI Quanta FEG 200-High Resolution Scanning Electron Microscope (SEM).

Determination of pH

It is important to note that despite changes in pH, there was no overall change in the measured mean diameter of the nanoemulsion oil droplets or the zeta potential. Although alterations in pH are generally known to affect droplet size and zeta potential, influencing the stability of the system, in this case, the pH variations did not lead to significant alterations. For safe ocular drug delivery, the desirable pH range is between 6.8 and 7.6. pH measurements can be accurately conducted using a Mettler Toledo (UK) pH meter.

Determination of Viscosity

Nanoemulsions have low viscosity, which facilitates easy handling, packaging, and administration. The continuous phase is crucial for emulsion stability, influencing factors like creaming rate, coalescence, and flocculation. Viscosity measurements are important, especially for ocular formulations, as high viscosity can cause discomfort in parenteral administration. The viscosity of nanoemulsions was measured using a Brookfield viscometer (DV-II+ Pro) at 25 ± 0.5 °C with spindle # CPE 18, which helps determine the drug's residence time

in the eye.

Refractive index
Refractive index of the nanoemulsion formulation was determined using a refractometer (Atago) at 20 °C according to the manual provided with the instrument.

RESULT:

Collection and authentication of plant material
The selected plant material ***Zingiber officinale, Allium cepa and Citrus limon*** were purchased from local market of Indore, (M.P) India. The specimens were identified and authenticated by the Botanist.

Macroscopic studies
Table : Organoleptic characters of plants Zingiber officinale, Allium cepa and Citrus limon

S.No	Parameters	Observations of Zingiber officinale	Observations of Allium cepa	Observations of Citrus limon
1.	**Shape**	Typically has a knobby, irregular shape with finger-like projections	Typically have a round or bulbous shape	Ellipsoidal or ovoid shape
2.	**Size**	Can vary	Various sizes, ranging from small to large bulbs	Typically small to medium-sized fruits.
3.	**Odour**	Distinctive and characteristic spicy, pungent, and slightly sweet aroma	Strong and pungent odor	Lemons have a fresh, citrusy, and tangy aroma
4.	**Taste**	Spicy	Distinctive, pungent taste	Lemons have a sour and tangy taste, attributed to the presence of citric acid
5.	**Colour**	Commonly tan or beige.	White, yellow, red, and purple	Pale yellow to a deeper, vibrant yellow
6.	**Foreign rganic atter**	Foreign organic matter such as soil, debris, or other plant materials	Soil, debris, or damaged scales	Dirt, debris, or damage to the peel

Physicochemical Standardization of Proposed Plant Drug:
The physicochemical properties of Zingiber officinale (Ginger), Allium cepa (Onion), and Citrus limon (Lemon) were determined, revealing the following: Ash values of 8.55%, 1.30%, and 7.44%; foreign organic matter content of 1.45%, 1.25%, and 1.02%; water-soluble ash of 5.00%, 2.00%, and 1.78%; acid-insoluble ash of 1.70%, 1.35%, and 1.05%; and moisture content of 13.50%, 7.05%, and 7.11%, respectively. These parameters provide insights into the mineral content, foreign material, and water retention of the samples, which are critical for assessing their quality and stability.

Table: Standardization parameters of *Zingiber officinale, Allium cepa and Citrus limon*

S.No	**Parameters % w/w**	***Zingiber officinale*** (%w/w)	***Allium cepa*** (%w/w)	***Citrus limon (%w/w)***
1	Ash value	8.55	1.30	7.44
2	Foreign organic matter	1.45	1.25	1.02
3	Water soluble ash	5	2.0	1.78
4	Acid insoluble ash	1.7	1.35	1.05
5.	Moisture content	13.50	7.05	7.11

Determination of Total Crude Fibre Content
The Total Crude Fibre Content of Zingiber officinale (ginger), Allium cepa (onion), and Citrus limon (lemon) was 10.24%, 6.02%, and 22.50%, respectively. The Total Volatile Matter content for these samples was 8%, 12%, and 10%, respectively. These parameters reflect the fiber content and the amount of volatile substances present in the samples.

Determination of heavy metal and mineral analysis
Heavy metal and mineral content analysis for all three plant extracts

Heavy metal	**Values***
Lead (pb)	Not detected
Chromium	0.37 ppm
Mineral Content	**Values***
Iron	270.25 mg/kg
Potassium	Not detected
Aluminum	Not detected
Copper	8..85 mg/kg
Magnesium	5.90 mg/kg

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Heavy metal	**Values***
Lead (pb)	0.05 ppm
Chromium	0.03 ppm
Mineral content	**Values***
Iron	20 mg/kg
Potassium	900 mg/kg
Aluminum	40 mg/kg
Copper	7.58 mg/kg
Magnesium	165 mg/kg

Heavy metal	**Values***
Lead (pb)	0.05 ppm
Chromium	0.06 ppm
Mineral content	**Values***
Iron	30.25 mg/kg
Potassium	1200 mg/kg
Aluminum	58.55 mg/kg
Copper	15.67 mg/kg
Magnesium	188.25 mg/kg

Behaviour of Powdered sample

Parameter	**Zingiber officinale (Ginger)**	**Allium cepa (Onion)**	**Citrus limon (Lemon)**
Color	Pale yellow to light brown	Light yellow to off-white	Light yellow to pale orange
Texture	Fine, slightly fibrous	Fine, smooth	Fine, smooth with slight granularity
Odor	Strong, spicy aroma	Strong, pungent onion smell	Fresh, tangy lemon aroma
Taste	Pungent, slightly sweet	Sharp, slightly sweet	Sour, tangy citrus flavor
Hygroscopicity	Moderately hygroscopic	Highly hygroscopic	Moderately hygroscopic
Flow Properties	Poor flowability; may require flow agent	Poor flowability; requires anti-caking agents	Generally good flowability; may require anti-caking agents
Bulk Density	Lower due to fibrous nature	Moderate	Generally higher due to finer particles
Powder Reconstitution	Forms slurry or paste, strong aroma	Forms thick paste, strong aroma	Quickly dissolves, tangy solution
Storage Requirements	Airtight container, cool, dry place	Airtight container, cool, dry place	Airtight container, cool, dry place
Aqueous Iodine Solution	Color Change: Brownish to reddish-brown. Indicates the presence of starch or polysaccharides.	Color Change: Brownish to reddish-brown. Similar reaction as ginger extract.	Color Change: Typically pale yellow to brown. May show less intense color change.
Aqueous Iodine Solution	Color Change: Brownish to reddish-brown. Indicates the presence of starch or polysaccharides.	Color Change: Brownish to reddish-brown. Similar reaction as ginger extract.	Color Change: Typically pale yellow to brown. May show less intense color change.
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Prelimnary Phytochemical Analysis Of Extracts:

The phytochemical analysis of Zingiber officinale (ginger), Allium cepa (onion), and Citrus limon (lemon) extracts showed varied results: none of the extracts tested positive for steroids, triterpenoids, glycosides, or proteins. Zingiber officinale and Allium cepa were positive for saponins, while Citrus limon was negative. Only Citrus limon tested positive for alkaloids and carbohydrates. All three extracts showed positive results for tannins, phenolic compounds, and flavonoids.

Table : Phytochemical Profile of *Zingiber officinale, Allium cepa and Citrus limon:*

S.no	Chemical Tests	*Zingiber officinale Extrac*	*Allium cepa Extract*	*Citrus limonExtract*
		Ethanol	Ethanol	Ethanol
1.	Tests for Steroids andTriterpenoids:			
	• Liebermann's Burchard Test	-	-	-
	• Salkowski Test	-	-	-
2.	Test for Saponins:			
	• Foam Test	+	+	-
3.	Tests for Alkaloids:			-
	• Hager's Test	+	+	+
	• Mayer's Test	+	+	+
4.	Tests for Glycosides:			
	• Borntrager's Test	-	-	-
	• Keller Killiani Test	-	-	-
5.	Tests for Tannins and Phenolic compounds:			
	• Gelatin Test	+	+	+
	• Ferric Chloride Test	+	+	+
6.	Tests for Flavonoids:			
	• Ferric chloride Test	+	+	+
	• Alkaline reagent Test	+	+	+
7.	Tests for Proteins:			
	• Biuret Test	-	-	-
	• Xanthoproteic Test	-	-	-
8.	Test for Carbohydrates:			
	• Fehling Test	-	-	+

Where + is Present and – is Absent

The total polyphenolic and triterpenoids content

The total polyphenolic and triterpenoids content for **Zingiber officinale** (ginger), **Allium cepa** (onion), and **Citrus Limon** (lemon) presented in a tabular form:

Plant Material	**Total Polyphenolic Content (mg GAE/g)**	**Total Triterpenoids Content (% w/w)**
Zingiber officinale (Ginger)	11.25	1.5
Allium cepa (Onion)	17.58	0.7
Citrus limon (Lemon)	12.5	1.22

Notes: GAE: Gallic Acid Equivalent, w/w: Weight/Weight

Physicochemical Properties of Nanoemulsions

In the particle size measurement, FE-5 exhibited the largest particle size at 20.5 nm, while FE-7 had the smallest at 10.5 nm. Smaller particle sizes, like FE-7's, are generally preferred for enhancing bioavailability in drug delivery, while larger particles, like FE-5's, may have specific applications depending on formulation needs. Regarding viscosity, FE-6 had the highest viscosity at 5.52 mPa s, while FE-7 had the lowest at 4.10 mPa s. Higher viscosity formulations like FE-6 may be suited for sustained release or better adhesion, whereas lower viscosity formulations like FE-7 are preferred where ease of flow is critical.

Table: Particle Size Measurement and Viscosity

Formulation Code	Size (nm)	Viscosity (mPa s)
FE-1	11.6	4.65
FE-2	12.5	4.47
FE-3	12.7	5.11
FE-4	15.2	4.22
FE-5	20.5	4.56
FE-6	13.5	5.52
FE-7	10.5	4.10
FE-8	12.5	4.75

Zeta Potential Measurement

Zeta potential, representing the electrostatic charge at the shear plane of nanoparticles, is a crucial parameter for assessing the stability of colloidal systems. The provided negative zeta potential values for formulations FE-1 through FE-8 indicate a predominantly negatively charged surface, suggesting electrostatic repulsion between particles. The similar magnitude of these values implies comparable levels of repulsion across all formulations, which contributes to preventing aggregation or flocculation. This stability is particularly important for maintaining the effectiveness and properties of nanoparticle suspensions.

Table : Zeta Potential Measurement

Formulation Code	Zeta Potential (mV)
FE-1	**-0.33**
FE-2	**-0.28**
FE-3	**-0.32**
FE-4	**-0.31**
FE-5	**-0.34**
FE-6	**-0.28**
FE-7	**-0.35**
FE-8	**-0.29**

Polydispersity Index (PDI) Measurement

The Polydispersity Index (PDI) quantifies the particle size distribution in a colloidal system, with values ranging from 0 to 1. Lower PDI values (close to 0.2 or below) indicate a more uniform size distribution, which is ideal for drug delivery or nanotechnology applications. FE-6 (PDI 0.18) and FE-7 (PDI 0.17) have the lowest PDI values, signifying relatively narrow size distributions. Regarding appearance, "Clear" formulations are transparent with no visible particles, whereas "Turbid" formulations are cloudy or hazy, indicating suspended particles. Clear formulations are preferred in applications like ophthalmic solutions, while turbid formulations may be suitable for controlled-release systems or suspensions.

Table : Polydispersity Index (PDI) Measurement

Formulation Code	PDI	Appearance
FE-1	**0.25**	**Clear**
FE-2	**0.20**	**Turbid**
FE-3	**0.27**	**Turbid**
FE-4	**0.25**	**Clear**
FE-5	**0.28**	**Clear**
FE-6	**0.18**	**Turbid**
FE-7	**0.17**	**Clear**
FE-8	**0.27**	**Clear**

Isotonicity or Tonicity adjustment

Osmolality measures the concentration of solute particles in a solution, typically in mOsm/kg, and isotonicity refers to a solution matching the osmotic pressure of bodily fluids. For ophthalmic or injectable formulations, the isotonic range is generally 280 to 320 mOsm/kg. FE-2, FE-7, and FE-6 have osmolality values within this range, making them potentially suitable for such applications. In contrast, FE-1, FE-3, FE-8, FE-4, and FE-5 have higher osmolality values, which may require tonicity adjustment to reduce irritation or discomfort during administration.

Table : Isotonicity or Tonicity adjustment

Formulation Code	Osmolality(mOsm/Kg)
FE-1	**450**
FE-2	**320**
FE-3	**401**
FE-4	**510**
FE-5	**509**
FE-6	**350**
FE-7	**301**
FE-8	**427**

Droplet size

Droplet size is a key parameter in formulations like emulsions or suspensions, affecting stability, bioavailability, and appearance. Smaller droplet sizes typically enhance surface area and stability. Among the formulations, FE-

7 has the smallest droplet size (30.27 nm), suggesting better stability and surface area, which is ideal for applications requiring fine droplets. FE-2, FE-6, FE-5, and FE-4 also have relatively small droplet sizes, offering similar benefits. However, FE-1, FE-3, and FE-8, with larger droplet sizes, may require optimization to improve their properties for specific applications.

Table: Droplet size

Formulation Code	Droplets size (nm)
FE-1	**45.05**
FE-2	**35.20**
FE-3	**45.17**
FE-4	**42.25**
FE-5	**41.28**
FE-6	**35.18**
FE-7	**30.27**
FE-8	**47.27**

Determination of pH

The pH values of formulations FE-1 through FE-8 range from slightly acidic to neutral. pH is crucial in determining the suitability of a formulation for various applications. FE-7, with a pH of 7.10, is slightly alkaline, which may be preferred for applications requiring an alkaline environment. FE-3, with a pH of 6.20, is slightly acidic, which may be suitable for specific formulations or ingredient compatibility. The remaining formulations (FE-1, FE-2, FE-4, FE-5, FE-6, and FE-8) have pH values within the slightly acidic to neutral range, making them versatile for various applications.

Table : Determination of pH

Formulation Code	**pH**
FE-1	6.33
FE-2	6.56
FE-3	6.20
FE-4	6.25
FE-5	6.22
FE-6	6.75
FE-7	7.10
FE-8	6.54

Determination of Drug content (%)

The drug content percentages for formulations FE-1 through FE-8 reflect the concentration of the active pharmaceutical ingredient (API) in each formulation. FE-1 has the highest drug content (95.02%), indicating a higher concentration of the active drug. FE-7 has the lowest drug content (90.57%), suggesting a slightly lower concentration. The other formulations (FE-2, FE-3, FE-4, FE-5, FE-6, and FE-8) exhibit varying drug content percentages, generally falling between these extremes. Higher drug content percentages are typically desired for efficacy.

Table : Determination of Drug content (%)

Formulation Code	**Drug content (%)**
FE-1	92.02
FE-2	94.42
FE-3	93.04
FE-4	94.05
FE-5	92.05
FE-6	94.75
FE-7	95.57
FE-8	93.05

Morphological analysis by SEM

Scanning Electron Microscopy (SEM) was used to analyze the surface morphology of drug-loaded nanoparticles. The SEM images revealed that the nanoparticles exhibited a smooth, oval shape with no fissures and a shiny surface. These characteristics indicate the successful removal of the solvent and suggest an effective formulation process. The particles were observed to have a size of approximately 100 nm, confirming the desired nanoparticle size and structure, which is essential for optimizing drug delivery. The SEM image visually corroborates these findings.

Shiny surface
Smooth Surface surface
Oval Shape

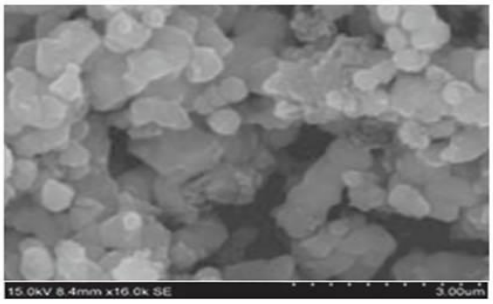

Figure: SEM image

CONCLUSION:

The study analyzed the organoleptic, physicochemical, and pharmaceutical properties of FE-1 through FE-8 and plant extracts from Zingiber officinale, Allium cepa, and Citrus limon. Organoleptic evaluation confirmed distinct characteristics, while physicochemical standardization and phytochemical profiling ensured consistent quality. Particle size, viscosity, zeta potential, and PDI analysis showed that smaller particles (e.g., FE-7) enhance bioavailability, and higher viscosity formulations are suitable for sustained release. Osmolality testing indicated that FE-2, FE-7, and FE-6 are isotonic, with others needing adjustment. pH values were within a suitable range for most formulations. Drug content varied, with FE-1 having the highest concentration. SEM imaging showed smooth, oval nanoparticles, confirming effective formulation. These findings support the optimization of formulations for pharmaceutical and cosmetic use.

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CONFLICT OF INTEREST:

The authors declare that there are no conflicts of interest regarding the publication of this research.

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