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Formulation of Anti-Bacterial Nanoemulsion Film Using *Chrysanthemum indicum. L* Flower Extract

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ABSTRACT

The rise of antibiotic-resistant bacteria underscores the need for innovative antimicrobial solutions. This study develops and characterizes a nanoemulsion film infused with Chrysanthemum indicum Linn flower extract (CIE), leveraging its antibacterial properties. Phytochemical analysis of CIE revealed a rich composition of bioactive compounds. A high-energy emulsification method yielded a stable nanoemulsion film with optimized droplet size, polydispersity index, and zeta potential. In vitro assays demonstrated the film's potent antibacterial activity against Staphylococcus aureus and Escherichia coli, surpassing that of the crude extract. This enhanced efficacy is attributed to improved solubility and bioavailability of CIE's phytoconstituents.

Keywords: *Chrysanthemum indicum.L*, Antibacterial nanoemulsion film, Phytochemical analysis, High-energy emulsification, Antibiotic-resistant bacteria, Topical antimicrobial agent, Wound healing, Skin infections.

INTRODUCTION

HERBAL COSMETICS

Herbal cosmetics, also known as botanical cosmetics, have been used for centuries to promote beauty, health, and wellness. These natural products harness the therapeutic properties of plants, herbs, and flowers to nourish and protect the skin, hair, and body. With the growing demand for natural and sustainable products, herbal cosmetics have gained popularity as a safer, more effective, and environmentally friendly alternative to synthetic-based cosmetics. By leveraging the power of nature, herbal cosmetics offer a holistic approach to beauty and wellness, providing a range of benefits for the skin, body, and mind.[1] **Fig.1.**

Fig.1 : Herbal Cosmetics

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NANOEMULSION FILM :

❖ A Nanoemulsion is a colloidal dispersion of two immiscible liquids (usually oil and water) stabilized by surfactants, with droplet sizes ranging from 20 to 200 nanometers.

❖ Nanoemulsion films are an advanced drug delivery system that combines nanoemulsions with film-forming materials to create thin, flexible films.

❖ These films are designed to improve the bioavailability, stability, and controlled release of active ingredients in pharmaceuticals, cosmetics, and food applications.

❖ These films can be transdermal, oral, or edible, depending on their application. **Fig.2.**

Types of Nanoemulsion Films :

1. Transdermal Films – Applied on the skin for drug absorption through the dermal layers.

2. Orodispersible Films (ODFs) – Dissolvable films placed on the tongue for rapid drug delivery.

3. Edible Films – Used in food packaging to enhance shelf-life and food safety.

4. Wound Dressing Films – Used for antimicrobial and wound healing applications.[2,3,4]

Fig.2: Nanoemulsion Film

***Chrysanthemum indicum.L* :**

❖ *Chrysanthemum indicum.L* is a flowering plant belonging to a genus of the dicotyledonous herbaceous annual flowering plant of the Asteraceae (Compositae) family.

❖ *Chrysanthemum indicum.L* (Compositae), distributed widely in China, is a well-known herb with small yellow flower.

❖ Flowers and buds of *Chrysanthemum indicum.L* have been traditionally used to treat various immune-related disorders, hypertension symptoms and several infectious diseases such as pneumonia, colitis, stomatitis, carbuncle and fever in folk medicine in China and Korea for thousands of years.

❖ Its flowers are also commonly used as tea to treat some eye diseases in traditional Chinese medicine.

❖ A series of studies have demonstrated that this plant has strong anti-bacterial, anti-viral, anti-oxidant, anti-inflammatory, and immunomodulatory properties.

❖ Phytochemical investigation on the title plant has shown the presence of flavonoids, terpenoids and phenolic compounds.[5,6,7] **Fig.3.**

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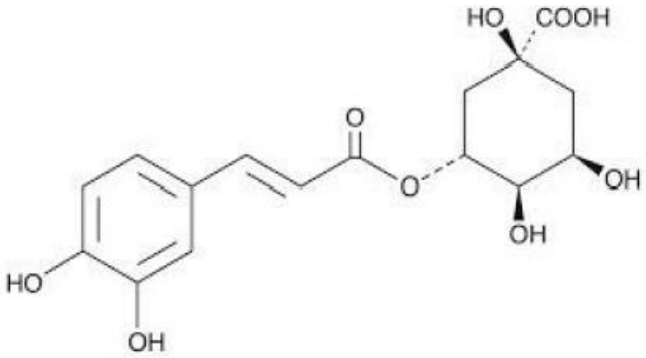
Fig.3: *Chrysanthemum indicum.L*

Phyto chemistry :

Phytochemicals are biologically active chemical compounds which are derived from plants. They have many health benefits for humans further than those attributed to macronutrients and *Chrysanthemum Spp.*. Flower contains octa-cosyl alcohol, β-sitosterol, lupeol, α-amyrin, daucosterol, ineupatorolide B, syringin,chlorogenic acid **Fig.4**, petasiphenol, physcion, acacetin, eupatilin, quercetin, diosmetin, luteolin, apigenin, apigenin- 7-O-β-D-glucopyranoside, quercetin-3-O-β-D-glucopyranoside **Fig.5**, luteolin-7-O-β-D-gluco pyranoside, apigenin-7-O-β-D- neospheroside, and acacetin-7-O-β-D-glucoside. Most of the Chrysanthemum flowers contain anthocyanins, cyanidin 3-glucoside and cyanidin 3-(3″-malonoyl) glucoside and carotenoids: lutein, zeaxanthin, β-cryptoxanthin, 13-cis-β-carotene, α-carotene, trans-β-carotene, and 9-cis-β-carotene. The major volatile compounds present in the plants are camphor **Fig.6**, α-pinene, chrysanthenone, safranal, myrcene, eucalyptol.[,9,10,22]



2,4,5,6,7,7ab-hexahydro-1H-indene, verbenone, β-phellandrene and camphene [3,4].

Fig.4: Chlorogenic Acid

Scientific classification

Kingdom: Plantae
Phylum : Tracheophyta
Class : Angiosperms
Clade : Asterids
Order : Asterales
Family : Astreaceae
Genus : Chrysanthemum
Species : C. Indicum L.

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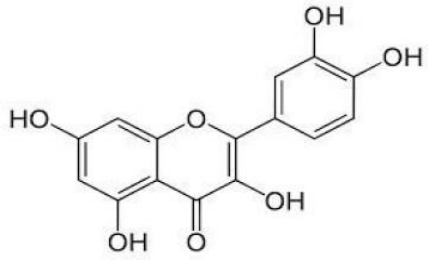

Fig.5: Quercetin

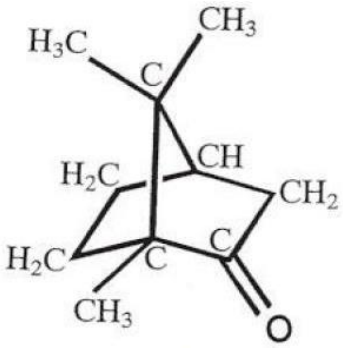

Fig.6: Camphor

LITRATURE REVIEW :

1.In **2020 Yan-Dong Shao, et al[31].,** reported as it reported to have anti oxidation activity we decided to choose *Chrysanthemum indicum* flower extract in skincare formulation due to its therapeutic benefits.

2. In **2021 kang H, et al[20].,** reported as *Chrysanthemum indicum.L* is widely used in traditional medicine for its anti-inflammatory, antioxidant, and antibacterial properties. These properties make it a valuable ingredient in cosmetic formulations aimed at improving skin health. Several bioactive compounds are present in *Chrysanthemum indicum. L*,such as flavonoids, sesquiterpenes, and phenolic acids, have been shown to contribute to its skincare benefits: Anti-inflammatory: Helps reduce redness and swelling, making it suitable for sensitive and irritated skin. Antioxidant: Protects the skin from free radical damage, which can lead to premature aging. Antimicrobial: Reduces bacterial growth on the skin, potentially preventing acne and other skin infections.

3. In **2021 S. Hotta., et al[22].,** reported as reported we decided to perform phytochemical screening analysis for *chrysanthemum indicum* flower extract.

4. In **2014 Manjit Jaiswal., et al[2].,** An advanced mode of drug delivery system": This article provides an in-depth overview of nanoemulsions, focusing on their potential to enhance the delivery of pharmaceutical agents.

5.In **2016 Ankur Gupta., et al[3].,**This review summarizes major methods for preparing nanoemulsions, theories predicting droplet size, factors affecting stability, and recent applications across various fields.

6. In **2017 Y. Singh,et,al[4].,**This article discusses popular techniques used to prepare nanoemulsions, their characterization, and their applications in cosmeceuticals and pharmaceuticals.

7. In **2023 Jeclin Inebel Dolongtelide et.al[17].,** as reported we decided to perform in vitro studies such as testing anti bacterial and antioxidant activity.

8. In **2005 Shunying Zhu et,al[14].,** we concluded that we can prepare nano emulsion flim due to presence of phenolic compounds and essential oils in *Chrysanthemum indicum* flower extract.

9. In **2020 F. Youssef ,et.al[5].,** we concluded that as the Nanoemulsion flim formulation have enhanced drug delivery,improved stability, Application in wound healing property if case of presence of antibacterial activity and also have application in skin care due to presence of antioxidant activity especially in our formulation of *Chrysanthemum indicum* flower extract nano emulsion flim.

10. In **2021 Weiming Zhong, et.al[29].,** we concluded that Nanoemulsion film formation offers several benefits across different applications, including drug delivery, cosmetics, and food packaging.So we decided to formulate *Chrysanthemum indicum* flower extract nano emulsion flim with their Charecterization.

11. In **2024 Sharma, Niharika, et al[30].,** We concluded that, *Chrysanthemum indicum.L* flowers demonstrate significant medicinal potential due to their rich phytochemical profile, including phenolic compounds with various health benefits such as antioxidant, anti-inflammatory, and antimicrobial properties.

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Conclusion :

- From this we conclude that this review outlines the potential of *Chrysanthemum indicum.L* in enhancing the formulation of nanoemulsion film, focusing on the plant's bioactive properties and the benefits of nanoemulsion technology for improved skin penetration and product efficacy.

- The incorporation of *Chrysanthemum indicum.L* extract into nanoemulsion film presents a novel approach to skincare, combining the benefits of traditional herbal extracts with modern nanotechnology. The enhanced delivery system provided by nanoemulsion ensures that the active compounds from *Chrysanthemum indicum.L* can effectively penetrate the skin, offering improved antioxidant, anti-inflammatory, and antimicrobial effects.

Aim and Objectives:

- This study aims to Develop a Nanoemulsion-based film incorporating *Chrysanthemum indicum* flower extract.
- Evaluate its antibacterial activity against common pathogens.
- Assess its antioxidant potential to determine its role in combating oxidative stress.
- Optimize the formulation for improved stability and bioavailability.

Plan Of Study :

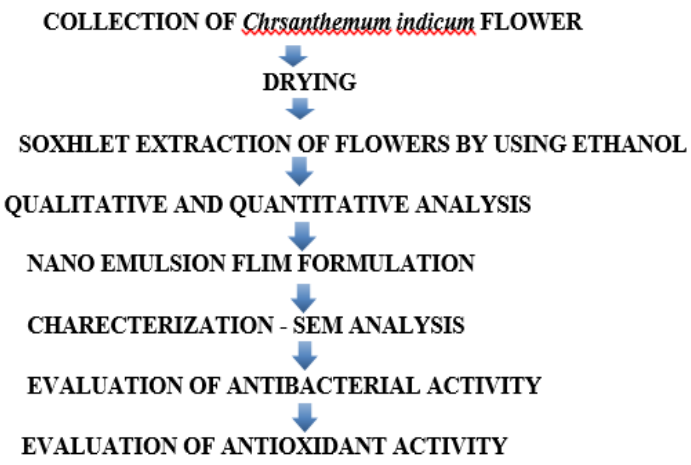

Fig.7: Soxhlet extraction (1)

METHODOLOGY :

Preparation of Plant Extract :

Materials needed:

- *Chrysanthemum indicum.L* flowers (dried or fresh)

- Ethanol (95% or 70%)

- Soxhlet apparatus

- Glass containers with lids

- Cheese cloth or filter paper

- Round-bottom flask

- Condenser

Procedure:

1. Preparation of Plant Material:

- If using fresh flowers, dry them thoroughly to remove excess moisture.

- Grind or chop the dried flowers into smaller pieces to increase surface area. [11,8]

2. Assembly of Soxhlet Apparatus:

- Place the ground flowers in the Soxhlet thimble.

- Attach the thimble to the Soxhlet apparatus.

- Connect the round-bottom flask to the Soxhlet apparatus.

- Add ethanol to the round-bottom flask.

- Attach the condenser to the Soxhlet apparatus. [11,8] **Fig.7.**

YELLOW COLOUR : DRY FLOWER EXTRACT

BROWN COLOUR: FERSH FLOWER EXTRACT

Fig.8: Soxhlet extraction (2)

Fig.9: Fresh and dry extract

3. Extraction:

- Heat the ethanol in the round-bottom flask using a heating mantle or water bath.

- The ethanol will vaporize, pass through the Soxhlet apparatus, and condense back into liquid.

- The condensed ethanol will extract the desired compounds from the plant material.

- The process is repeated continuously for 2-6 hours. [11,8] **Fig.8.**

4. Collection of Ethanol Extract:

- After the extraction process, collect the ethanol extract from the round-bottom flask.

- Filter the extract through cheesecloth or filter paper into a clean glass container. [11,8] **Fig.9.**

5. Concentration (Optional):

- If desired, concentrate the extract using a rotary evaporator or by simply leaving the container open to allow solvent evaporation. [11,8]

6. Storage:

- Store the *chrysanthemum indicum.L* extract in a clean, dark glass container with a tight-fitting lid. [11,8]

Soxhlet extraction is a continuous process that allows for efficient and thorough extraction of desired compounds from plant material. This method is particularly useful for extracting high-yield extracts. [11,8]

Phytochemical evaluation : [Fig.13,14] [TABLE.2]

1. Test for Alkaloids :

The extracts of flower were warmed separately with 2% H2S04 for 2 minutes. It was filtered and few drops of following reagents were added, which indicated the presence of alkaloids. Dragendroff's reagent: A red precipitation indicated the positive test. Mayer's reagent: A creamy white colored indicated the positive test. Picric acid: A yellow precipitation indicates the positive test. [8,9,10,22]

2. Test for Flavonoids :

A small quantity of the extract was heated with 10ml of ethyl acetate in boiling water for 3mins. The mixture was filtered and the filtrates were used for the following tests. The filtrate was shaken with 1ml of dil. Ammonia solution(1%). The layers were allowed to separate. A yellow coloration was observed in ammonia layer indicating the presence of flavonoid. The filtrate was shaken with 1ml of 1% Ammonium Chloride solution, where light yellow colour was observed. It indicated the presence of flavonoid. [8,9,10,22]

3. Test for carbohydrates :

The extracts were shaken vigorously with water and filtered. A few drops of molisch's reagent was added to the aqueous filter, followed by vigorous shaking again. Concentrated H2SO4 (1ml) was carefully added to form a layer below the aqueous solution. A brown ring at the interface indicated the positive test. [8,9,10,22]

4. Test for Saponins :

A small quantity of different extracts was diluted with 4ml of distilled water. The mixture was shaken vigorously and then observed on standing for stable brake, which indicated positive test. [8,9,10,22]

5. Test for steroids :

2 ml of Acetic anhydride and 2ml H2SO4 were added to the extracts. The color changed from violet to blue or green, which indicated the presence of steroids. [8,9,10,22]

6. Test for Anthraquinone glycosides (Borntrager's test) :

Dil.H2SO4 was added to the extracts and boiled. Then filtered and cooled. To the cold filtrate, 3 ml of benzene was added and mixed. The benzene layer was separated and ammonia (2ml) solution was added to it. A rose pink to red color in ammonical layer was observed, which indicated positive test. [8,9,10,22]

7. Test for cardiac glycosides (Legal's test) :

To each extract, 1ml of pyridine and 1ml of sodium nitroprusside solution were added and observed. A deep red color was observed indicating the positive test. [8,9,10,22]

8. Test for terpenoids (salkowski test) :

Each extract was mixed with 2ml of chloroform and then concentrated H2SO4 (3ml) was carefully added to form a layer. A reddish brown coloration at the interface indicated positive result for the presence of terpenoids. [8,9,10,22]

9. Test for gum and mucilages:

Each extract was dissolved in 10ml of distilled water and 25 ml of absolute alcohol was added to it with constant stirring. White or cloudy precipitate indicated the presence of gum and mucilages. [8,9,10,22]

10. Test for Amino acids and proteins :

Each extract was dissolved in 10 ml of distilled water and the filtrate was subjected to test the presence of proteins and amino acids.

a) Biuret Test :

2 ml filtrate was treated with one drop of 2% Copper Sulphate solution and then 1ml of ethanol (95%) was added to it followed by excess potassium hydroxide pellets .Pink color in the Ethanolic layer indicated the presence of proteins. [8,9,10,22]

b) Ninhydrin Test :

Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) were added to 2ml of aqueous filtrate. A characteristic purple color indicated the presence of amino acids. [8,9,10,22]

QUANTITATIVE ANALYSIS OF PHYTOCHEMICALS :

HPTLC ANALYSIS FOR FLAVONOIDS DETERMINATION:

Materials required :

1. Sample:

Plant extract rich in flavonoids (e.g., *Chrysanthemum indicum* extract).

2. Mobile phase solvents:

Toluene : ethyl acetate: formic acid

3. Flavonoid standards:

Quercetin, kaempferol, rutin, etc., as reference standards.

4. Stationary phase:

Merck, HPTLC Silica gel 60 F_{254}

5. Reagents for derivatization (optional):

10% Sulphuric acid

6. UV/Visible scanner:

For detection and quantification.

Procedure :

1. Preparation of Sample and Standards :

- Prepare the plant extract by Soxhlet extraction or other suitable methods.
- Dissolve a small portion of the extract in a solvent (e.g., methanol).
- Prepare flavonoid standards in methanol at known concentrations. [12]

2. Application on HPTLC Plate :

- Spot the plant extract and standards on the HPTLC plate using an applicator (e.g., CAMAG Linomat 5).
- Apply spots at regular intervals and maintain uniform quantities (typically 2-10 µL) to allow good separation. [12] **Fig.10,15.**

3. Development of the Plate :

- Prepare a mobile phase optimized for flavonoid separation, such as Toluene :Ethyl Acetate:Formic acid.
- Place the HPTLC plate in a development chamber pre-saturated with the mobile phase.
- Develop the plate until the solvent front reaches about 80-90% of the plate height.
- Remove the plate and dry it in a fume hood. [12] **Fig.11,16.**

4. Derivatization : [Fig.12]

- For better visualization, spray the plate with a derivatizing reagent like 10%sulfuric acid.
- Heat the plate at 100-120°C for a few minutes to develop colored bands for flavonoids.[12]

5. Detection and Quantification :

- Analyze the plate under UV light at 254 nm and 366 nm to observe flavonoid bands.
- Record Rf values for each band and compare them to standards.
- Use an HPTLC densitometer to measure the intensity of each band for quantification.
- Calculate the concentration of flavonoids in the sample by comparing the sample band intensities to the standard calibration curve. [12] **Fig.17,18,19.**

6. Calculation of Flavonoid Content :

- Use the densitometric data to quantify the flavonoid content, usually expressed as micrograms per milligram of extract or as a percentage. [12]

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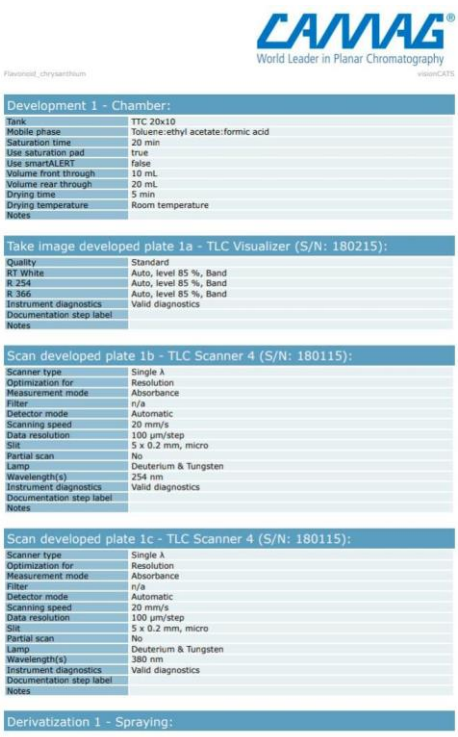
CAMAG
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Development 1 - Chamber:

Tank	TTC 20x10
Mobile phase	Toluene:ethyl acetate:formic acid
Saturation time	20 min
Use saturation pad	true
Use smartALERT	false
Volume front through	10 mL
Volume rear through	20 mL
Drying time	5 min
Drying temperature	Room temperature
Notes	

Take image developed plate 1a - TLC Visualizer (S/N: 180215):

Quality	Standard
RT White	Auto, level 85 %, Band
R 254	Auto, level 85 %, Band
R 366	Auto, level 85 %, Band
Instrument diagnostics	Valid diagnostics
Documentation step label	
Notes	

Scan developed plate 1b - TLC Scanner 4 (S/N: 180115):

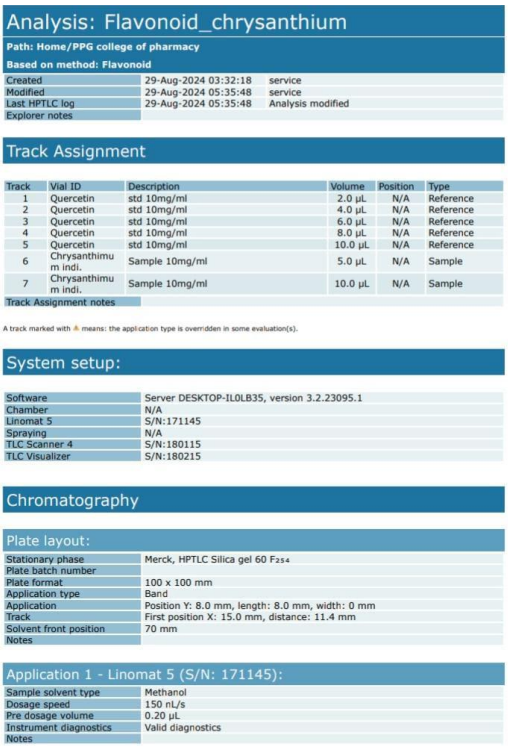
Scanner type	Single λ
Optimization for	Resolution
Measurement mode	Absorbance
Filter	n/a
Detector mode	Automatic
Scanning speed	20 mm/s
Data resolution	100 µm/step
Slit	5 x 0.2 mm, micro
Partial scan	No
Lamp	Deuterium & Tungsten
Wavelength(s)	254 nm
Instrument diagnostics	Valid diagnostics
Documentation step label	
Notes	

Scan developed plate 1c - TLC Scanner 4 (S/N: 180115):

Scanner type	Single λ
Optimization for	Resolution
Measurement mode	Absorbance
Filter	n/a
Detector mode	Automatic
Scanning speed	20 mm/s
Data resolution	100 µm/step
Slit	5 x 0.2 mm, micro
Partial scan	No
Lamp	Deuterium & Tungsten
Wavelength(s)	380 nm
Instrument diagnostics	Valid diagnostics
Documentation step label	
Notes	

Derivatization 1 - Spraying:

Fig.10: HPTLC Procedure (1)


Analysis: Flavonoid_chrysanthium

Path: Home/PPG college of pharmacy
Based on method: Flavonoid

Created	29-Aug-2024 03:32:18	service
Modified	29-Aug-2024 05:35:48	service
Last HPTLC log	29-Aug-2024 05:35:48	Analysis modified
Explorer notes		

Track Assignment

Track	Vial ID	Description	Volume	Position	Type
1	Quercetin	std 10mg/ml	2.0 µL	N/A	Reference
2	Quercetin	std 10mg/ml	4.0 µL	N/A	Reference
3	Quercetin	std 10mg/ml	6.0 µL	N/A	Reference
4	Quercetin	std 10mg/ml	8.0 µL	N/A	Reference
5	Quercetin	std 10mg/ml	10.0 µL	N/A	Reference
6	Chrysanthimum indi.	Sample 10mg/ml	5.0 µL	N/A	Sample
7	Chrysanthimum indi.	Sample 10mg/ml	10.0 µL	N/A	Sample

Track Assignment notes

A track marked with ⚠ means: the application type is overridden in some evaluation(s).

System setup:

Software	Server DESKTOP-IL0LB35, version 3.2.23095.1
Chamber	N/A
Linomat 5	S/N:171145
Spraying	N/A
TLC Scanner 4	S/N:180115
TLC Visualizer	S/N:180215

Chromatography

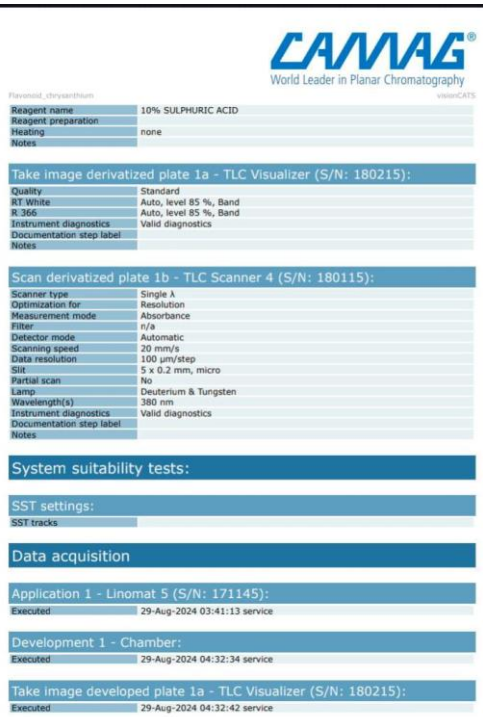
Plate layout:

Stationary phase	Merck, HPTLC Silica gel 60 F_{254}
Plate batch number	
Plate format	100 x 100 mm
Application type	Band
Application	Position Y: 8.0 mm, length: 8.0 mm, width: 0 mm
Track	First position X: 15.0 mm, distance: 11.4 mm
Solvent front position	70 mm
Notes	

Application 1 - Linomat 5 (S/N: 171145):

Sample solvent type	Methanol
Dosage speed	150 nL/s
Pre dosage volume	0.20 µL
Instrument diagnostics	Valid diagnostics
Notes	

Fig.11: HPTLC Procedure (2)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Reagent name	10% SULPHURIC ACID
Reagent preparation	
Heating	none
Notes	

Take image derivatized plate 1a - TLC Visualizer (S/N: 180215):

Quality	Standard
RT White	Auto, level 85 %, Band
R 366	Auto, level 85 %, Band
Instrument diagnostics	Valid diagnostics
Documentation step label	
Notes	

Scan derivatized plate 1b - TLC Scanner 4 (S/N: 180115):

Scanner type	Single λ
Optimization for	Resolution
Measurement mode	Absorbance
Filter	n/a
Detector mode	Automatic
Scanning speed	20 mm/s
Data resolution	100 µm/step
Slit	5 x 0.2 mm, micro
Partial scan	No
Lamp	Deuterium & Tungsten
Wavelength(s)	380 nm
Instrument diagnostics	Valid diagnostics
Documentation step label	
Notes	

System suitability tests:

SST settings:

SST tracks

Data acquisition

Application 1 - Linomat 5 (S/N: 171145):

Executed	29-Aug-2024 03:41:13 service

Development 1 - Chamber:

Executed	29-Aug-2024 04:32:34 service

Take image developed plate 1a - TLC Visualizer (S/N: 180215):

Executed	29-Aug-2024 04:32:42 service

Fig.12: HPTLC Procedure (3)

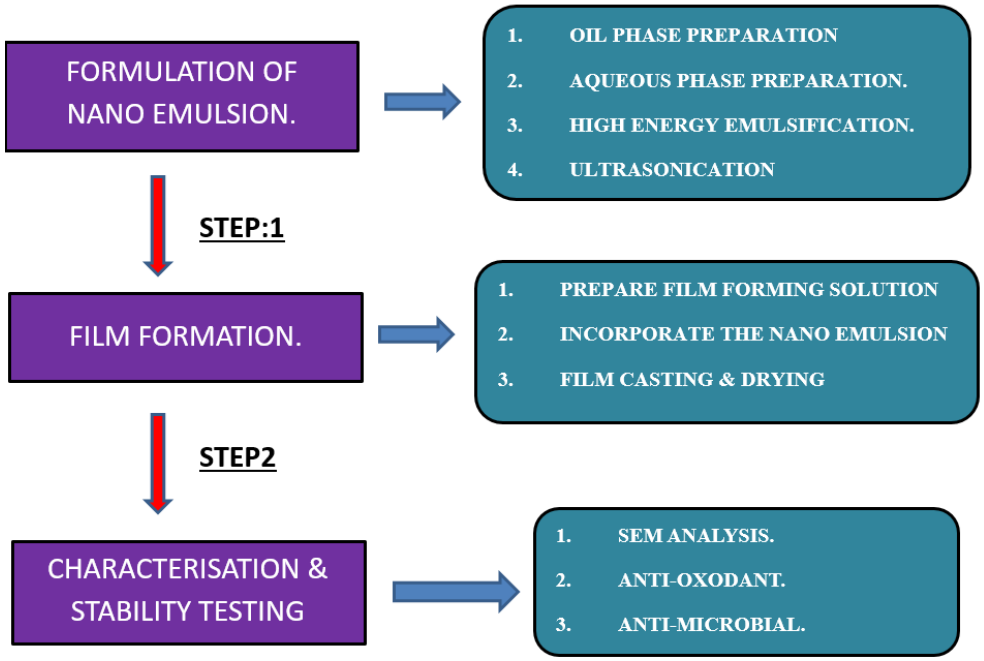
Preparation of Nano Emulsion Film :

- The Nano Emulsion Flim was developed by the modified method Polyvinyl alcohol (5%) is mixed with distilled water and stirred at room temperature.

- Under stirring conditions, 5% of Chrysanthemum indicum flower extracts were added drop wise.

- 5% citric acid is added to the solution and stirred for 30mins. The films were poured into a plastic petri dish and kept at 60°C for overnight.

- Nano emulsion Film will be formed.[26]



SCANNING ELECTRON MICROSCOPY :

❑ Scanning electron microscopy is a powerful tool for characterizing their morphology, size and surface features. The size of our nanoemulsion were estimated by the scanning electron microscopy. Small particles with size of less than 1mm can be detected by scanning electron microscopy.

❑ An electron gun emits out a focused beam of high energy electrons that scans across the sample surface present in the sample chamber, when the electrons interact with the sample they produce secondary electrons, detectors collect the secondary electrons and construct the high resolution image.[24,25] **Fig.20,21.**

EVALUATION OF ANTI-BACTERIAL ACTIVITY FOR NANO EMULSION FILM : [Fig.22,23] [Table.1,3]

The antibacterial activity of the nano emulsion flim film was evaluated against the three test organisms- *Escherichia coli*, *Staphylococcus aureus* . The Muller-Hinton agar was prepared and sterilized and poured into plates. Test pathogens of the overnight cultures were grown and 0.1% of liquid culture of each test organism was streaked with the help of a cotton swab throughout the Petri plate by rotating the plate at different angles. Films were cut into 2cmX2cm sizes and placed on the plates. The plates were incubated in an incubator at 37ºC for 48hrs. [13,14,22,28]

Table.1

Drug Used	**Concentration**
standard : Gentamycin	5 µg
SAMPLE : *Chrysanthemum indicum.L* Flower Extract	10 mg|ml

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EVALUATION OF ANTI-OXIDANT ACTIVITY FOR NANO EMULSION FILM :

DPPH(2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay:[Table.4]

The free radical scavenging potential of different extracts were determined according to the procedure of Kulisic with some modifications [16]. An aliquot of 50 µL of sample solution of various concentrations (25–400 µg/mL) were mixed with 950 µL of methanolic solution of DPPH (3.4 mg/100 mL). The reaction mixture was incubated at 37°C for 1 hrs in the dark. The free radical scavenging potential of the extracts were expressed as the disappearance of the initial purple color. The absorbance of the reaction mixture was recorded at 517 nm using UV–Visible spectrophotometer (Agilent 8453, Germany). Ascorbic acid was used as the positive control. DPPH scavenging capacity was calculated by using the following formula.[15,16,17,18,19,20,22]

$$\text{Scavenging activity (\%)} = \frac{ABSORBANCE\ CONTROL\ \ ABSORBANCE\ SAMPLE}{ABSORBANCE\ CONTROL} \times 1OO$$

RESULTS : FOR PHYTOCHEMICAL EVALUATION:

TABLE. 2

S.NO	PHYTOCHEMICAL CONSTITUENTS	ETHANOL
1.	Carbohydrates	+
2.	Saponins	_
3.	Alkaloids	_
4.	Cardiac glycosides	_
5.	Amino acids and proteins	+
6.	Gums and mucilage	_
7.	Terpenoids	+
8.	Flavonoids	+
9.	Steroids	_

"+" Indicates Positive and "-" Indicates negative

Fig.13: Dried Extract Result

Fig.14: Fresh Extract Result

DISCUSSION : The extract was analysed for the presence of key phytochemicals like Amino acids, proteins, terpenoids, flavonoids

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FOR QUANTITATIVE ANALYSIS OF PHYTOCHEMICALS (FLAVANOIDS) :

HPTLC ANALYSIS FOR FLAVONOIDS DETERMINATION:

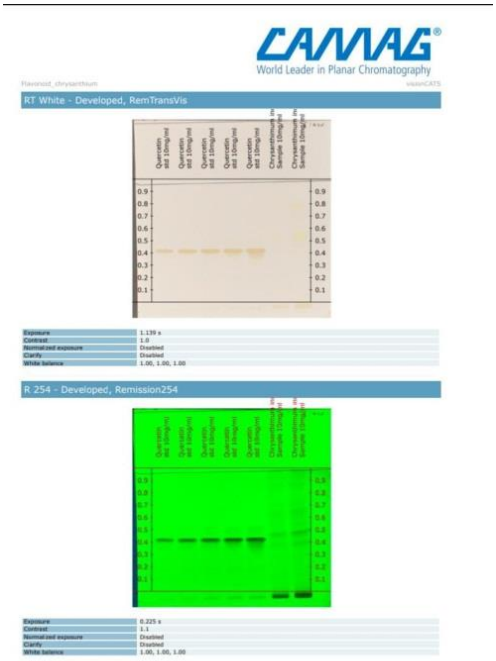

Fig.15:HPLC Result (1)

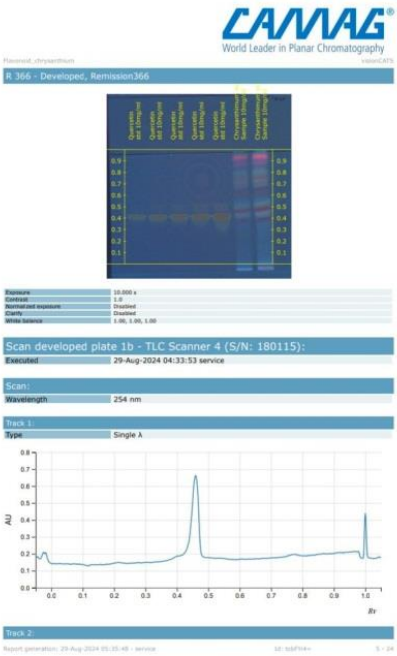

Fig.16:HPLC Result (2)

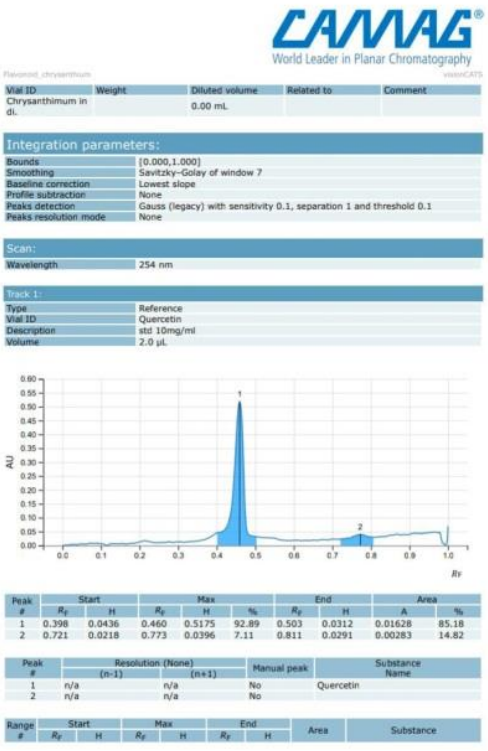

Fig.17:HPLC Result (3)

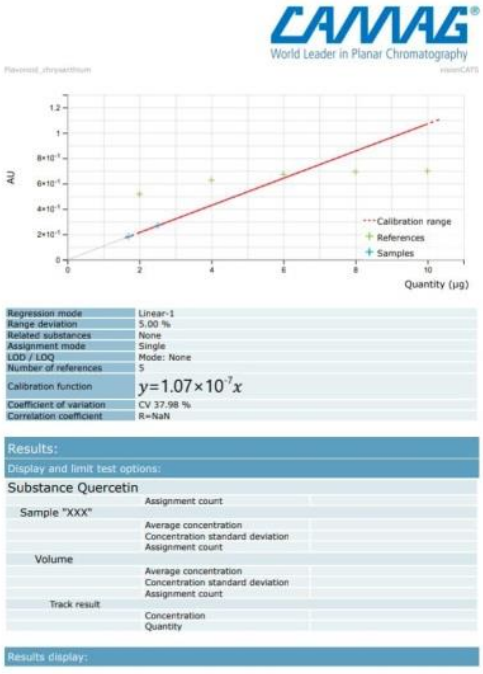

Fig.18:HPLC Result (4)

Fig.19:HPLC Result (5)

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Discussion

- The HPTLC analysis successfully identified and separated multiple bioactive compounds mainly flavonoids in *Chrysanthemum indicum* flower extract.

- The observed Rf values and peak intensities provide insights into the extract's chemical profile and pharmacological potential.

SCANNING ELECTRON MICROSCOPY :

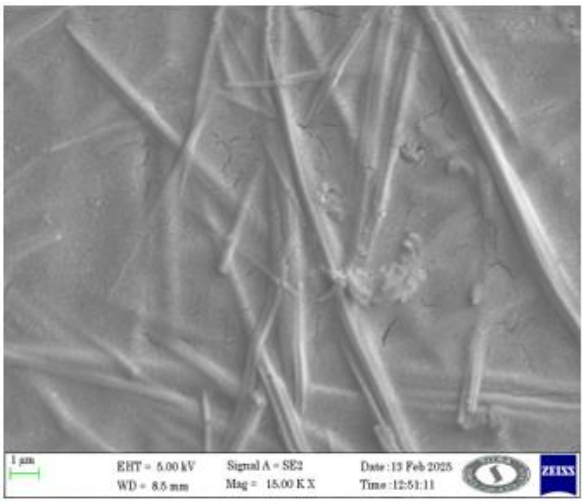

Fig.20:SEM Result (1)

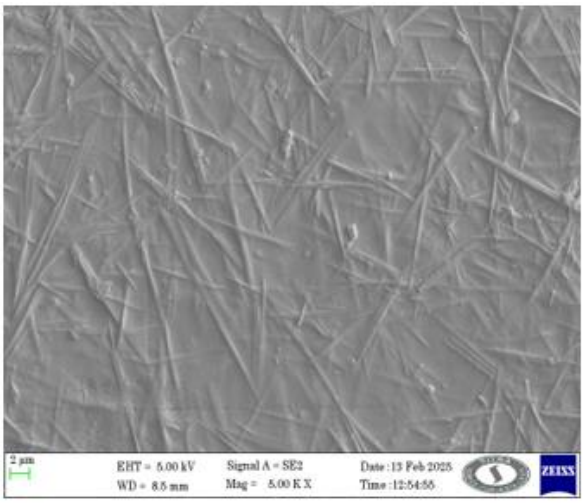

Fig.21:SEM Result (2)

Discussion :

- The SEM images confirm a smooth, homogeneous film with well-dispersed nanoemulsion droplets, the formulation is success and suitable for further testing.

- Any structural irregularities, phase separation, or large droplet formations should be addressed by optimizing the formulation process (e.g., emulsification technique, surfactant type, or polymer concentration).

EVALUATION OF ANTI-BACTERIAL ACTIVITY OF NANO-EMULSION FILM :

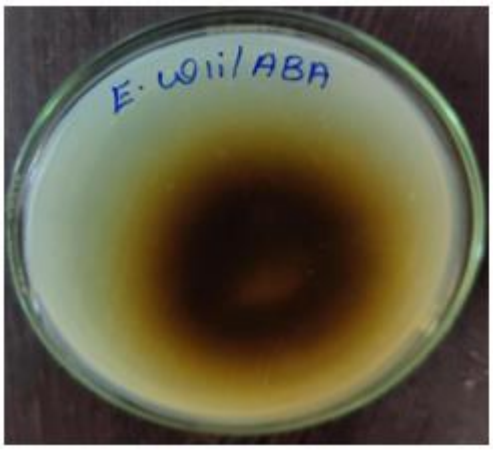

Fig.22: ***E.Coli***

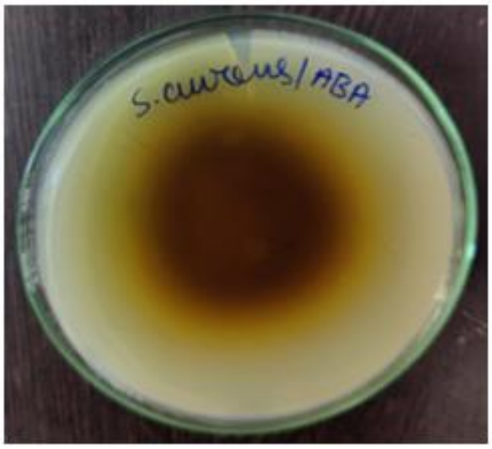

Fig.23: ***Staphylococcus.Aureus***

TABLE.3

Organism	Standard (in mm) (Gentamycin)	Sample (in mm) (Nano-emulsion film)
E. coli	**15.6**	**No zone inhibition**
S. aureus	**20.5**	**11**

Discussion :

- If the activity is comparable to standard antibiotics, it could be further developed for pharmaceutical or cosmeceutical applications.

- The study confirms the antibacterial potential of *Chrysanthemum indicum* flower extract against *Staphylococcus aureus*.

EVALUATION OF ANTI-OXIDANT ACTIVITY OF NANO-EMULSION FILM :

1. DPPH (2,2-Diphenyl-1-Picrylhydrazyl) Free Radical Scavenging Assay :

Discussion :

- The nanoemulsion film exhibited significant DPPH radical scavenging activity, indicating the presence of bioactive compounds such as flavonoids, phenolics, and terpenoids from the *Chrysanthemum indicum* extract.

- The Percentage inhibition of DPPH radicals was found to be dose-dependent, meaning higher concentrations of the film extract showed stronger antioxidant activity.

TABLE.4

Concentration (µg/ml)	Standard (Ascorbic Acid)	Sample
25	**11.47**	**1.05**
50	**31.95**	**5.21**
75	**47.79**	**10.47**
100	**65.71**	**13.68**
250	**74.83**	**20.32**
500	**88.37**	**34.16**
750	**96.00**	**49.88**
1000	**99.07**	**56.33**

CONCLUSION :

❑ Thus by formulating the *chrysanthemum indicum* flower extract nano emulsion film which performs antibacterial and antioxidant activity this can be used for wound healing property against bacterial infection and also can be used for skin care by formulating those film as Face mask due to antioxidant activity which helps in anti-aging activity. But for those specifications formulation like formulation of a film or patch which produce anti bacterial activity against only that particular bacteria.

❑ So we have just formed a trial piece of *Chrysanthemum indicum* flower nano emulsion flim which can be developed in Future along with skin irritancy test and so on.

❑ We conclude our project work by Formulating a trial piece of Nano Emulsion Flim using ***Chrysanthemum indicum*** flower extract which provides anti bacterial activity against ***staphylococcus aureus*** and also provide anti oxidant activity which produce wound healing and anti aging properties respectively.

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DECLARATION OF CONFLICTING INTERESTS:

The authors declared no potential conflicts of interest conceming the research, authorship and / or publication pf this article.

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