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A Research of Flavonoids in Inflammation: Exploring Their Role in Modulating Pro-Inflammatory Pathways

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ABSTRACT

The objective of this study was to develop and evaluate the in-vitro and in-vivo efficacy of Citrus limon peel extract loaded silver Nano gel for the treatment of inflammation. To achieve this, the alcoholic extract of Citrus limon peel was first screened for the presence of phytochemical constituents. A silver Nano gel was then developed using the Citrus limon peel extract, which was subsequently evaluated for its anti-inflammatory activity using in-vitro and in-vivo models. The use of Citrus limon peel extract loaded silver Nano gel aims to overcome the limitations of conventional formulations, such as poor absorption and bioavailability, limited ability to reach the target site, and expensive production and formulation processes. By developing a nanogel-based formulation, the study seeks to improve the delivery and efficacy of the Citrus limon peel extract. The potential benefits of this developed silver nanogel include improved anti-inflammatory activity, better penetration, and reduced costs, making it a promising treatment option for inflammation. By leveraging the therapeutic properties of Citrus limon peel extract and the advantages of Nano gel technology, this study aims to provide a novel and effective solution for the treatment of inflammation. **Future Prospects:** *Citrus limon* peel extract based silver Nano gel were successfully formulated and this could be a promising approach for treatment of inflammation.

Keywords: *Nano particles, Gel, Extraction, treatment of inflammation*

1. INTRODUCTION

A distinctive feature of inflammatory response is that damage to the body's own tissues is unavoidable. Inflammation involves multiple cell types, chemical signals, and pathways, making it a complex biological response. Chronic inflammation can have severe consequences, including tissue damage and scarring, disease development, and an increased risk of cancer. Conditions like arthritis, diabetes, and cardiovascular disease have been linked to chronic inflammation. Management of inflammation typically involves medications, lifestyle changes, and alternative therapies. Anti-inflammatory medications, such as NSAIDs or corticosteroids, can help reduce inflammation. Maintaining a healthy diet, exercising regularly, and managing stress can also help alleviate symptoms. Some alternative therapies, such as acupuncture or herbal supplements, may also be beneficial in reducing inflammation. Several synthetic pharmaceutical products in various dosages form are available in the market for arthritis treatment but are less preferred because of their elevated allergic reactions, repeated therapy, and side effects. Herbal products provide relief with comparatively less side effects. Now days, for effective treatment, more and more search is diverted towards herbals. Although a number of herbal products are available for topical admistration like creams, ointment, gel etc, and these conventional formulations have less effect to the body and have little percutaneous absorption. In this respect, the newer approaches like silver nanogel are developed as these formulations are stable and with high drug loading capacity and increased percutaneous absorption.

Methodology

Preliminary Phytochemical screening: The total flavonoids content in the Citrus limon peel extract was determined using a calorimetric method, which revealed that the extract contained 20.12% w/v of flavonoids. A calibration curve was plotted using rutin as the standard, and the λmax of rutin was found to be 510 nm. This study provides valuable information on the phytochemical composition of Citrus lemon peel extract, which can be useful for further research and development of potential therapeutic applications.

Formulation and evaluations of silver nanoparticles: The silver nanoparticles of peel extract of *Citrus limon* were formulated by chemical and microwave method. On the basis of various parameters like time, concentration of $AgNO_3$ microwave method was conducted. Further, the batch A4 from *Citrus lImon* was optimized on the basis of particle size, entrapment efficiency, and *in-vitro* release of silver nanoparticles.

Formulation and evaluations of silver nanogel: Optimized batch of silver nanoparticles containing *Citrus limon* peel extract were incorporated into gel separately. The optimization of topical gel was carried out using different gelling agents with varying concentrations, like Carbopol 940, CMC, and HPMC. The pH of the final formulations was adjusted using triethanolamine. The selected batch of both the plants *Citrus limon* (A4) was of light yellow colour, semisolid consistency, and neutral pH without any grittiness.

Pharmacological screening: Wistar albino rats of either sex, weighing between 150-300 g, were used for the anti-inflammatory and anti-arthritic activity studies. One day study for inflammation was recorded by using 0.1 mL of 1% carrageenan as inflammation inducer rat paw.

Summary of Findings: The silver nanoparticles of *Citrus limon* peel extract were formulated and evaluated on the basis of various available methods. Further, the microwave method was selected on the basis of time duration. Among all batches of *Citrus limon*, A4 was obtained as optimized batch with a particle size 78.7 nm, polydispersity index 0.256, zeta potential -37.8, entrapment efficiency 91.95%, and *in-vitro* release of 90.1% . A4 were incorporated into topical gel separately and found effective in inflammation.

Research Application: Novel herbal formulations were developed to deliver silver nanoparticles of Citrus lemon peel extract for the treatment of inflammation and arthritis. These formulations aim to provide more efficient therapy with no side effects, higher bioavailability, and cost-effectiveness compared to synthetic drugs. The use of silver nanoparticles in these formulations offers several benefits, including anti-microbial activity and improved bioavailability. Silver itself has been shown to reduce the growth of micro-organisms during inflammation, while the nano-formulation enhances the delivery of the active compounds, leading to better absorption and utilization.

2. PLANT PROFILE

2.1 LEMON *(Citrus Lemon)* Tendon et al. [82]

Synonym: Citrus fruit, Baranimbu, Gulgul, Paharinimbu, Paharikaghzi.

Fig.2.1: Citrus limon.

Taxonomical classification:

Kingdom **:** Plantae

Order **:** Sapindales

Family **:** Rutaceae

Genus **:** *Citrus*

Species **:** *Lemon*

Binomial Name: *Citrus limon*

Common Name

Hindi : Nimbu

English : Lemon

Sanskrit : Ruchika

Habitat : *Citrus limon* is found in tropical and subtropical climates.

Morphology :

The fruit is a fleshy hesperidium known as a lemon. The exocarp and the mesocarp make pericarp known as peel or rind. Lemon fruit is oblong to ovate, with a nipple-like a protuberance at the apex. It is light yellow or golden in color. The fruit is sour in taste. It is a small perennial tree (3-6cm in height) with a number of branches armed with hard thick thorns. Branches are angular, rounded and smooth. Leaves are evergreen, entire, coriaceous and glossy green, although young leaves may be reddish. Flowers are hermaphrodite and every flower has 20-24 stamens arranged in small groups.

Part of plant used : Dried peel of *Citrus limon*.

Chemical Constituents:

The peel or rind of the fruit contains an essential oil (up to 2.5%). Limonene, geranial, neral, and citronellal are present in the essential oil. Flavonoids (rutin, hesperidin, naringin, and quercetin), Pectin, Pigments (carotenes and xanthophylls) are present and other active principles are vitamin C, mucilage, calcium oxalate and coumarins (limettin, bergapten, aurapten, and bergamottin). The pulp of citrus fruit contains organic acids (citric acid, malic acid, glycolic acid, lactic and pyruvic acid), vitamin C or ascorbic acid and Carbohydrates (glucose, fructose, and sucrose). The other active principles of lemon pulp also contain minerals (magnesium, calcium, phosphorus, potassium, and iron).

Uses : Flowers and fruits are used in natural medicine and is traditional diet. Peel and pulp of lemon fruits are used in vessel-protection and venotonic activities, such as flavonoids are used in the treatment of blood vessels disorders such as varices, chronic venous insufficiency (CVI), and low capillary resistance.

Topical uses : It is useful to treat injuries from traumatism, skin burn or surgery. It is also helpful in new tissue formation. Citrus flowers and rind are well known for different dermo-cosmetic applications. It is also used in inflammation.

2.2 POLYMER PROFILE

2.2.1 Carbopol

Non-Proprietary Names: Carbomer

Synonym – Polyacrylic acid, carboxy polymethylene

Chemical Name – Carbomer

Empirical Formula – $(C_3H_4O_2)_n$

Functional Category: Bioadhesive, suspending agent, emulsifying agent, reaction-modifying agent, tablet binder, viscosity-increasing agent.

Description: Carbopol is white colored, acidic, hygroscopic, fluffy powders with a slight characteristic odor.

Melting point – 260^{o}c

Specific gravity – 1.4

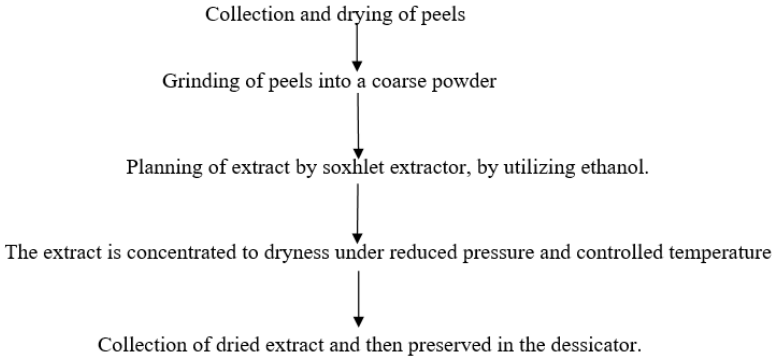
Solubility – Soluble in water, glycerin and after neutralization in ethanol (95%).

Application in pharmaceutical formulation and technology: used as viscosity or suspending increasing agents in various formulations like- gels, creams, and ointments for use in an ophthalmic, rectal and topical preparation. **Rowe et al. [68]**

3. PROPOSED METHODOLOGY

A. Preparation of extract:

1. *Citrus lemon* (Soxhlet extraction method)



B. Development of silver nanoparticles:

1. Chemical method.
2. Microwave method.

C. Formulation development of silver nanogel

D. METHODOLOGY

3.1 Collection, Identification and Authentication of Plant Material

3.1.1 Collection of Plant Material

The peel of *Citrus limon* were collected.

3.1.2 Drying of Plant Material

The plant material was shade dried at room temperature.

3.1.3 Storage of Plant Material

The peels of *Citrus limon* were pulverized and sieved through sieve no. 23 and stored in a container.

3.2 Extraction Procedure

3.2.1 Preparation of crude extract of *Citrus lemon* peel

Conventional hot soxhlet extraction was used to get the ethanolic extract of peel. 100g of powered peel were extracted with 2500ml of ethanol at a temperature range of 70-80^{0}C successively for 42 days. The extract was filtered and removed off the solvent using a vacuum rotary evaporator (Buchi type). After the complete extraction, obtained residue was kept in desiccator and color, odor and yield were recorded. **Jacobosan et al. [29], Sood et al. [77]**

3.2.1.1 Colour of extract = Dark brown

3.2.1.2 Odour of extract = Aromatic, Pleasant

3.2.1.3 Yield obtained = 11.29 g

3.3 Preliminary Phytochemical Studies of extract

3.4 Preliminary Phytochemical Studies of extracts

Table 3.1: Phytochemical screening of *Citrus lemon* and *Citrus limetta* peel extract.

S.No	Phytochemical Constituents	Name of the test	Observation	Result
				Citrus lemon
1	Alkaloid	**Dragendroff's Test**	The test solution was mixed with Dragendroff's reagent (solution of potassium bismuth iodide). It gives reddish brown precipitate indicating the presence of alkaloids.	+
		Wagner test	The test solution was mixed with Wagner reagent (Iodine Potassium Iodide). Formation of reddish brown precipitate confirmed the presence of alkaloids.	+

		Hager test	The test solution was mixed with sodium hydroxide solution. Formation of yellow color precipitate confirmed the presence of alkaloids.	+
2	Steroids	**Salkowski Test**	The test solution was mixed with 1mL of chloroform. Then carefully added 1mL of concentrated sulphuric acid and shaken gently. A reddish brown color in the chloroform layer and green fluorescence in the acid layer confirm the presence of the steroidal ring.	+
3	Flavonoids	**Alkaline reagent test**	Test solution mixed with 20% sodium hydroxide solution. Then added dilute HCl, a yellow color forms and disappears on the addition of HCL which indicate the presence of flavonoids.	+
4	Saponin	**Foam test**	The test solution was mixed with 5mL of distilled water in a test tube and shaken vigorously. The formation of stable foam indicates the presence of Saponin.	-
5	Glycosides	**Baljet Test**	The test solution was mixed with picric acid. The formation of an orange color indicates the presence of glycosides.	+
		Keller-Kiliani test	One ml of plant extract was treated with 2mL glacial acetic acid containing a drop of $FeCl_3$. A reddish brown color indicates the confirmation of Keller-Kiliani test.	+
		Legal test	One mL test solution was dissolved in Pyridine and sodium nitroprusside solution added and made alkaline. A pink to red color is produced.	+
6	Tannins	**Ferric Chloride test**	The test solution was mixed with 2mL of ferric chloride. Formation of a dark brown color indicates the presence of tannins.	-

7	Proteins	**Xanthoproteic test**	The test solution was mixed with 1mL of concentrated nitric acid and boiled. A yellow color precipitate was formed. After cooling, sodium hydroxide solution was added. Formation of orange color conflicts proteins	+
8	Phenolic compounds	**Ferric chloride test**	The test solution was mixed with 2mL of ferric chloride. Formation of blue or green color indicates the presence of phenolic compounds.	+
9	Fixed Oils	**Spot test**	The test solution was pressed between filter papers. Oil stains on the filter paper confirm the presence of fixed oils.	-
10	Carbohydrates	**Molish Test**	The test sample was mixed with α-napthol and conc. H_2SO_4. Ring formation occurs which indicate the presence of carbohydrates.	+
11	Terpenoids	The test solution was mixed with chloroform and conc. H_2SO_4. Formation of red-brown color indicates the presence of Terpenoids.		+

(+) indicates present, (-) indicates absent **Ortuno et al. [57]**

Estimation of total flavonoids:

The total amount of flavonoids in the *Citrus limon* peel concentrate was resolved by Colorimetric technique. Rutin was utilized as standard and all-out flavonoids substance considered as mg of rutin identical (RE) per gram strong concentrate of citrus peels. For the planning of rutin stock solution, in a 100 ml volumetric flask, 100 mg of rutin in 10ml of ethanol was dissolved and volume made up with water. 0, 2, 4, 6, 8, and 10 ml of standard solution were included in 10 ml volumetric flasks, and volume made up with distilled water. The absorbance was taken at 510nm with spectrophotometer and a standard graph was plotted between concentration and absorbance.

Calorimetric method: In a tube consisting of 1 ml of double distilled water, 0.25 ml of optimally diluted sample was added. Further, at a sequence interval of 0, 5 and 6 min, introduced 0.075 ml of 5% $NaNo_2$, 0.075 ml of 10% $AlCl_3$ and 0.5 ml of 1 M NaOH. Finally, adjusted the volume of the reacting mixture with 2.5 ml of double distilled water. Then, using a spectrophotometer measured the absorbance of the solution at 510 nm. Then, with the standard curve achieved from rutin, the total quantity of flavonoids in every sample was calculated and stored in terms of milligrams of rutin equivalents (RE) per gram of solid extract of citrus peel. **Sivakumar et al. [75]**

Table3.2: Absorbance of Rutin.

Sr. No.	Concentration (µg/ml)	Absorbance
1	0	0.000
2	2	0.107
3	4	0.244
4	6	0.385
5	8	0.502
6	10	0.616

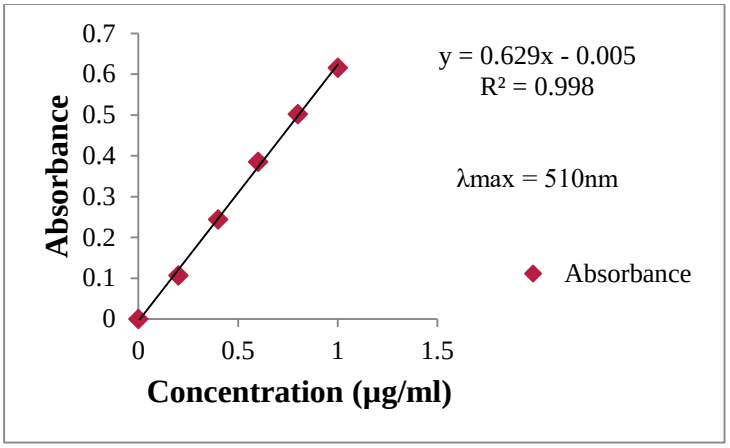


Fig.3.1 Standard curve of Rutin

Table 3.3: Flavonoids concentration in *Citrus lemon* peel extract.

Scientific Name	Local Name	Extraction yield (%)	Absorbance	λmax	Total Flavonoids (mgRE/g solid extract)
Citrus limon	**Lemon**	**11.29**	**0.545**	**510nm**	**9.28s**

3.5 Functional group analysis by IR spectroscopy

The FTIR spectra of ethanolic extract of *Citrus limon* peel, Carbopol polymer, was analyzed by FTIR (PerkinElmer spectrum version) spectrophotometer.

3.5.1 FTIR spectra of *Citrus limon* peel extract

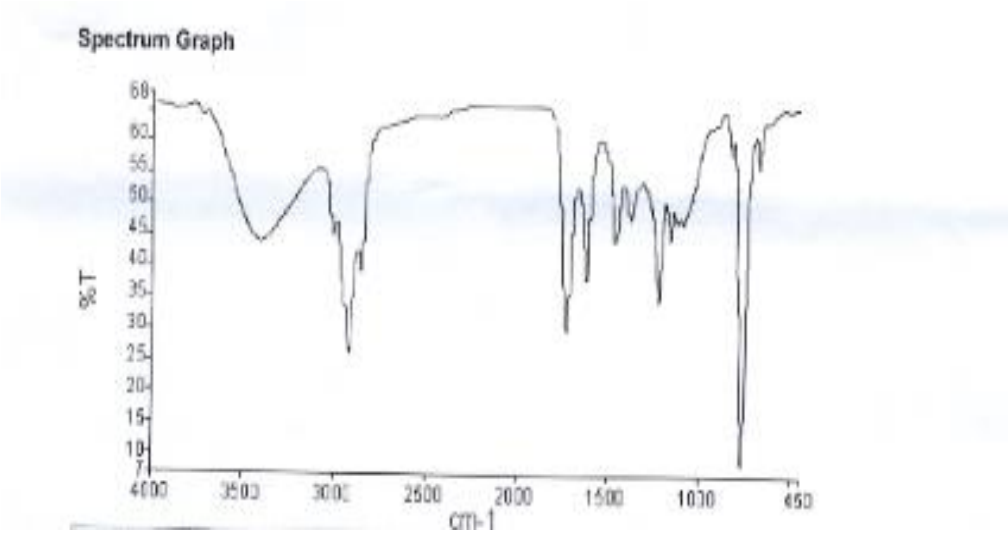


Fig. 3.2 FTIR of *Citrus limon* peel extract.

3.5.3 FTIR spectra of Carbopol polymer

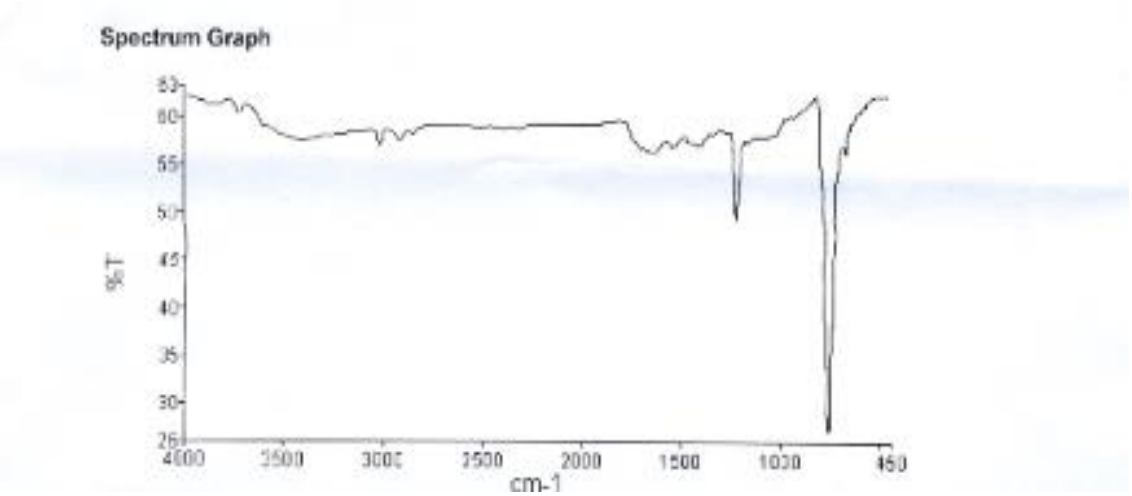


Fig. 3.4 FTIR of Carbopol polymer.

Table 3.6 FTIR spectrum peaks of Carbopol polymer.

2. Theoretical peak 3.	4. Observed peak	5. Groups
6. 900-690 7.	8. 758.85	9. C-H bending (out of plane)
10. 1260-1000 11.	12. 1214.81	13. C-O stretching
14. 1650-1600 15.	16. 1637.68	17. C=C stretching
18. 3100-2400	19. 3020.57	20. C=C stretching (broad)

3.5.4 FTIR spectra of Carbopol polymer with *Citrus lemon* peel extract

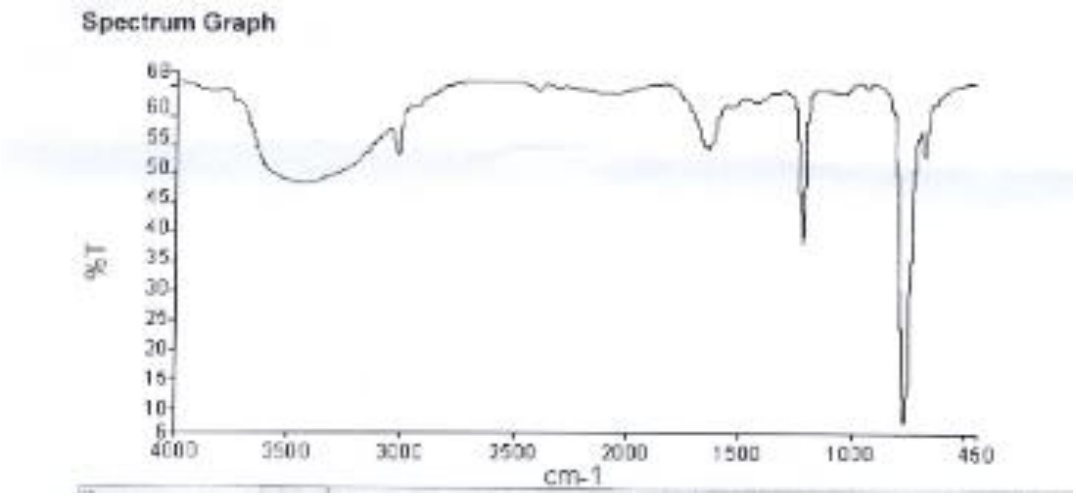
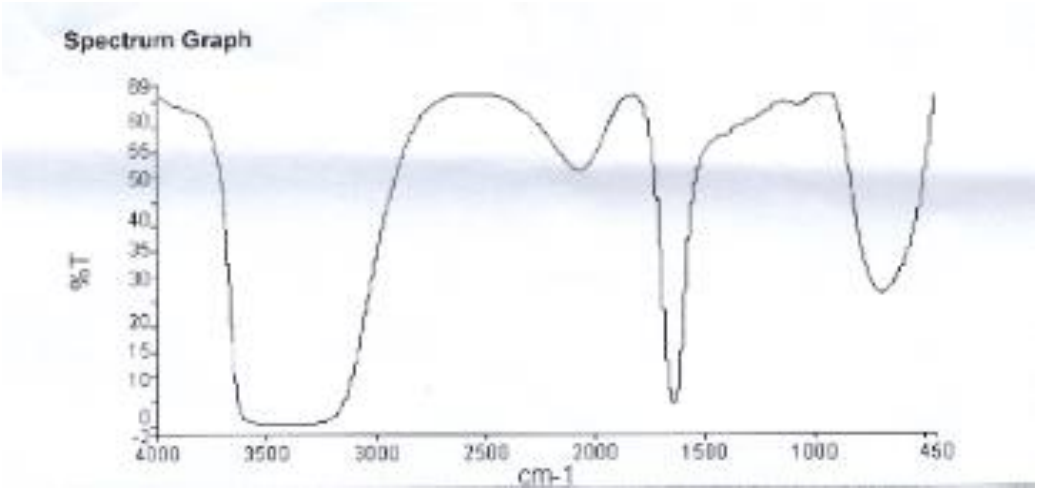


Fig. 3.5 FTIR of Carbopol polymer with *Citrus limon* extract.

Table 3.7 FTIR spectrum peaks of Carbopol polymer with *Citrus limon* peel extract.

21. Theoretical peak 22.	**23. Observed peak**	**24. Groups**
25. 900-690 26.	27. 791.12	28. C-H bending (out of plane)
29. 1260-1000 30.	31. 1215.92	32. C-O stretching
33. 1650-1600 34.	35. 1637.42	36. C=C stretching
37. 2100-1650 38.	39. 2068.06	40. C-H bending
41. 3500-3200 42.	43. 3435.46	44. O-H stretching (strong)

3.5.5 FTIR spectra of Carbopol polymer with *Citrus limon* peel extracts



3.6 Selection of methodology for silver nanoparticles loaded with *Citrus limon* peel extract

3.6.1 Chemical method: Chemical reduction is the most common technique used to prepare AgNPs as a stable, colloidal dispersion in water or organic solvent. Initially, the decrease of multiple complexes with silver ions leads to the creation of silver atoms, followed by agglomeration into oligomeric clusters. These clusters ultimately lead to the formation of silver colloidal particles. After 24 hours at room temperature, the light yellow color changes into darker brown structure. **Huang S et al. [27**]

3.6.2 Microwave method : In this methodology, plant concentrate and $AgNO_3$ were taken in 50 mL round bottom flask and put in a microwave that worked at half intensity of 350 W for the 90S (3 cycles of 30 sec). The light yellow shading arrangement immediately went to the darker shading, demonstrating the development of silver nanoparticle of plant extract. This colloidal arrangement was centrifuged at 1000r.p.m at 4^0C for 1 hr. The benefit of microwave-interceded combination over the customary warming is improved energy of the response for most part, quick warming and the age of limited high-temperature zones at response sites. **Prathna T. C et al. [65]**

Fig. 3.7 Colour before the formulation.

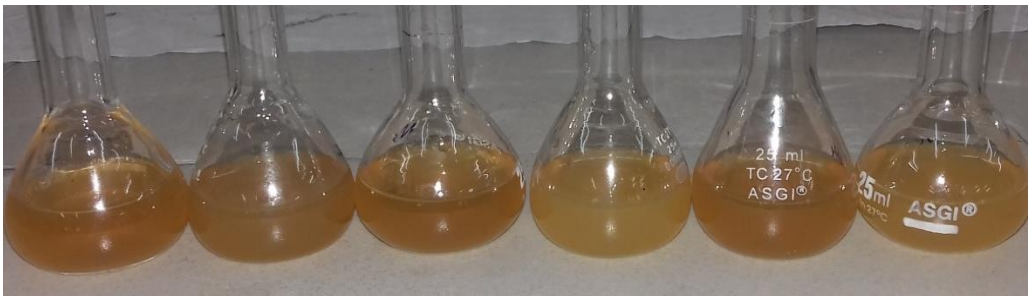


Fig. 3.8 Colour after formulation.

Table 3.9 3^2 Factorial designs for the optimized of microwave-assisted silver nanoparticles. Is Fatimah et al. [28], Loriz Francisco et al. [41]

Sr. No	Conc· of $AgNO_3$ (X1)	Power (X2)	Time (X3)
1	+1	+1	+1
2	+1	0	0
3	+1	-1	-1
4	0	+1	+1
5	0	0	0
6	0	-1	-1
7	-1	+1	+1
8	-1	0	0
9	-1	-1	-1

Actual value: X1,(+1)=20mL,(0)=15mL, (-1)=10mL , X2,(+1)=450W,(0)=350W,(-1)=250W, X3,(+1)=120 Sec, (0)=90 Sec, (-1)=60 Sec.

Table 3.10 Formulation parameters of silver nanoparticles loaded *Citrus lemon* extract.

Formulation Code	Concentration of plant extract (mM)	Concentration of $AgNO_3$ (mM)
Citrus limon		
A1	10	0.2
A2	10	0.2

A3	10	0.2
A4	10	0.2
A5	10	0.2
AS6	10	0.2

3.7 Evaluation of microwave assisted synthesis of silver nanoparticles using *Citrus lismon* extract.

3.7.1 UV-Visible absorption of silver nanoparticles: The development of Ag-NPs stacked with *Citrus limon* concentrate was checked with a UV-Visible spectrophotometer (Shimadzu 1700, Japan) for λmax at a wavelength between 200 to 600 nm. Distilled water was utilized as blank for performing UV-Visible absorption. **Yen San Chan et al. [92]**

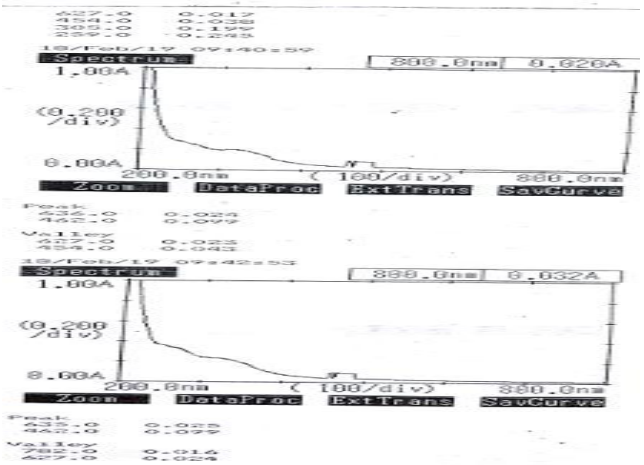


Fig. 3.9 U.V-Visible analysis of *Citrus lismon* from chemical and microwave method.

Table 3.10 Formulation parameters of silver nanoparticles loaded *Citrus limon* peel extract.

Formulation Code		**Concentration of plant extract (mM)**	**Concentration of $AgNO_3$ (mM)**
Citrus lemon			
A1		10	0.2
A2		10	0.2
A3		10	0.2
A4		10	0.2
A5		10	0.2
As6		10	0.2

3.7 Evaluation of microwave assisted synthesis of silver nanoparticles using *Citrus limon* peel extract.

3.7.1 UV-Visible absorption of silver nanoparticles: The development of Ag-NPs stacked with *Citrus limon* peel concentrate was checked with a UV-Visible spectrophotometer (Shimadzu 1700, Japan) for λmax at a wavelength between 200 to 600 nm. Distilled water was utilized as blank for performing UV-Visible absorption. **Yen San Chan et al. [92]**

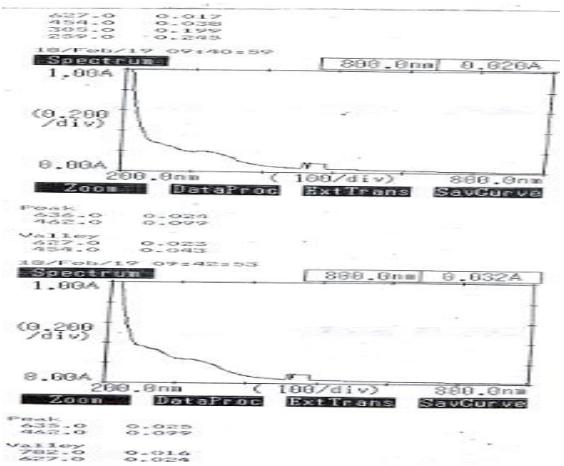


Fig. 3.9 U.V-Visible analysis of *Citrus limon* from chemical and microwave method.

3.7.2 FTIR of silver nanoparticles

3.7.2.1 FTIR of *Citrus limon* peel extract loaded AgNPs

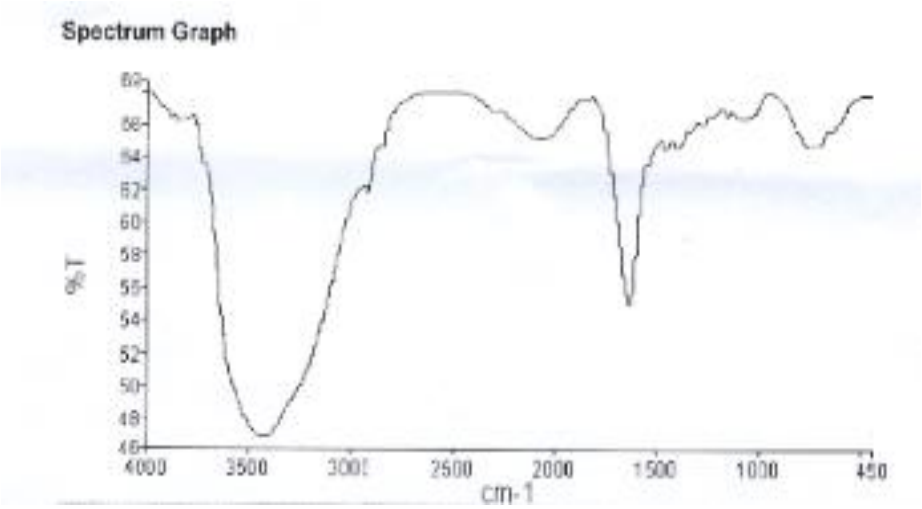


Fig. 3.11: FTIR of *Citrus limon* peel extract loaded AgNPs.

Table 3.11: FTIR of *Citrus limon* peel extract loaded AgNPs.

45. Theoretical peak 46.	47. Observed peak	48. Groups
49. 900-650 50.	51. 764,52	52. C-H bending (out of plane)
53. 1650-1600 54.	55. 1635.08	56. C=C stretching
57. 2100-1650 58.	59. 2069.88	60. C-H bending (aromatic)
61. 3500-3200 62.	63. 3433.71	64. O-H stretching (strong)

3.7.3 Particle size and polydispersity index (PDI): The size and distribution of nanoparticles are critical factors in their formulation. To assess these characteristics, dynamic light scattering (DLS) was utilized, measuring both particle size and polydispersity index (PDI). Particle size and size distribution are the most significant characteristics of the nanoparticle scheme. Differaction light scattering was used to measure nanoparticles size and pdi. The polydispersity index was researched to determine the narrowness / broadness of the distribution of particle size. Before measurement, the sample was sonicated. Each sample was diluted with distilled water to prevent multi-scattering phenomena and put in a dispsable size cuvette. The measurement was performed at 25ºC. The mean diameter and size dissemination of resultant homogenous suspension was evaluated. **Vasile et al. [88]**

3.7.3 Surface Charge: Zeta potential estimations were done by ZetaSizer (Nano ZS, (Malvern Instruments, UK) with an expendable slim cell. Each example was appropriately weakened with water and set in a dispensable zeta cell.**Panacek et al. [59]**

3.7.4 Entrapment Efficiency of Silver nanoparticles: Capture proficiency of silver nanoparticles was controlled by utilizing UV-Visible Spectrophotometer (Shimadzu 1700, Japan) at 510nm. About 1 mL of nanosuspension was sonicated for 2 minutes and diluted up to 10 mL with water. It was centrifuged at 1000 rpm for 1 hr at 4^0c. The absorbance of the free drug in the supernatant was resolved utilizing relapse condition from adjustment bend at 510 nm and the measure of entrapment efficiency was determined. Nanosuspension without medication was taken as reference and the optical absorbance was subtracted from the absorbance of each sample. % entrapment efficiency was determined by utilizing the following equations. **Ahmed V et al. [4]**

$$\%\ \mathbf{Entrapmentefficiency} = \frac{(\mathbf{Totalamountofdrug - Freedrug})}{\mathbf{Totalamountofdrug}} \times \mathbf{100} \qquad \mathbf{(3.1)}$$

3.7.5 *In-vitro* release of microwave assisted synthesis of silver nanoparticles using *Citrus limon* extract: Drug release in-vitro was performed using an open ended tube technique. One end of the open ended tube was attached to cellophane paper earlier soaked in glycerin for about 20 minutes, which acts as a semi-permeable membrane. The open ended pipe was solved in a stand and the end attached to cellophane was immersed in 100 mL of phosphate buffer pH 7.4 at 37 ± 0.5ºC and stirred at 50rpm. *Citrus limon* peel extract loaded with AgNPs were transmitted to the pipe. Drug release was evaluated by removing 5 mL of the sample at 15-minute periods for the first hour and one hour periods for 8 hours and lastly at 24 hours. The removed (5ml) specimens were transmitted to 10ml standard flasks and produced with Phosphate buffer pH 7.4 up to volume. The resulting solutions were analyzed by measuring the absorbance at 272nm using UV Visible spectrophotometer. **Prasad S. et al. [62**]

Table 3.13 Evaluation parameters of the various batches of Ag-NPs of *Citrus limon* peel extract.

Sr. No	Formulation code	Particle size (nm)	PDI	Zeta potential (-mV)	Entrapment efficiency (%)	*In-vitro* release study (%)
1	A1	157.9	0.272	-32.8	97.9	85.91
2	A2	175.4	0.341	-48.2	96.43	87.90
3	A3	81.7	0.253	-37.8	97.95	82.12
4	**A4**	**78.7**	**0.256**	**-37.8**	**91.95**	**90.1**
5	A5	618.4	0.294	-49.5	96.48	89.0
6	A6	234.2	0.461	-39.8	92.39	88.9

3.8 Development of nanogels using silver nanoparticles of *Citrus limon* peel extract

3.8.1 Selection of polymer: The optimization of topical silver nanogel was completed utilizing diverse gelling specialists like Carbopol 940, HPMC, and CMC. Each clump of the gel was detailed with 0.50%, and 1% of gelling agents. The clumps were assessed for physical appearance, pH, spreadability, extrudability, and thickness. Among the three polymers, the best outcomes were obtained with Carbopol 940. **Swathi V et al. [81]**

Table 3.15: Formulation of gel using different polymers at different concentration.

Batch	Carbopol(g)	HPMC(g)	CMC(g)	Propylene glycol(mL)	Triethanolamine	Distilled water (mL)
E1	0.5	0	0	2.25	q.s.	25
E2	1	0	0	2.25	q.s.	25
F1	0	0.5	0	2.25	q.s.	25
F2	0	1	0	2.25	q.s.	25
G1	0	0	0.5	2.25	q.s.	25
Gs2	0	0	1	2.25	q.s.	25

Table 3.16: Evaluation parameters of the various gelling agents at different concentrations.

Parameters	Carbopol 940	HPMC	CMC
Appearance	Clear, Transparent	White, Viscous	White, Viscous
pH	7.0	6.5	6.8
Spreadibility	79 second	38 second	43seconds
Extrudability	15.9 cm	13.8cm	12.2cm
Viscosity (Cp)	1,42,000	78,000	66,000
	89,000	52,000	51,000
	67,000	48,000	42,000
	51,000	31,000	29,000

3.8.2 Formulation of silver nanogel of the optimized batch of *Citrus limon* peel extract loaded AgNPs

Table 3.17: Development of silver nanogels using optimized batch of A4.

Ingredients	Batch NA4
Citrus limon peel extract	0.2%
$AgNO_3$	0.2 mM
Carbopol 940	1%

Propylene glycol	2 ml
Triethanolamine	q.s.
Methyl paraben	0.01%
Distilled water	Upto 100g

Carbopol 940 was chosen for the gel on account of its gelling property and high thickness. Advanced clums of silver nanoparticles of plant (A4) was fused into gel based on different assessment parameters. Propylene glycol was utilized as entrance enhancer and Triethanolamine was utilized to change the pH of the gel. The groups were assessed for physical appearance, pH, spreadability, extrudability, consistency, and *in-vitro* release.

3.8.3 Evaluations of microwave assisted synthesis of silver nanogels using peel extract of *Citrus limon* separately.

3.8.3.1 pH: The pH meter was calibrated at pH 4 and 7 with phosphate buffer solution, and was used to evaluate pH. One gram of each formulation was dissolved in 10 mL of purified water. The experiment was repeated three times and reported as average. Sample pH was measured 48 hours, 1 week, 2 weeks, 1 month, and 3 months after preparation.

3.8.3.2 Viscosity: Brook field viscometer (Model. RV) was used for the determination of viscosity of the sample. 5gm gel was taken in a beaker and spindle No. 7 was dipped for 5 min, rotated 10rpm and dial readings were taken.

(Centipoise) η = Factor × dial reading (3.2

3.8.3.3 Spreadability

The spreadability of each batch of silver nanogel was resolved by estimating the measurement of 1 gm gel between level plates (20×20 cm^2) for 1 minute. The standard weight tied on the upper plate was 125 g.

S= M.L/T (3.3)

Where, S= Spreadability, M= weight tied to slide, L= length of the slide, T= time taken

3.8.3.4 Surface morphology: The Nanogel's shape and surface morphology prepared with optimized parameters was noted by scanning electron microscopy. The research reveals that most of the Nanogel particles were mildly spherical in form, the particle surface showed a distinctive smoothness, and the particle size was in the nanometric range as shown by SEM. Some of the particles were discovered to be in clusters and mostly the general formulation reveals uniform dispersion of extract throughout the gel.

3.8.3.5 Texture analysis of gel: Texture analysis was done using Texture analyzer model TA-XT2, with load sensitivity 5g. Texture Expert Exceed (version 2.61 Exceed) from stable micro systems was used to collect and display the data.

3.8.3.7 Extrudability: A closed collapsible tube containing approximately 20 g gel was strongly pressed at the crimped end and a clamp was applied to avoid any back roll. Removed the cap and extruded the gel. Collected and weighed the quantity of the extruded gel. Calculated the proportion of the extruded gel.

3.8.3.8 Drug content : 1 g gel was dissolved in 10 ml of ethanol in a 10 mL volumetric flask. The flask was shaken at intervals to allow the drug to release. The sample was filtered and suitable dilutions were prepared. The absorbance of the sample was taken by UV-Visible spectroscopy at 272nm and drug content was determined. The drug content of gel was 91.1% for A4.

3.8.3.9 *In-vitro* release study: The *in-vitro* release study was performed in the Franz diffusion cell. The receptor compartment consisted of phosphate buffer of pH 7.4. Dialysis membrane was mounted between the donor and receptor compartment. 0.5 g gel was taken in the donor compartment and stirred on a magnetic stirrer. The temperature was maintained at 37^0C. 1mL of the sample was withdrawn at regular and replaced by 1 mL phosphate buffer. The sample was analyzed by U.V-Visible spectrophotometer. **J Patel et al. [30], K.Sumalatha et al. [33]**

3.8.3.10 Skin irritation study: One g silver nanogel were applied onto the dorsal skin of rats weighing 150-300 g and occluded with gauze and bandages. After 24 hrs, the gel was removed and the score of erythema was determined according to the method of Draize as follows 0, no erythema, 1, mild erythema, 2, moderate erythema, 3, severe erythema. **Aiyalu R et al. [5]**

Table 3.18: Evaluation studies of silver nanogel (A4).

Evaluation parameters	Batch
	A4
Physical appearance	Light yellow, translucent
pH	7.0
Extrudability	14.8cm
Gel strength	52g
Spreadability	49 seconds
Viscosity (Cp)	72000
	3100
	23500
	9800
Drug content	91.1%
In-vitro release	90.1%

4. PHARMACOLOGICAL ACTIVITY

4.1. Animal: Wistar rats, (either sex) weighing 150-300g were used for the evaluation of the anti-inflammatory and anti-arthritic activity of *Citrus limon* peel extract loaded silver nanogel. The animals were kept in propylene cages with a steel net, in a room maintained under standard living conditions of temperature 25±5^{0}C and relative humidity of 55±5%, with regular 12 hour light and 12-hour dark cycles and allowed free access to standard laboratory food and water.

4.1.1. Experimental groups: Rats were divided into three groups randomly, with five animals in each group.

Table 4.1 Experimental group with respective treatment.

Sr. No.	**Experimental group**	**Treatment**
1	Group I (Inducer group)	Carrageenan Solution (.1 mL of 1% solution for inflammation)
2	Group II (Standard group)	Voveran gel (Diclofenac sodium)
3	Group III (Test-I (A4) group)	Test Formulation I (Silver nanogel-I)

4.1.1.1 Anti-inflammatory activity: The carrageenan-induced paw edema method was used for anti-inflammatory activity evaluation. Wistar albino rats were divided into 3 groups (n=5). The first group (negative control) group, second group was treated with Diclofenac sodium gel, third group were treated with test formulation-I (A4). All treatments were applied to the plantar surface of the left hind paw of rats by gentle rubbing of 0.5 g with the index finger. After one hour, a subplantar injection of 0.1 mL of 1 % carrageenan in normal saline injected into the treated paw of all rats. The volumes of injected paws were measured by using plethysmometer immediately, before and 3 hours following carrageenan injection.

The percent inhibition was calculated using the formula as follows:

$$\% \text{ edema inhibition} = [1-(V_t/V_c)] \times 100 \quad \textbf{[4.1]}$$

Where V_t= volume in the drug-treated group and V_c= volume in the drug control group **Hasan** Soliman Yusufogly et al.

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Table.4.2: Anti-inflammatory activity of *Citrus limon* peel extract formulated silver nanogel by carrageenan-induced hind paw edema in rats.

Treatment	Oedema volume (cm) reduction after 3 hr	Percentage inhibition
Control	0.32±0.02	-
Voveran gel	0.06±0.02	79.21
A4	0.10±0.0	61.70

Values are Mean±SEM. n=5 in each group. ***p<0.01, when compared to the gel base

4.1.1.1.1 Statistical analysis: Results are expressed as Mean±SEM. The data compared between experimental groups by One-way ANOVA, Dunnett's *post hoc* test using GraphPad Prism 5.0 software. p<0.01 was considered significant.

4.1.1.2.1 Body weight: Reduction in the body weight was observed for arthritic control group whereas gain in the body weight was observed in Voveran gel (standard group), test formulation 1 (A4s) .

4.1.1.2.2 Paw Volume: The arthritic control groups showed signs of arthritis development as seen by increases in paw volume. Reduction in paw volume was observed in Voveran gel (standard group), test formulations(A4).

4.1.1.3 Skin irritation test of silver nanogel containing *Citrus limon* ssextract: The prepared silver nanogel containing *Citrus limon* peel extract was evaluated for its irritant effect. Where no erythema or edema was observed for all the formulations, even after 24 hrs of study, indicating that the prepared silver nanogel formulations was found to be safe. **Aiyalu. R *et al.* [5]**

Pharmacological Screening: The result of this study indicated that the local application of *Citrus limon* peel extract loaded silver nanogels can be an effective medication for inflammation and arthritis.

5. CONCLUSION

The present study was based on the novel, a fast, accurate, feasible and convenient technique was utilized for the formulation of silver nanogel containing *Citrus limon* peel extract for high yield, good drug loading, and increased drug efficiency, etc. Silver nanogel provides faster and prolonged action, increases product efficiency. *In-vitro* release studies of silver nanogel containing *Citrus limon* peel extract showed that silver nanogel provide faster action and prolonged activity as compared to normal topical gel.

6. FUTURE PROSPECTIVE

Development and *In-vitro* and *In-vivo* evaluation of *Citrus lismon* peel extract loaded silver nanogel was successfully formulated and can be a promising approach for the treatment of inflammation.

7. CONCLUSION

A silver Nano gel was then developed using the Citrus limon peel extract, which was subsequently evaluated for its anti-inflammatory activity using in-vitro and in-vivo models. The use of Citrus limon peel extract loaded silver Nano gel aims to overcome the limitations of conventional formulations, such as poor absorption and bioavailability, limited ability to reach the target site, and expensive production and formulation processes. By developing a nanogel-based formulation, the study seeks to improve the delivery and efficacy of the Citrus limon peel extract. Novel herbal formulations were developed to deliver silver nanoparticles of Citrus lemon peel extract for the treatment of inflammation and arthritis. These formulations aim to provide more efficient therapy with no side effects, higher bioavailability, and cost-effectiveness compared to synthetic drugs. The use of silver nanoparticles in these formulations offers several benefits, including anti-microbial activity and improved bioavailability. Silver itself has been shown to reduce the growth of micro-organisms during inflammation, while the nano-formulation enhances the delivery of the active compounds, leading to better absorption and utilization.

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