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Development and Evaluation of Herbal Nanogel for the Treatment of Oral Candidiasis

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Keywords

Nanoparticles, Nanogel, Eudragit S100, Carbopol 934.

Abstract

The primary goal of the current study is to create Nanogel using Amaranthus viridis and Azadirachta indica extract nanoparticles. Because of its antibacterial qualities, Amaranthus viridis and Azadirachta indica extract was crucial in the creation of Nanogel for the topical distribution of the active ingredient. Nanoparticles are created with Amaranthus viridis and Azadirachta indica extract to increase medication penetration rates when administered topically and the formulation with the highest level of entrapment effectiveness is chosen to create Nanogel. In order to increase the effectiveness of the active ingredient's entrapment, Eudragit S100 is employed in the formulation of nanoparticles. The F5 formulation, which contains a higher concentration of Eudragit S100 and is transformed into gel by the gelling ingredient Carbopol 934, is chosen as the optimised formulation. There have been good outcomes observed in all of the evaluation trials for the gel.

1. Introduction

Medicine made from plants is crucial for both medicinal and financial reasons. Up to 80% of people worldwide still use herbal remedies as their main form of treatment, primarily in developing nations. This is because they are more widely accepted, better suited to human physiology, and have less adverse effects. Herbal medicines have a number of advantages over allopathic treatments, but they also have some drawbacks that make them less popular with patients. It is not shocking that one-fourth of the global population uses herbal and conventional medicines to cure a range of diseases. Herbal medicines are manufactured from plant material or any part of a plant that is used to heal wounds, treat infections, and promote overall health. It refers to a product or preparation made from a plant or plant parts and used for any such function. Herbal medicine is the oldest sort of medicinal therapy that has been identified[1].In the past, the majority of people in the world have relied on traditional medicine, but in recent years, attention has shifted to the use of biodegradable and ecologically friendly plant-based medications for the treatment and prevention of numerous ailments. India has many plants with therapeutic characteristics. Synthetic medications can be effectively replaced with medicinal herbs.

A procedure known as topical medicine distribution is used to apply a drug-containing formulation to the skin in order to treat a cutaneous ailment. This practise is employed when other methods of medicine administration, such as oral, sublingual, rectal, and parental, are ineffective or when a local skin condition, such as a fungal infection, manifests1. Topical drugs are regularly used to treat ailments of both the system and the immediate area. The topical administration system absorbs the medication being administered and transports it to the site of action, where it exerts its therapeutic effect. The physiological properties of the carrier have a direct bearing son how quickly a medication releases from a topical preparation. One of the main advantages of a topical administration

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approach is first-pass metabolism. On the basis of particle size, the term "microemulsion" is used. The drug easily diffuses through the skin and reaches the site of action because of the small particle size. In addition to keeping the microemulsion in place for a very long time, the gel will aid in the medication's delayed release. A number of fungal infections are currently spreading more widely and pose a serious threat to society. Tinea corporis, Tinea pedis, and Tinea capitis are three dangerous types of fungi that can cause skin infections. With the use of a technique like nanogel, the medicine can be easily absorbed into the skin and quickly begin to work.[2,3]

Topical medication delivery techniques have advantages.:

- Easy application and practical use;
- Inhibition of the first pass metabolism.
- Terminating the drugs is simple.
- Drug provided specifically to a certain location.
- Drug distribution at a particular location [4]

Topical medication delivery techniques have drawbacks.:-

- There may be a chance of localised skin irritation when the product is applied.
- Some medications are difficult for the skin to absorb because of their low permeability.
- It is difficult to permeate drugs with bigger particle sizes.
- Medication activities can be performed with very low plasma concentrations[5].

Nanogel

The word "nanogel" refers to nanoscale particles made of physically or chemically crosslinked polymer networks that enlarge when exposed to an appropriate solvent.

The word "nanogel" was first used to describe cross-linked bifunctional networks of a polyion and a nonionic polymer for the transport of polynucleotides.

Nanogels are cross-linked hydrophilic polymer-based submicron-sized hydrophilic polymers. Although they are soluble in water, they differ from linear macromolecules with equal molecular weights in terms of their characteristics. These structures and their larger equivalents. Hydrogel nanoparticles, more recently known as nanogels, are a class of nanoscale particulate materials that have attracted a lot of attention from researchers looking into drug delivery systems. It's intriguing that the properties and characteristics that both NPs and hydrogels separately possess would coexist in hydrogel nanoparticulate materials. The pharmacy industry appears to be profitable due to the hydrophilicity, adaptability, versatility, high water absorptivity, and biocompatibility of these particles as well as all the benefits of nanoparticles, particularly their long half-life in circulation and the ability to be actively or passively targeted to the desired bio phase, such as tumour sites. Several methods have been utilised to create nanoparticles that resemble hydrogels. in addition to the typical synthetic polymers. Research is still being done on the production of nanoparticles using hydrophilic polymers that are present in nature. [6,7]

Characteristics of nanogel

1.Biological compatibility and biodegradability

2. The water's ability to expand.

3. An improved ability to load medications

Size of the particles

5. Liquability

6.Colloid stability

7. Non-immunologic reactivity

Nanogels are divided into two categories

The first is determined by how responsive they are, which can be either stimulus-sensitive or nonresponsive.

1.In the case of non-responsive nanogels, they simply swell when they take in water.

2. Stimuli-responsive nanogels fluctuate in size in response to changes in environmental variables as temperature, pH, magnetic field, and ionic strength. A range of environmental cues cause multiresponsive microgels to respond.

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Based on the types of connections present in the network chains that form the gel structure, the following four classifications for polymeric gels have been established:

1. Gels with physical cross links

2. Nanogels with liposome modifications

3. Nanogels in micelles

4. Hybrid nanogels

ORAL CANDIDASIS-

The oral mucosa is frequently infected with an opportunistic fungal disease known as oral candidiasis, also known as "thrush." A commensal microbe called Candida albicans, which lives on people and is very adaptable, is the main perpetrator. However, modifications to the milieu of the host can encourage the transition from commensalism to pathogen status. The remarkable diversity of virulence factors required for this transformation includes cell surface adhesins, proteolytic enzymes, morphological changes, and the evolution of drug resistance. It must stick to oral cavity bacteria in order for C. albicans to endure. It has been proven that a variety of synergistic interactions with other oral species make colonisation in the host easier. Local innate immune defences are crucial for keeping Candida in its commensal state because it frequently colonises the oral mucosa and the host immune response in the oral cavity is focused on a more tolerogenic state. Saliva is particularly rich in anti-candidal peptides, which are thought to be a component of the host's innate defence and which also hinder Candida from sticking to epithelial cells. The T helper 17 (Th17) subtype of adaptive immune responses is predominantly involved in mucosal host defences, regulating early Candida development and preventing later tissue invasion. Our understanding of Candida virulence factors and the variables affecting host susceptibility to infections depends heavily on animal models, particularly the rat model of denture stomatitis and the mouse model of oropharyngeal candidiasis. Novel therapeutic approaches are focused on discovering bioactive molecules that target pathogenic processes to stop C. albicans from transitioning from a benign commensal to a pathogen in light of the rising prevalence of resistance to the few few conventional antifungal medications.

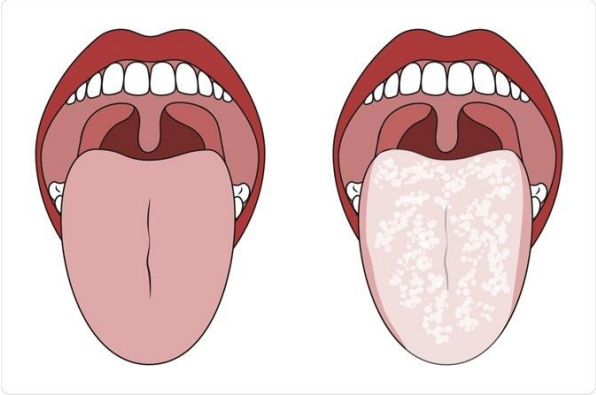

Figure 1: Oral Candidiasis

Drug profile:

Figure No.2-*Amaranthus viridis*

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Figure No.3-*Azadirachta indica*

	Amaranthus viridis L	***Azadirachta indica***
Kingdom	Plantae	Plantae
Subkingdom	Viridiplantae	Tracheobionta Vascular
Division	Tracheophyta	Spermatophyta Seed
Subdivision	Spermatophytina	Magnoliophyta Flower
Class	Magnoliopsida	Magnoliopsida Dicotyledonous
Superorder	Caryophyllanae	
Order	Caryophyllales	Rutales
Family	Amaranthaceae	Meliaceae
Genus	*Amaranthus*	Azadirachta
Species	Viridis	indica
Binomial name	*Amaranthus viridis L*	*Azadirachta indica*
Synonyms	Amaranthus ascendens Loisel, Euxolus viridis Moq., Viride Pyxidium Moench, Glomeraria viridis Cav, Amaranthus emarginatus Salzm, and Amaranthus gracilis Desf	Melia azadirachta L., Melia indica (A. Juss.), Azadirachta indica var. minor, Azadirachta indica var. siamensis, Azadirachta indica subsp.

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Pharmacological Activities-

- Antioxidant & Antimicrobial Activities
- Antiphytopathogenic Activity
- Hepatoprotective Activity
- Anthelmintic Activity
- Antinociceptive and Antipyretic Activities
- Antidiabetic and Antihyperlipidaemic Activities
- Antifungal Activities[8,9,10]

2. Materials and Equipment:

Materials:

Table 1 : Materials

Sr. No.	Materials	Suppliers
1	Amranthus viridis extract	Hubali,Chennai
2	Azadiractica indica leaf extract	Hubali,Chennai
3	Cabropol 934	Mumbai's Fine Chem Research Lab
4	Propylene glycol	Mumbai's Fine Chem Research Lab
5	Triethanolamine	Mumbai's Fine Chem Research Lab
6	Methyl paraben	Mumbai's Fine Chem Research Lab
7	Propyl paraben	Mumbai's Fine Chem Research Lab
8	Eudragit S100	Mumbai's Fine Chem Research Lab
9	Poloxamer407	Mumbai's Fine Chem Research Lab
10	Glycerine	Mumbai's Fine Chem Research Lab

Equipments:

Table 2 : Equipments

Sr. No.	Equipment	Model/Maker
1	Electronic balance	Analytical balance
2	pH meter	Hanna instruments
3	FT-IR	JASCO4100, Japan
4	UV- Visible Spectrophotometer	Shimadzu UV 1700, Japan
5	Brookfield Viscometer	LVDV-2

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6	Sonicator	Lag Enertech electronics pvt. Ltd, Vasai
7	Centrifuge	Remi centrifuge, R-8C
8	Mechanical Stirrer	Remi – Lab Stirrer
9	Hptlc	VisionCATS(3.1)

3. Experimental work

Preformulation study :

Preformulation is the research of the physical and chemical characteristics of a pharmacological ingredient, both on its own and when combined with an excipient. The first step in developing a drug's dosage form rationally is preformulation testing. The goal of preformulation testing is to collect data that will be helpful in developing stable, bioavailable, and mass-producible dosage forms.

a. Estimation of *Amaranthus viridis* and *Azadirachta indica* extract using UV spectroscopic method :

A. Preparation of stock solution :

The extracts of Azadirachta indica and Amaranthus viridis were transferred to separate 100 ml volumetric flasks after being carefully weighed at 10 mg each. They were then diluted in 20 ml ethanol and 70 ml distilled water. The flask was treated to 15 minutes of sonication to produce a clear solution, and the volume was then adjusted to the desired objective to produce a solution with 100 ug/ml.

B. Extract calibration curve in distilled water:

To determine the maximum wavelength, individual scans were performed on the working standard solution of extract (30 ug/ml). *Amaranthus viridis* and *Azadirachta indica* were discovered to have maximum wavelengths of 413 nm. By adding 0.5 to 2.5 ml of the stock solution to each of five volumetric flasks (10 ml), standard solutions of the extract in the range of 5 to 25 ug/ml were created. Distilled water was used to fill the volumetric flask. Beer's Lambert law was used to calculate the absorbtivity of these solutions after their absorbance was measured[11].

b. Drug excipient computability studies :

A. Physical compatibility test :

A physical compatibility test was used to determine how well drug excipients worked together. The medication and excipient were combined in a 1:1 ratio. Different glass vials with room temperature were used to store the mixes. In order to verify the compatibility of the medicine with the excipients, samples were tested for colour and appearance after 15 days.

B. FT-IR Analysis

The possibility of a solid-state interaction between the medication and excipient was investigated using Fourier Transform Infrared (FT-IR) Spectroscopy. Using a potassium bromide (KBr) backdrop and an FT-IR spectrophotometer, it was detected. The spectra were scanned across a frequency range of 4000-400 cm-1.

k. HPTLC Fingerprint of *Amaranthus viridis* and *Azadirachta indica*:-

Analysis of the provided sample using fingerprints was done. Using R white, R 254 nm, and R 366 nm, photo documentation was done after development. After derivatizing the plate with the Vanillin Sulfuric Acid Reagent and heating it for three minutes at 110°C. At R White R 254 and R 366nm, photo documenting and scanning are carried out after derivatization**[**12**,**13**].**

Table no. 3-Optimized HPTLC condition

Stationary Phase	Merck, TLC AI plate silica gel 60 F 254
Plate format	100 x 100 mm
Application type	Band

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Application	Position Y: 8.0 mm, length: 8.0 mm, width: 0.0 mm
Track	First position X: 21.5 mm, distance: 11.4 mm
Solvent front position	70 mm
Software	VisionCATS(3.1)
ATS 4	S/N:250243 (NEW ATS4)
Mobile Phase	Toluene:Ethyl acetate
Saturation time	20 min
Mode of Separation	Normal Phase
Sample applicator	Camag Linomat V
Sample Solvent Type	Methanol

g.Description: The sample's appearance, colour, and odour were assessed visually.

h. Melting point: The capillary tube method was used to evaluate the melting points of Amaranthus viridis and Azadiractica indica. Its observed value was contrasted with that of Azadiractica indica and Amaranthus viridis.

i. Analysis of solubility: Amaranthus viridis and Azadiractica indica were studied for solubility in both water and organic solvents. Three test tubes containing 10 mg of the medication were filled with the necessary amount of reputable solvent. Shaking the test tubes to check for solution clarity was done.

Formulation of Herbal nanogel: [Nanoprecipitation method]

The nanogel was prepared using carbapol 934,EudragitS100,Poloxamer 407, propylene glycol, triethanolamine, methyl paraben, propyl paraben and distilled water in quantity sufficient to prepare 50 gm of herbal gel as per table no. The formulation procedure was,

- Drug: Ethanol is used to dissolve the polymer (Eudragit S100 and Poloxmer) [Phase 1].
- Carbopol 934 has been submerged in water.
- After swelling, the carbopol has been kept on a magnetic stirrer for stirring.
- The combination of carbopol has been stirred continuously while the prescribed amount of nanoparticle dispersion or isolated nanoparticle has been added.
- Triethanolamine is added for pH adjustment.

Ingredients	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Amaranthus viridis extract (gm)	0.2	0.2	0.2	0.2	**0.2**	0.2	0.2	0.2	0.2
Azadiractica indica leaf extract (gm)	0.2	0.2	0.2	0.2	**0.2**	0.2	0.2	0.2	0.2
Carbapol 934 (gm)	0.45	0.3	0.5	0.5	**0.45**	0.45	0.4	0.4	0.45
Eudragit S100	0.015	0.065	0.03	0.1	**0.065**	0.065	0.03	0.065	0.065

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Poloxmer 407	0.015	0.065	0.03	0.1	**0.065**	0.065	0.03	0.065	0.065
Propylene glycol (ml)	1	1	1	1	**1**	1	1	1	1
Methyl paraben (gm)	0.1	0.1	0.1	0.1	**0.1**	0.1	0.1	0.1	0.1
Propyl paraben (gm)	0.05	0.05	0.05	0.05	**0.05**	0.05	0.05	0.05	0.05
Triethanolamine	2 Drops	2 Drops	2 Drops	2 Drops	**2 Drops**	2 Drops	2 Drops	2 Drops	2 Drops
Ethanol	10	10	10	10	**10**	10	10	10	10
Distilled water	Q.S	Q.S	Q.S	Q.S	**Q.S**	Q.S	Q.S	Q.S	Q.S

Table 4: Formulation of Herbal nanogel

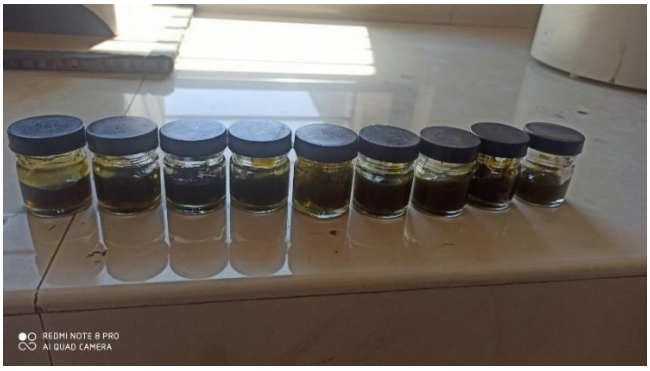

Figure No.4 -All 9 Batches of Nanogel

Evaluation of Herbal Nanogel:

- **Organoleptic properties :**
- **Physical apperance**

Herbal nanogel was examined for appearance and colour.[14] Referring to Table no.11,

- **Homogeneity-**

Visual checks were made to ensure that each generated formulation was homogeneous after the nanogel had been added to the container. We looked at the homogeneity of the formulations to see if there were any aggregates and whether they were visible.(In Table 11)

- **Measurement of pH –**

On an EQUI-TRONICS MODEL 614" digital pH metre, the pH of the gel was determined.One gramme of nanogel was dissolved in 100 millilitres of filtered water, and the mixture was left undisturbed for two hours. Calculating the average of the three measurements of each formulation's pH was done.[15,16] Table 12 demonstrates this.

- **Spreadability –**

A circle with a 1 cm diameter was created on a glass plate, and 0.5 g of gel was added to it. The glass plate was then placed over another plate to determine the spreadability. A 50 g weight was allowed to rest on the upper glass plate for five minutes.[17,18].The results are shown in Table 19.

o It was determined using the formula.

$$S = ML/T$$

- M is the weight fastened to the upper slide;
- L is the length of the glass slides; a
- T is the time required to separate the slides.

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- **Viscosity**

At 25°C, the viscosity of several gel formulations comprising both leaf extracts was tested. A Brookfield viscometer (Model LMDV 60) was used to measure the nanogel's viscosity. The 50 g of nanogel that had been precisely measured was put to the 50 ml glass beaker. Spindle number 4 was chosen, and it is submerged in the nanogel. The viscometer was run at 10 rpm until the reading stabilised and was recorded in Pa.s. [19] (See Table 19)

- **Research on the absorbing of moisture**

In this test, a desiccator is filled with one gramme of nanogel. In addition, distilled water-filled beakers are positioned adjacent to the nanogel in the same desiccator. The nanogel should then be weighed and checked 24 hours later. A nanogel formulation's weight would increase if it were to absorb any liquid.

- **Drug content**

The mixture of 20 ml of phosphate buffer solution with a pH of 7.4 and 1 g of nanogel was then filtered through paper. The absorbance at 255 nm was then measured using a Japanese Shimadzu UV 1700 instrument[20]. (See Tables 14 and 15)

$$\text{Drug content} = \frac{\text{Theoretical concentration} - \text{practical concentration}}{\text{Theoretical concentration}} \times 100$$

- **Test for centrifugation**

All nine of the nanogel batches underwent centrifuged testing in a Remi centrifuge. Two phases were separated when the device was turned on for an hour at a speed of 1000 rotations per minute. (View Table 16).

- **Freeze thaw test**

The freeze-thaw test involved freezing herbal nanogels at -10 0C for 24 hours, followed by a 24-hour period of defrosting at normal temperature. Changes were noted by ocular observation after this cycle had been repeated five times.

• Gel durability

The strength of the nanogel was measured as the time in seconds required for a weight to pass through it. A 5 gramme sample was delivered to each of the successful batches. 3.5gm of weight were placed to the nanogel's surface. the time it takes for the weight to stab the gel for 0.5 cm.(See Table 17)

• Extrudability

The nanogel mixtures were contained and sealed in standard-capped collapsible aluminium tubes. In order to measure extrudability, thumb pressure was used. Excellent +++, Good ++, and Satisfactory + are the grades that were assigned. [21] (See the Table 18)

- **Zeta potential-**

Zetasizer (Malvern Zetasizer) measures the nanogel preparation's zeta potential by placing the formulation in a transparent, single-use zeta cell and measuring the outcome. Using methanol to clean the cuvettes, the sample is added before doing the experiment[22].

- **PARTICLE SIZE**

The nanogel was recognised using FESEM thanks to its specified particle size. The average SLN diameter in the dispersion was determined using field emission scanning electron spectroscopy. The nanogel's surface morphology was described by scanning electron microscopy. [23,24]

- **ENTRAPMENT EFFICIENCY**

The centrifugation method was used to evaluate the success of the SLN dispersion's entrapment. The supernatant liquid from the SLN dispersion, which was prepared by centrifuging the mixture at 20,000 rpm for an hour while it was refrigerated, contained the equivalent of 5 mg of medication. Before filtering, the recovered liquid was properly diluted with a new phosphate buffer solution of saline pH 7.4 to determine the concentration of free medication. The absorbance at 420 nm in a UV spectrophotometer was determined using the following method to determine the entrapment efficiency: (Take note of table No. 13)

- **Stability Study**

The stability of both closed and open containers was tested. In this experiment, nanogel spent three months at ambient temperature.[25,26,27] (See the table 23)

• Drug Release Studies in Vitro

The drug release studies were conducted using Franz diffusion cells, which had an effective diffusion area of 3.14 cm 2 and a cell volume of 16.5 mL. A thin (1 g) layer of Nanogel was placed to the surface of the Cellophane membrane. A cellophane membrane was clamped in the diffusion cell's donor and receptor chambers. Phosphate buffer solution (pH 6.8) was

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newly prepared and poured into the receptor chamber. Using a magnetic stirrer, the receptor chamber was stirred. Samples were collected at the necessary intervals. Samples were analysed for drug content using a UV visible spectrophotometer at max (nm) following the necessary dilutions. The drug was then entirely replaced with new buffer after computing the overall amount of drug released for each suitable time interval as a function of time.[28]

• Functions against fungi

The Cup-plate method was used to compare all batches of produced formulations' antifungal activity to commercially available antifungal formulations as well as formulations without drug-containing gel (blank formulation). The cultures utilised were of the bacterium Candida Albicans. In order to conduct the antifungal test, the agar well diffusion method was employed. The prepared food was taken inside and placed in sterile petri dishes where it was left to cool and dry. The distribution of each bacterial culture was then carried out utilising a micron wire loop. With a sterile cork borer of 6 mm in diameter, 4 mm-deep holes were made. the holes with 0.5 grammes of gel from each batch. Following that, plates were maintained at 27 degrees Celsius for the next 48 hours. Next, the diameter of the zone of inhibition (measured in centimetres) for each chemical and each fungal strength was determined.[29,30]Look at table 22.

4. Result and Discussion:

Phytochemical tests of *Amranthus virdis* extract

Table 5 : Phytochemical test of *Amranthus viridis*. extract

Sr. No.	Phytoconstituents	Test performed	Results
1.	Alkaloid	Hager's test	+
2.	Flavonoid	Shinoda test	+
3.	Carbohydrate	Fehling's test	+
4.	Glycoside a. Cardiac glycoside b. Anthraquinone glycoside	 Legal's test Borntrager's test	 - +
5.	Tannins	Gelatin test	+
6.	Saponin	Froth formation test	-
7.	Steroid	Libermann -Buchard test	+
8.	Protein	Xanthoproteic test	-
9.	Phenol	Ferric chloride test	+

Table 6: Phytochemical tests of *Azadiractica indica* extract

Sr. No.	Phytoconstituents	Test performed	Results
1.	Alkaloid	Hager's test	+

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2.	Flavonoid	Shinoda test	+
3.	Carbohydrate	Fehling's test	+
4.	Glycoside a. Cardiac glycoside b. Anthraquinone glycoside	 Legal's test Borntrager's test	 + +
5.	Tannins	Gelatin test	+
6.	Saponin	Froth formation test	+
7.	Steroid	Libermann -Buchard test	-
8.	Protein	Xanthoproteic test	+
9.	Phenol	Ferric chloride test	+

UV analysis of *Amaranthus viridis* extract:

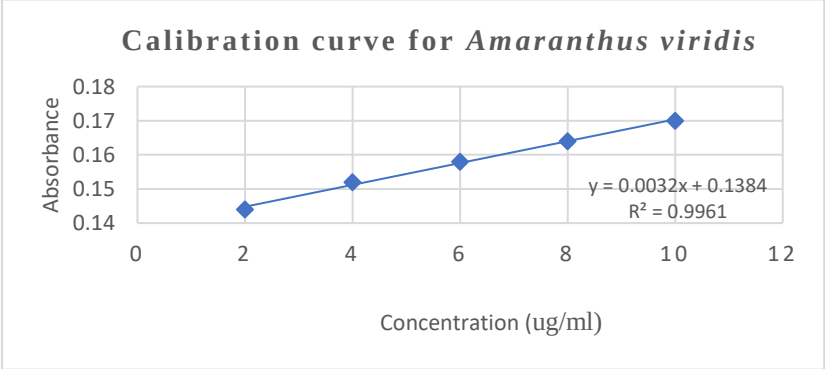

Figure No.5: Calibration curve of *Amaranthus viridis* extract

UV analysis of *Azadirachta indica*. extract :

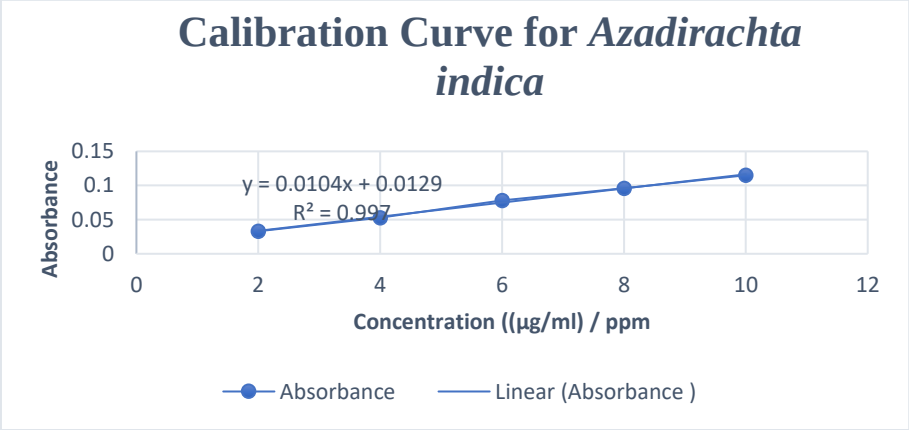

Figure No.6: Calibration curve of *Azadirachta indica leaf* extract

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IR spectra of drugs

a.*Amaranthus viridis*

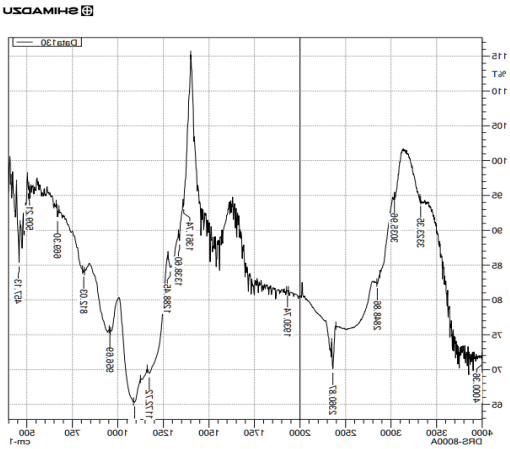

Figure No.7: Infrared spectroscopy of *Amaranthus viridis*

b.Azadirachta indica

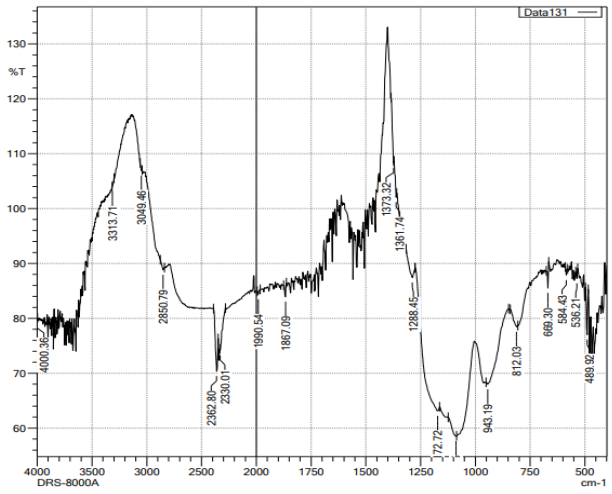

Figure No.8: Infrared spectroscopy of *Azadirachta indica*

Evaluation of herbal nanogel :

Organoleptic properties

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Table No. 11 : Observation of organoleptic properties of herbal nanogel

Formulations	**Physical appearance**		
	Colour	**Tetxure**	**Homogeniety**
F1	Greenish	Smooth	Homogenous
F2	Greenish	Smooth	Homogenous
F3	Greenish	Smooth	Homogenous
F4	Greenish	Smooth	Homogenous
F5	**Greenish**	**Smooth**	**Homogenous**
F6	Faint Greenish	Smooth	Homogenous
F7	Faint Greenish	Smooth	Homogenous
F8	Greenish	Smooth	Homogenous
F9	Greenish	Smooth	Homogenous

pH of herbal nanogel:

The following table, number 12, has pH readings in triplicate. Any pH is acceptable for the oral cavity.

Table No.12: pH

Formulations	**pH**
F1	6.20
F2	6.31
F3	5.23
F4	6.25
F5	**6.49**
F6	6.37
F7	6.56
F8	6.87
F9	6.77

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Particle size of Nanogel:

The size and dispersion of globules in nanogel

The formulation's zeta potential was 24.6 mv, and the formulation's typical globule size was 100 nm. Photomicrography Emulsions from the correctly diluted optimised batches were examined under a 40X light microscope. Emulsion globules that were almost spherical could be seen in the photomicrograph. Despite the fact that this study does not provide an accurate size estimate, it does provide an overview of the generation of nanogel and the effectiveness of the method employed**.**

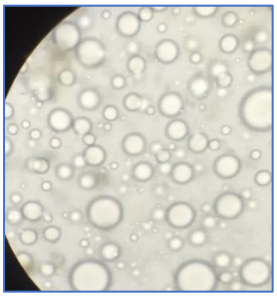

Figure No:9 Photomicrograph of Formulation

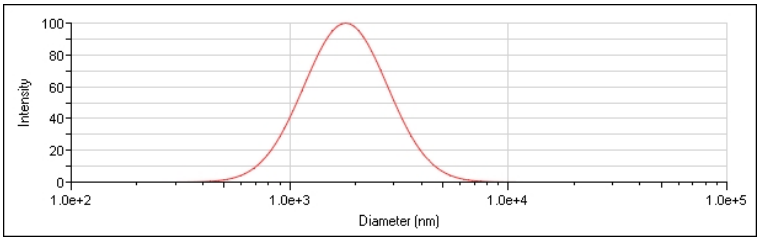

Figure 10 The formulation's optimised particle size.

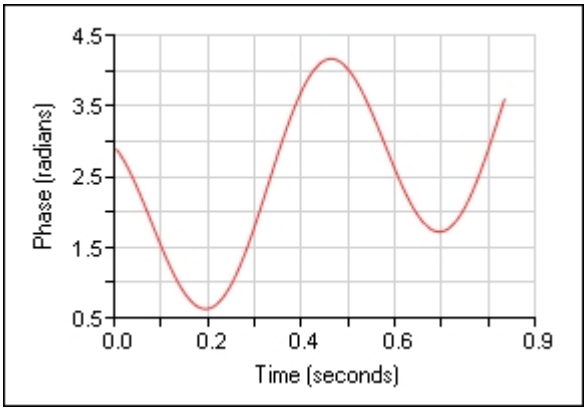

Figure 11 Optimised batch's zeta potential

Entrapment Efficiency-

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Table No.13: Entrapment Efficiency

BATCHES	ENTRAPMENT EFFICIENCY	PARTICLE SIZE
F1	75.36 %	85±0.56
F2	66.18%	88±0.44
F3	67.55 %	95±0.58
F4	77.99 %	98±0.55
F5	**87.12%**	**100±0.45**
F6	79.81 %	94±0.43
F7	81.02 %	96±0.39
F8	85.69 %	99±0.25
F9	83.67 %	106±0.35

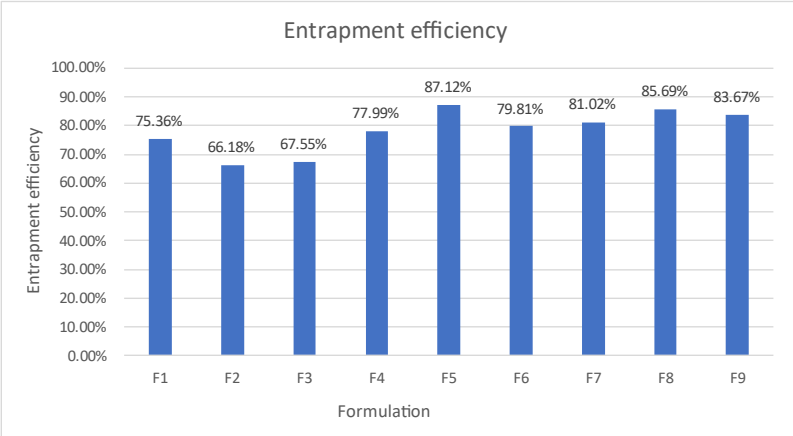

Fig No.12: Entrapment efficiency

SEM-

The nanogel (F5) was SEM-ed using an IMINA apparatus with a 100x magnification and a 10,000 electron volt energy range. It was discovered that the 78.66 nm size of the nanogel formulation F5 SEM image reveals that there is no fracture of nanogel.

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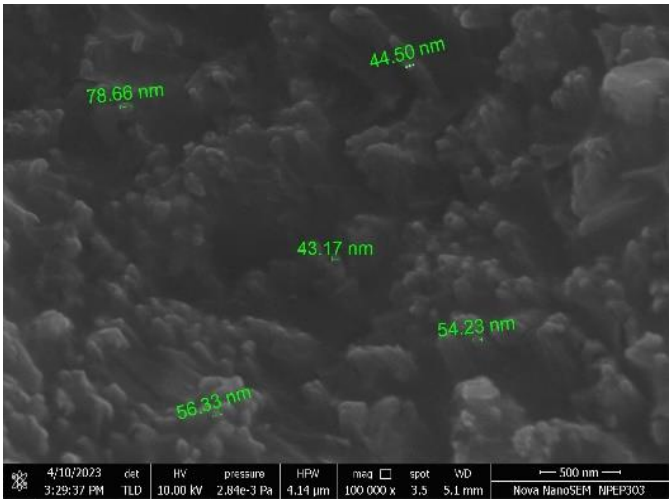

Figure 13-Scanning electron microscopy of nanogel formulation(F5)

Moisture absorption studies:

The formulation batches F1, F2, F3, F4, F5, F6, F7, and F8 pass the test since there are no weight changes when the nanogel is placed next to a beaker that is filled with water in the desiccator. These formulations maintain their stability over time. However, batch F9 of the formulation exhibits a modest weight gain, possibly as a result of the higher amount of propylene glycol in this formulation than in other formulations. It has an impact on stability.

% Drug content of *Amaranthus viridis and Azadiractica indica* leaf extract

The accompanying table number 14 and figure 13 both provide the percentage of drugs present in each gramme of Amaranthus viridis extract nanogel.

Table No.14: % Drug content of *Amaranthus viridis*

Formulation	**% Drug content**
F1	81.64%
F2	84.89%
F3	92.48%
F4	93.93%
F5	**98.26%**
F6	96.82%
F7	87.42%
F8	90.67%
F9	89.23%

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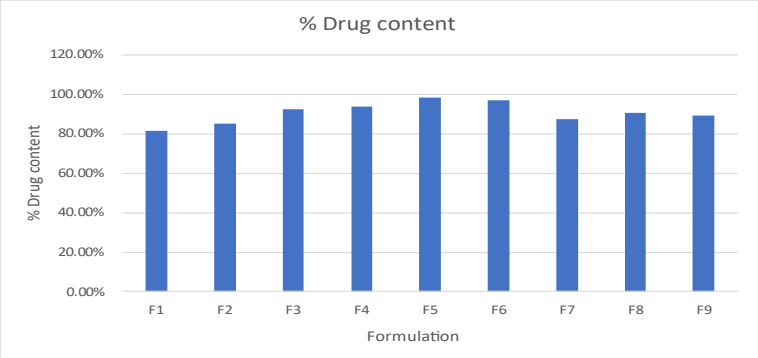

Figure 14: % Drug content of Amaranthus viridis extract

Table 15 and picture 14 provide information on the percentage of drugs present in each gramme of Azadiractica indica leaf extract nanogel.

Table No. 15: % Drug content of *Azadiractica indica* leaf extract

Formulation	**% Drug content**
F1	82.17%
F2	84.88%
F3	91.47%
F4	95.73%
F5	**98.44%**
F6	96.89%
F7	86.82%
F8	91.08%
F9	88.75%

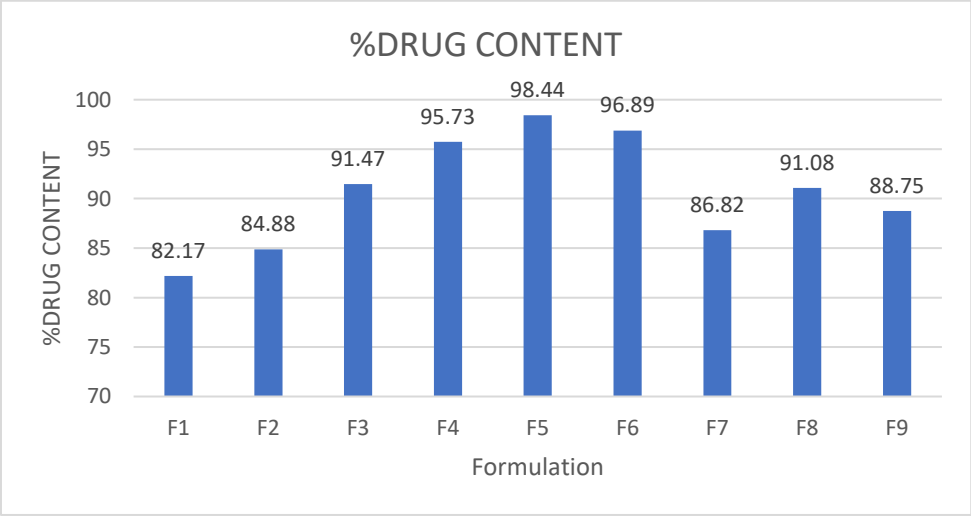

Figure 15: % Drug content of *Azadiractica indica* leaf extract

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Test of centrifugation:

When the nanogels were placed for centrifugation, the findings are displayed in Table 8.10.

Table No.16: Centrifugation test

Formulation	Observation
F1	No phase separation
F2	No phase separation
F3	No phase separation
F4	No phase separation
F5	No phase separation
F6	No phase separation
F7	No phase separation
F8	No phase separation
F9	Phase separation

Freeze thaw testing:

All of the nanogels were kept at ambient temperature and in the freezer. There is no change in the appearance, colour, or texture of any nanogels, and phase separation cannot be seen. So, the freeze-thaw test is passed by all nanogels.

Gel strength:

Table No. 17 : Gel strength

Formulation	Gel strength (Seconds)
F1	19
F2	22
F3	35
F4	16
F5	**25**
F6	32
F7	27
F8	39
F9	30

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Extrudability

Table No.18: Extrudability

Formulation	**Extrudability**
F1	++
F2	+++
F3	++
F4	+
F5	**+++**
F6	++
F7	++
F8	++
F9	+

HPTLC of *Amaranthus viridis and Azadiractica indica*.leaf extract

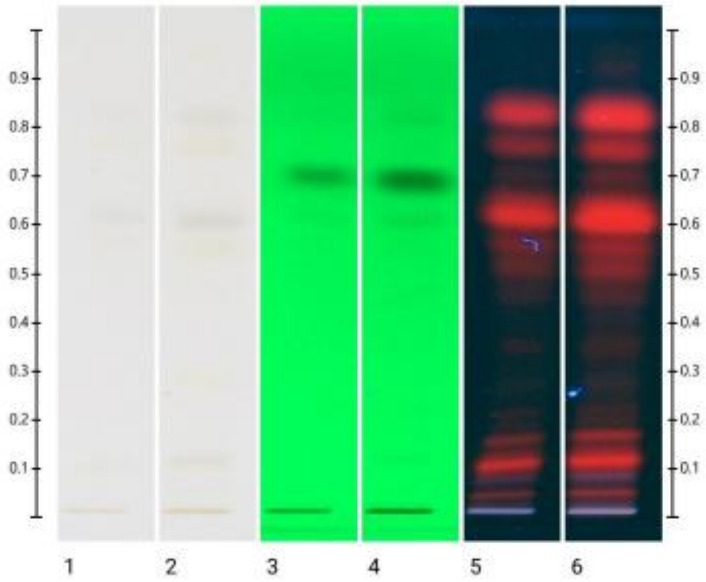

Figure 16*:* HPTLC fingerprint of *Amaranthus viridis* leaf extract at R 366nm

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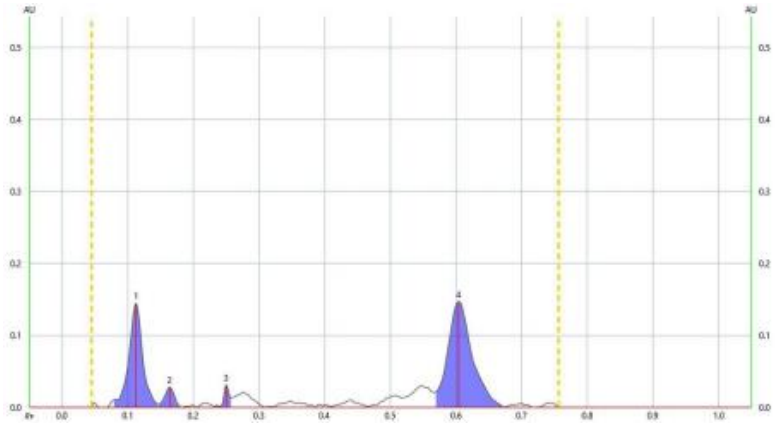

Figure 17: Densitogram of *Amaranthus viridis* extract

B]Azadirachta indica-

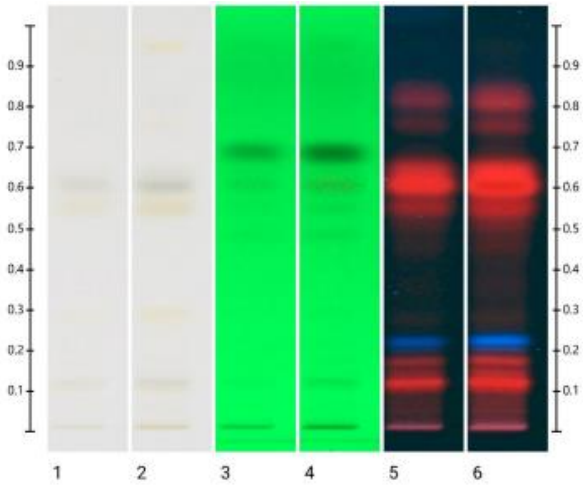

Figure 18*:* HPTLC fingerprint of *Azadiractica indica* leaf extraxt at R 366nm

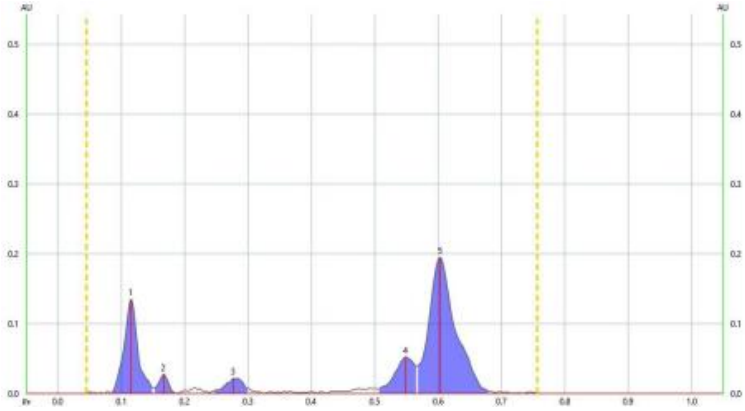

Figure 19: Densitogram of *Azadiractica indica* extract

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Viscosity and Spreadability :

Dependant and independent factors for formulation of nanogels

Table No. 19: Dependant and independent factors for formulation of nanogel

Sr. No.	Carbapol 934 (%)	Eudragit S100(%)	Viscosity (cps)	Spreadability (gm.Cm/sec)
F1	0.45	0.0155	3745.2	7.8
F2	0.3792	0.065	2988.5	6.8
F3	0.5	0.03	2671.2	6.8
F4	0.5	0.1	3779.9	7.7
F5	**0.45**	**0.065**	**2435.1**	**7.9**
F6	0.4	0.03	2766.8	7
F7	0.4	0.1	3078.9	7.18
F8	0.520	0.065	3547.7	6.9
F9	0.45	0.114	3845.3	7.6

% Drug release of Amranthus viridis extract

Table 20 and figure 19 below show the percentage of drugs released by an amranthus viridis extract nanogel.

Table No.20: % Drug release of *Amarnthus viridis* extract nanogel

Time (min)	F1 (%)	F2(%)	F3(%)	F4(%)	F5(%)	F6(%)	F7(%)	F8(%)	F9(%)
0	0	0	0	0	0	0	0	0	0
0.5	17.27	12.72	12.72	14.09	10.9	13.18	8.63	9.54	14.09
1	22.27	17.27	24.09	20.9	15.9	25.45	15	14.54	24.09
2	29.54	22.27	34.09	28.18	19.18	32.72	25.9	23.63	35.45
3	40.45	33.63	40	35.9	29.54	42.72	34.63	35.9	43.63
4	49.54	40	47.27	43.63	39.09	51.81	41.81	42.27	51.81
5	53.18	47.27	53.63	52.4	53.72	56.95	54.09	52.27	57.27
6	55.9	57.72	55.45	58.63	64.77	67.27	64.54	57.72	61.36
7	59.54	62.27	62.27	71.36	80.36	74.09	75	64.09	67.7
8	63.18	65.45	66.36	78.63	88.18	82.4	80.9	69.09	73.63

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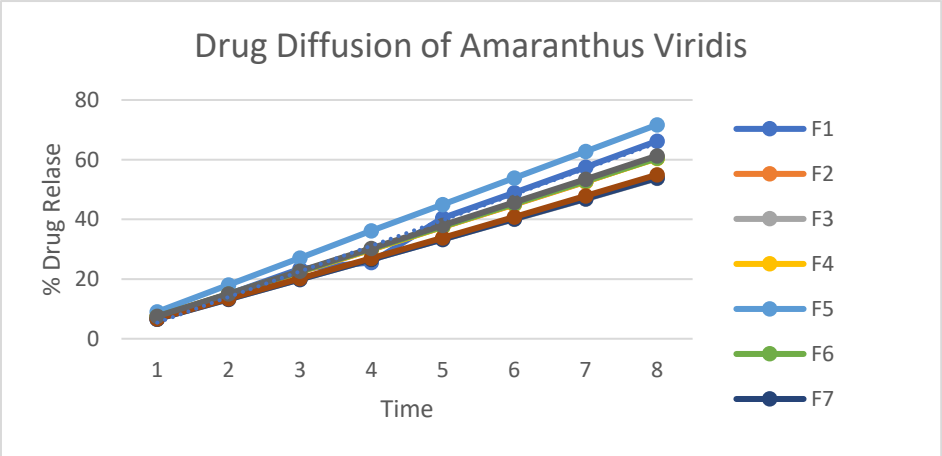

Figure 20: % Drug release of Amaranthus viridis extract nanogel

% Drug release of *Azadiractica indica leaf* extract

The percentage of drug release from Azadiractica indica leaf extract nanogel is shown in figures 20 and 21 below.

Table No. 21: % Drug release of *Azadiractica indica leaf* extract

Time (min)	**F1 (%)**	**F2(%)**	**F3(%)**	**F4(%)**	**F5(%)**	**F6(%)**	**F7(%)**	**F8(%)**	**F9(%)**
0	0	0	0	0	0	0	0	0	0
0.5	18.36	14.67	14.56	15.67	11.75	15.69	10.56	11.96	16.5
1	22.27	17.27	24.09	20.9	15.9	25.45	15	14.54	24.09
2	29.54	22.27	34.09	28.18	19.18	32.72	25.9	23.63	35.45
3	40.45	33.63	41.78	35.9	29.54	42.72	34.63	35.9	43.63
4	49.54	42.5	47.27	43.63	39.09	51.81	41.81	42.27	51.81
5	53.18	47.27	53.63	52.4	53.72	56.95	54.09	52.27	57.27
6	55.9	57.72	55.45	58.63	64.77	67.27	64.54	57.72	61.36
7	59.54	62.27	62.27	71.36	80.36	74.09	75	64.09	67.7
8	65.36	66.8	67.5	80.46	90.5	84.2	82.75	71.56	75.89

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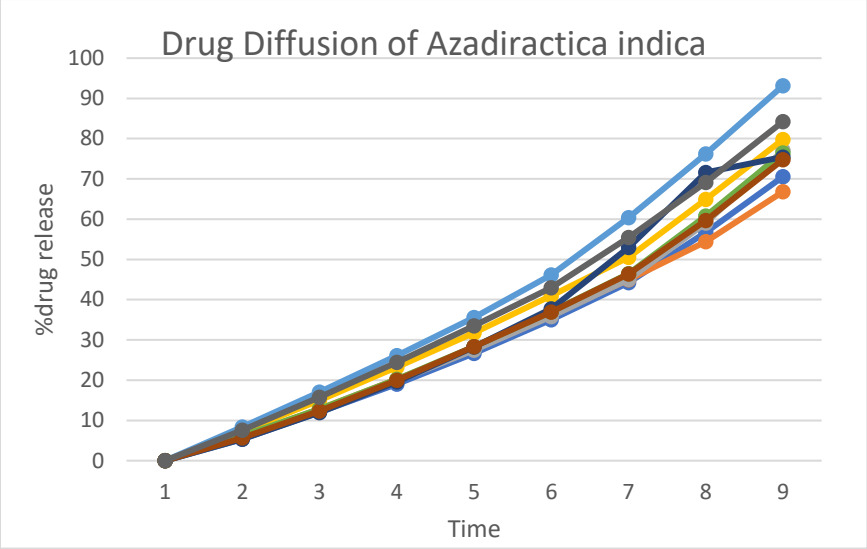

Figure 21: % Drug release of *Azadiractica indica leaf* extract

Analysis of antimicrobial activity:

In the batch that had been optimised (F5), the formulation's effectiveness against fungi was assessed. The antimicrobial test was conducted using agar well diffusion, and Candida albicans was employed as the culture. The table presents the findings. The Cup plate method was utilised, and sabouraud dextrose agar used the agar medium.

Table No. 22 -Zone of Inhibition Study

Formulation sample	Observed zone of inhibition
Marketed formulation	12±1
Optimized batch F5	14.12±1.50mm

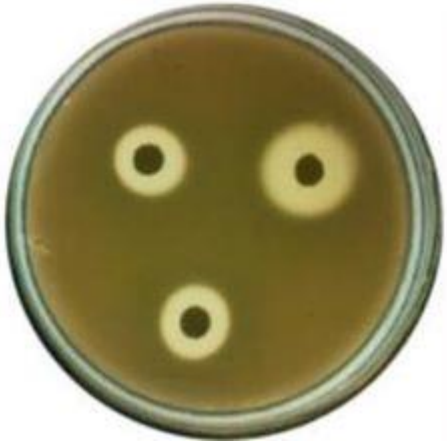

Figure No 22– Antimicrobial study

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Stability study:

Table No.23: Stability study of herbal nanogel

Temperature and humidity	**Parameter**	**Observation (in months)**	
		Stability data for 1 month	Stability data for 2 month
	pH	6.49	6.52
	Colour	Greenish	Greenish
30 ± 2°C /65 ± 5% RH	Texture	Smooth	Smooth
	Viscosity (CPS)	2435.1	2436.2
	Spreadability (gm.cm/sec)	7.9	8.1
	pH	6.49	6.56
40 ± 2°C /75 ± 5% RH	Colour	Greenish	Greenish
	Texture	Smooth	Smooth
	Viscosity (Pa.s)	2435.1	2438.3
	Spreadability (gm.cm/sec)	7.9	8.1

5. Conclusion

This project's main goal is to make a herbal nanogel. The right blend of medicinal plants, their extract in the ideal concentration, and a suitable formulation can all have positive medical effects on the body. These factors may also boost the potency of medications and formulations. Studies on the extracts of *Amaranthus viridis* and *Azadiractica indica* leaves may promote drug penetration from the affected area for the effective treatment of oral candidiasis, potentially displaying both antifungal and antibacterial action. The extract of *Amaranthus viridis*, which has antifungal properties, may be more stable with the addition of propylene glycol. Additionally, it possesses antioxidant qualities that aid in protecting the mouth's surface from oxidative damage. *Azadiractica indica* leaf extract contains phenolic acids, flavonoids, terpenoids, glycosides, and saponins, all of which have antibacterial properties. As a result, the nanogel that is used to treat oral candidiasis contains the herbal combination of *Amaranthus viridis* extract and *Azadiractica indica* leaf extract. Stat Ease® Design-Expert v13.0.2.0 provided the most accurate estimates of the parameters for nanogel synthesis using the Central Composite Method. The batch that was optimised and contained Carbapol 934 0.7% and Eudragit S100 3.5% produced the best results in terms of spreadability and drug release. Consequently, the batch with the F5 described features would be the best batch out of these 9 batches.

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