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FORMULATION AND EVALUATION OF HERBAL EXTRACT LOADED NANOGEL OF *MORINGA OLEIFERA*

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Abstract: The present study focuses on the formulation and evaluation of a herbal extract-loaded nanogel using *Moringa oleifera* leaves for enhanced therapeutic efficacy and controlled drug delivery. Nanogels are nanoscale hydrogel systems known for their high stability, biocompatibility, controlled release properties, and improved bioavailability. *Moringa oleifera*, a medicinal plant rich in flavonoids, phenols, alkaloids, tannins, saponins, and glycosides, possesses significant pharmacological activities including antioxidant, anti-inflammatory, antidiabetic, antimicrobial, and anticancer properties.

Ethanolic extraction of *Moringa oleifera* leaves was carried out by maceration, followed by qualitative phytochemical screening to confirm the presence of bioactive constituents. Nanoparticles were prepared using sodium alginate and calcium chloride via ionic gelation, and subsequently incorporated into a Carbopol 934-based nanogel formulation. Four formulations (F1–F4) were developed with varying polymer concentrations and evaluated for physicochemical parameters including appearance, homogeneity, pH, viscosity, particle size, drug content, spreadability, extrudability, and zeta potential.

Among the formulations, F4 demonstrated optimized characteristics with a particle size of 296.0 nm, drug content of 98.2%, and zeta potential of –35.6 mV, indicating good stability and enhanced bioavailability. The nanogel exhibited suitable pH, satisfactory spreadability, and acceptable viscosity for topical application. The study concludes that *Moringa oleifera* extract-loaded nanogel is a promising drug delivery system capable of providing controlled and sustained release, improved cellular penetration, and enhanced therapeutic potential.

***IndexTerms* :*Moringa oleifera*, Nanogel, Herbal extract, Sodium alginate, Carbopol 934, Controlled release, Zeta potential, Phytochemical analysis.**

INTRODUCTION:

Nanogel is produced by physically and chemically cross-linking polymers to hydrogel at the nanoscale. Nanogels usually range in size from 20 to 200 nm. In addition to their bulging and degrading properties, nanogels are distinguished by their size, increased surface area, and hygroscopicity. Nanogels enable the regulated and extended release of pharmaceuticals. Their three-dimensional structure makes it simple to use nanogels to capture drugs, polymers, and liquid phases in suspension [1]. These include exceptional thermodynamic stability, a high solubilisation capacity, a comparatively low viscosity, and the ability to withstand harsh sterilisation procedures [2]. Targeting delivery ,Low level of toxicity , Controlled and Sustained Medication delivery, High Encapsulation Stability are the features of nanogel [3]. It is suitable for certain target and transport attributes. It is very biocompatible and biodegradable [4]. But it is expensive technique to remove all solvents and surfactants at the end of the arrangement. Some particles are micrometer-sized [5].

The most commonly grown species of the monogeneric Moringaceae family, which is indigenous to the sub-Himalayan regions of India, Pakistan, Bangladesh, and Afghanistan, is Moringa oleifera. The plant provides a great provider of minerals including calcium and potassium as well as vitamins A and C. Moringa formulations have been found to have potential antihypertensive, anti inflammatory, hypolipidemic and antidiabetic effects[6]

BOTANICAL CLASSIFICATION

➢Kingdom: Plantae
➢Division: Magnoliophyta
➢Class: Magnoliopsida
➢Order: Brassicales
➢Family: Moringaceae
➢Genus: Moringa
➢Species: oleifera.[7]

Several pharmacological effects, including antibacterial, antifungal, anti-inflammatory, antioxidant, anticancer, fertility, and wound healing, are displayed by Moringa oleifera. Phytoconstituents present in moringa oleifera such as, Kaempferol : Flavonoid - Oxidative damage protective activity. Quercetin : Flavonoid - Exerts an excellent effect as anti-diabetic agent. coumaric acid : Phenolic acid - Antioxidant and anti-microbial .Myricetin : Flavonoid - Potential prevention of diabetes mellitus and other diabetic

complications .Ellagic acid : Polyphenol - Prevents viral and bacterial infections, potential antioxidant .Ferulic acid: Phenol - Promising results as anti- cancer, antioxidant, antithrombotic, anti-arrhythmic, and anti-inflammatory.Caffeic acid : Phenol - Boosts athletic performance, reduces fatigue, helps weight loss, protects against herpes, HIV, cancer. Sinapic acid : Phenol - Cardioprotective, renoprotective, anxiolytic, neuroprotective. Gallic acid : Phenol - Anti-inflammatory, anti-neoplastic,anti-oxidant .Syringic acid : Phenol - Anti-oxidant, antimicrobial. Isorhamnetin : Flavonoid - Anti-oxidant[8]

The presence of phytochemicals makes it a good therapeutic agent [9]. It is commonly used for Anti diabetic, Anticancer, Antiulcer, Neuroprotectant, Immunomodulator, Antimicrobial [10], Hepatoprotectant, Hypotension, Ocular disease [11].

MATERIALS AND METHODS

SHADE DRYING

To facilitate preservation of contents, size reduction purposes and preventing the other physiochemical reactions occur due to presence of water. The collected plants and shade dried for 2-3 days.

SOLVENT SELECTION

- Aliphatic alcohols with up to 3 carbon atoms or mixtures are the solvents with the greatest extractive power.
- According to the pharmacopoeia, Ethyl alcohol (Ethanol) is the solvent of choice for obtaining classic extract.

ETHANOLIC EXTRACTION OF *Moringa oleifera*

- 500 ml of 100 percent pure ethanol was added to 50 grams of the powdered leaves.
- It was macerated for 72 hours.
- Filter the mixture using whatman no.1 filter paper and collect the filtrate.
- Evaporate the ethanol by keeping the extract at room temperature until a semi solid extract is obtained.

PHYTOCHEMICAL ANALYSIS:

QUALITATIVE ANALYSIS

Test for alkaloids:

Alkaloids are found by dissolving the extracts in diluted hydrochloric acid, filtering each one separately, and testing for alkaloids.

- **Mayers test:** The Mayers reagent is mixed with the extraction. Alkaloids are indicated by the production of a yellow cream precipitate. [12]
- **Wagner's test:** If a brown-reddish-brown development is seen, which suggests the presence of alkaloids, Wagner's reagent is added to the extraction. [13]

Test for flavonoids:

- **Lead acetate test:** The extracts are mixed with a few drops of lead acetate solution. The presence of flavonoids is indicated by a yellow precipitate.
- **Sulphuric acid test:** The presence of flavonoids is shown by the production of an orange hue when a few drops of sulphuric acid are added to the extracts.[14]

Test for steroids:

- 4 ml of acetic anhydride was added to 1 g of each crude extract (separately), and then 2 ml of H2S04 was added. A shift in hue from violet to blue or green suggested the presence of steroids.[15]

Test for terpenoids :

- **Salkowski's Test :**To create a layer, 5 mg of the chosen plant part extract is combined with 2 mL of chloroform and 3 mL of strong sulphuric acid. Terpenoids are indicated by a reddish-brown hue.[16]

Test for anthraquinone:

- **Bontrager's Test:** In a water bath, 5 mg of the extract is cooked with 10% HCl for a short while. After filtering, it is left to cool. The filtrate is mixed with an equivalent volume of CHCl3. The liquid is heated after a few drops of 10% NH3 are added. Anthraquinones are indicated by the development of a pink hue.[17]

Test for phenols :

- **Ferric chloride test:** Add a few drops of ferric chloride to the 10 mL extract to detect phenols. Phenol is indicated by a bluish-black hue**.[b]**
- **Lead acetate test:** Ten milligrammes of extract are combined with a few drops of lead acetate solution. The presence of phenol is indicated by a yellow hue.[17]

Test for saponins:

- After adding 0.5 g of extract to 2 ml of water and shaking it constantly for 10 minutes, foaming developed, indicating the concentration of saponins.[17]

Test for tannins:

- Tannin detection involves heating a few millilitres of the extract in a water bath after mixing it with a few millilitres of water. Filtration is done on the mixture. The filtrate is mixed with ferric chloride. The presence of tannins is indicated by the dark green colour.[17]

Test for carbohydrate:

- Molicsh's reagent was applied to the aqueous solution and 70% ethanol MoLE. A purple ring emerged after dissolving in five millilitres of distilled water. To demonstrate the presence of carbs, add a few drops of alcoholic α naphthol solution to a test tube.[17]

Test for glycosides:

- Legal's reagent was used to dissolve sodium nitropruside in pyridine and NaOH after mixing MoLE with diluted HCl. The development of a blood-red colour indicated the presence of glycosides.[17]

PREPARATION OF *Moringa oleifera* EXTRACT NANOPARTICLES:

- Weigh Calcium chloride dihydrate and dissolve in sterile water.
- Weigh sodium alginate, dissolve in sterile water and mix it for 15 mins in magnetic stirrer until fully hydrated.
- Add the extracted sample to the sodium alginate solution and stir it for 5 mins.
- Add tween 80 to the solution to maintain the homogeneity.
- By stirring the alginate -extract solution, add the calcium chloride solution dropwise using burette. Continue stirring for 10 mins for the formation of small particles.
- Place the beaker in an ice bath and sonication was performed.

FORMULATION OF NANOGEL:

- Carbopol 934 was added to sterile water while stirring at low speed for 20 mins.
- Add glycerol and stir it until dissolved.
- To maintain pH , triethanolamine was added while stirring.
- Then the drug phase is added to the aqueous phase drop wise and stirred until nanogel is formed.

COMPOSITION	F1	F2	F3	F4
Moringa oleifera HERBAL PLANT EXTRACT (g)	2	2	2	2
SODIUM ALGINATE (g)	0.80	1.3	1.8	2.3
TWEEN -80 (ml)	2	2	2	2
GLYCEROL (ml)	4	4	4	4
CARBAPOL(g)	0.6	1.1	1.6	2.1
WATER (ml)	Q.S	Q.S	Q.S	Q.S
TRIETHANOLAMINE (ml)	1	1	1	1

EVALUATION:

Appearance:

To look for any particle appearance, colour, or clarity, we visually inspected the nanogel bases.[18]

Homogeneity:

The uniformity was established by visually inspecting the nanogel formulation. They were examined for aggregate presence and appearance.[19]

Determination of pH:

The Electro lab R digital pH tester was used to measure the pH of the nanogel mixture. A little amount of the mixture was added to a beaker filled with a fixed volume of filtered water. The electrode was submerged in the formulation to determine its pH.[20]

Measurement of particle size:

The average size of the nanogels was measured and recorded using a Zeta sizer and a Malvern MasterSizer 2000 MS.[21]

Drug content:

Nanogel was dissolved in 10 millilitres of ethanol to evaluate the drug concentration. For one hour, the mixture was centrifuged at a speed of 448 relative centrifugal force (RCF). After the liquid supernatant was removed, the samples were analysed using a UV spectrophotometer with a wavelength of 452 nm.[22]

Spreadability:

Mutimer's recommended equipment is used to determine spreadability. A specific formula was used to calculate spreadability.

$S=M.L/T$

Where, S=Spreadability,
L=Length of glass slide,
M=weight tied to higher slide,
T=Time taken to separate the slides.[23]

Extrudability:

Flexible aluminium tubes were filled with the mixes. After compressing the tubes to extrude a 0.5 cm gel ribbon in 10 seconds, the formulations' extrudability was evaluated.[22]

Viscosity :

A Brookfield rheometer with spindle number 64 running at 10 rpm was used to measure the viscosity of the formulation. The assembly was equipped with a water bath that was controlled by a thermostat and maintained at 25°C. After the viscosity was determined, it was placed in the beaker while wearing a thermostatic jacket. The values were noted after the spindle had passed through the nanogel.[24]

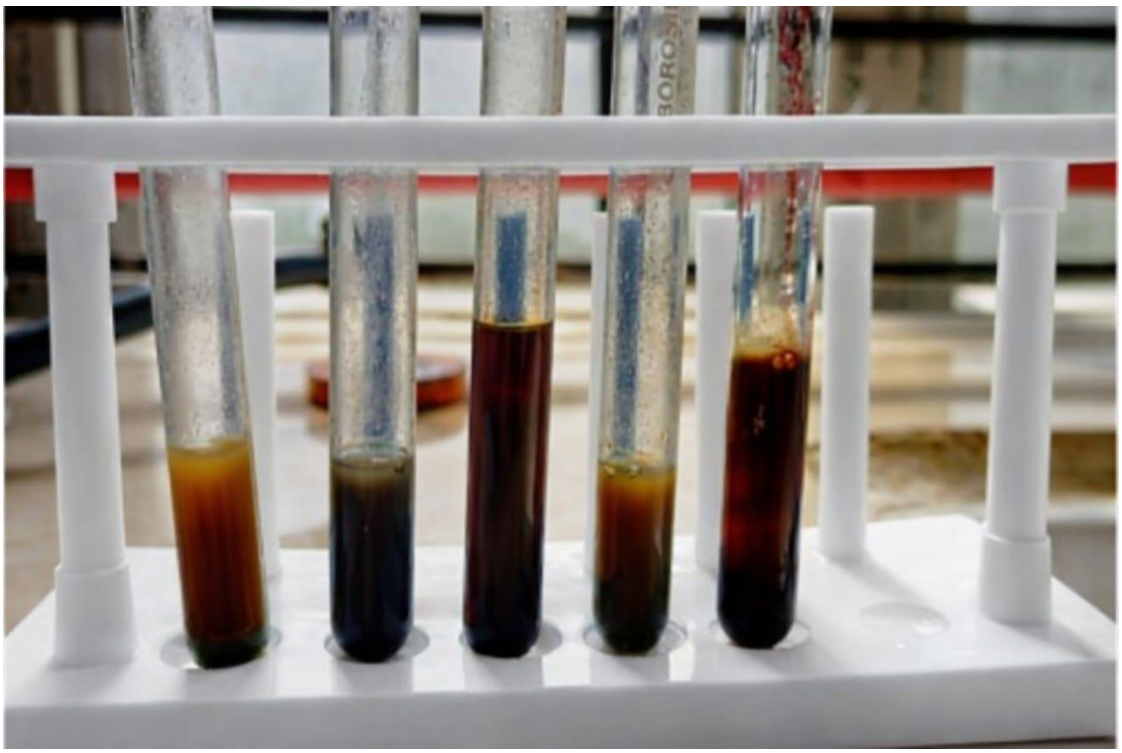
Zeta potential:

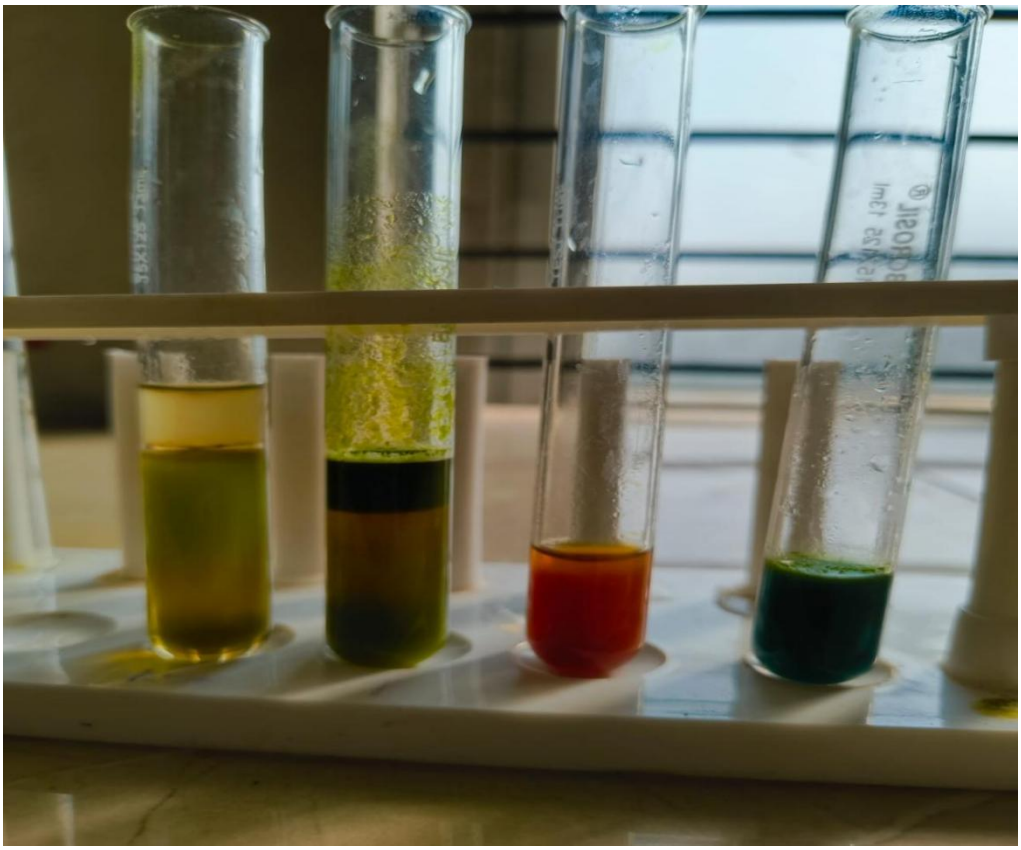
The zeta potential indicates the overall charge a partical acquires in a specific medium stability of the nanogel during storage can be predicted from ZP value. ZP indicates the degree of repulsion between close and similarly charged particals in the dispersion, the suitable dilutions of the dispersion are made using distilled water and it is scanned by using disposable sizing cuvette the electrophoretic mobility (zeta potential measurement of nanogel was done by using zetasizer (Nano ZS90,Malvern instruments). The samples were placed in polystyrene cuvette(at 25°C) combined with zeta dip cell was used to measure the potential.

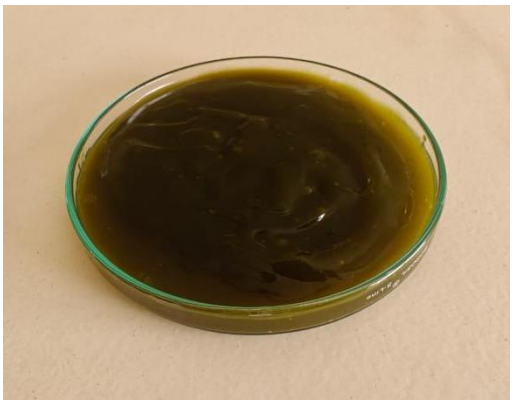
RESULTS & DISCUSSION

Qualitative phytochemical analysis

S.NO	Plant constituents	Inference
1	TEST FOR ALKALOIDS	PRESENT
2	TEST FOR FLAVONOIDS	PRESENT
3	TEST FOR STEROIDS	PRESENT
4	TEST FOR TERPENOIDS	PRESENT
5	TEST FOR ANTHRAQUINONES	PRESENT
6	TEST FOR PHENOLS	PRESENT
7	TEST FOR SAPONINS	PRESENT
8	TEST FOR TANNINS	PRESENT
9	TEST FOR CARBOHYDRATE	PRESENT
10	TEST FOR GLYCOSIDES	PRESENT





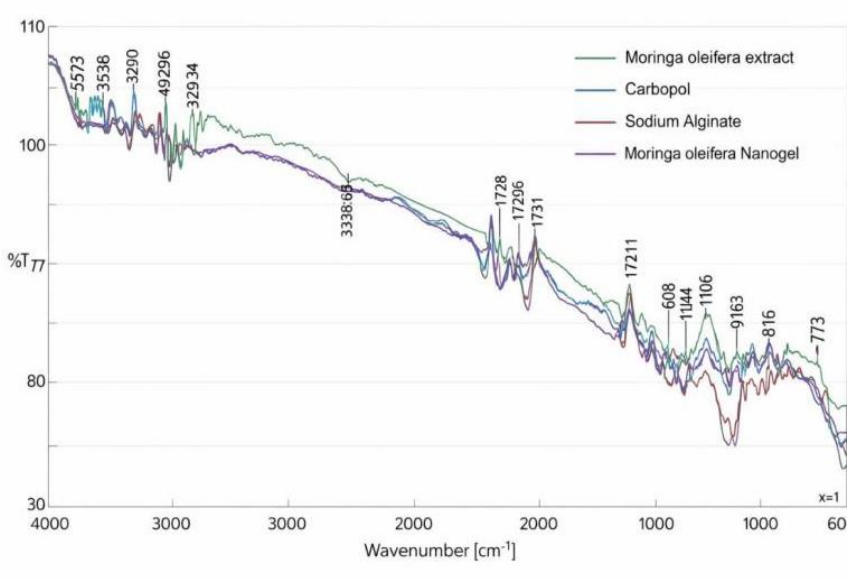


EVALUATION PARAMETERS	F1	F2	F3	F4
APPEARANCE	Clear	Clear	Clear	Clear
HOMOGENICITY	Homogenous	Homogenous	Homogenous	Homogenous
pH	5.4	5.9	6.3	6.8
VISCOSITY in cp at (50 rpm)	750	655	758	498
PARTICLE SIZE (nm)	350.3	325.1	290.2	296.0
DRUG CONTENT	87.2	89.4	93.5	98.2
SPREADABILITY (g.cm)	4.12	5.06	6.4	7.8
EXTRUDABILITY	76	65	52	48

EVALUATION PARAMETERS
CHARACTERIZATIONS

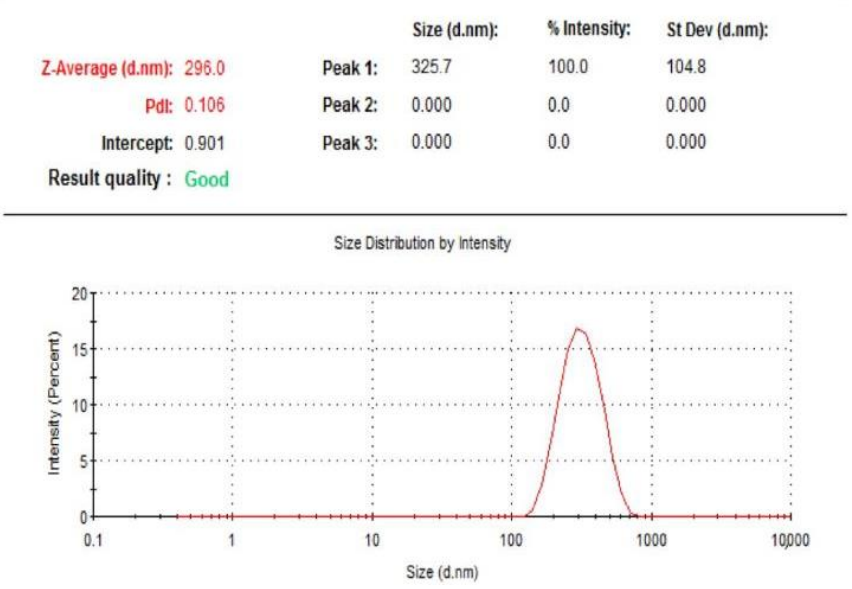
FT -IR REPORT OF *Moringa oleifera* LOADED NANOGEL

FTIR obtains the infrared spectrum of any solid, liquid, or gas using the principle of infrared radiation absorption and emission. It was used to identify the unique functional groups that were present in the extract. A tiny amount of M. oleifera loaded nanogel was utilized for the mid-IR spectrum using an infrared spectrometer . The scan range for the sample was 4000 to 400 cm-1. The FTIR peak values were noted.



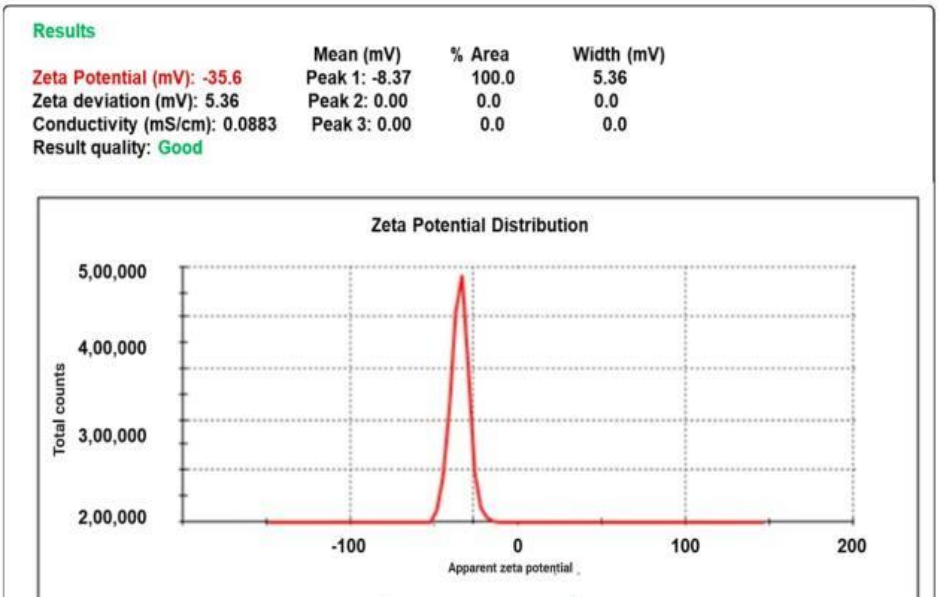
PARTICLE SIZE

The optimized formulation's particle size is 296.0 nm, which is the lower particle size.



ZETA POTENTIAL

The produced nanogel has a greater level of long-term stability, as demonstrated by the decreased Zeta Potential value of -35.6 mV.



CONCLUSION

The present research study highlights the formulation and evaluation of Moringa oleifera (Moringaceae)which will be having more natural therapeutic components. The plant is easily available based on the ethnopharmacological survey, it has been used for curing variety of diseases. The major traditional uses of the moringa oleifera are antidiabetic, anticancer,anti ulcer,,neuro protective,immunomodulater,anti-cataract so it had been selected for our research work.

The morphological evaluation and it's character shows the general character to the plant . Preliminary phytochemical showed the presence of alkaloids, flavonoids, steroids, terpenoids, anthraquinones, phenols, saponins, tannins, carbohydrates, glycosides .

The project aimed to formulate and evaluate a nanogel loaded with *Moringa oleifera* plant extract for its potential therapeutic properties. Nanogels have gained attention in recent years as a promising drug delivery system due to their small size, high stability, and ability to encapsulate various bioactive compounds.

The project began with the extraction of *Moringa oleifera* plant extract using a suitable solvent. The extract was then loaded into a nanogel formulation using a specific technique. The formulation process involved optimization of various parameters such as polymer concentration, surfactant type, and stirring speed to achieve a stable and effective nanogel. The formulated nanogel was subjected to various characterization tests to assess its physical and chemical properties, such as particle size, zeta potential, drug loading efficiency. These tests confirmed the successful encapsulation of *Moringa oleifera* extract within the nanogel and its controlled release behaviour.

The level of Alkaloids, saponin, phenol, flavonoids, tannins in ethanolic extract of Moringa oleifera leaf were 10.73 %, 18.46%, 8.53%, 9.31%,11.43% respectively. The FTIR profile of ethanolic extract of moringa oleifera was studied. The presence of kaempferol, quercetin, myricetin, ferulic acid, caffeic acid, coumaric acid was identified and quantified as per above mentioned ratio. The most frequently studied phenolic content will be gallic acid ,it has been shown to have biological properties and there are positioned in the effective pharmacological property, the anti-inflammatory and anti neoplastic property, The oxidative damage protective activity is due the presence of kaempferol, the anti diabetics activity of the plant is due to the presence of quercetin, the antioxidant and antimicrobial activity of the plant extract is due to the presence of syringic acid, the anti bacterial as well as antiviral activity of the plant is due to the presence of elagic acid.

The findings of this project suggest that *Moringa oleifera* extract-loaded nanogel holds great promise as a potential therapeutic option . The nanogel formulation can provide controlled and sustained release of active compounds, improving the efficacy and duration .

So we can conclude that *Moringa oleifera* extract loaded nanogel facilitate higher cellular penetration and possess high bioavailability and sustained release.

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