Formulation and Characterization of Hydrogel from Aloe vera and Moringa Oleifera leaf

Pinki Gupta*, Anupam Kr. Sachan, Kiran Kumari and Shubham Ojha
Dayanand Dinanath Collage, Institute of Pharmacy, Ramaipur, Kanpur, Utter Pradesh, India
Email: pinkigupta842@gmail.com

Abstract
The goal of the research is to use sodium carboxy methyl cellulose (SCMC) as a gelling agent to formulate and characterize hydrogel from Aloe vera and Moringa oleifera leaves. Moringa oleifera contain isothiocyanates, phenolic, flavonoids and Aloe vera contain anthraquinones glucomannan. These are responsible for anti-inflammatory activity. Aloe vera and Moringa oleifera have antioxidant properties. Moringa oleifera showed high antioxidant than Aloe vera. Hydrogel was made with 1% and 2% concentrations of sodium carboxymethyl cellulose (SCMC) acting as the gelling agent. The hydrogel with 2% SCMC, it showed the best physicochemical characteristics, such as ideal viscosity, extrudability, and viscosity, among all the formulations.
Compared to the other formulation, the F4 formulation (2% SCMC, Moringa oleifera, and Aloe vera extract) shown superior anti-inflammatory effect. Aloe vera and Moringa oleifera extract can both be used to create formulations that are useful for developing medicinal and cosmetic applications.

Keywords: Aloe vera leaves, Moringa oleifera leaves, Sodium carboxy methyl cellulose (SCMC), Hydrogel.

Introduction
Plant parts such as leaves, bark, seeds, roots, and flowers are used to make herbal medication. Phytomedicines is another name of its. These are treated numerous medical disorders. Synthetic medicines have only single active compound. whereas, herbal medicines have a variety of bioactive ingredients that can be combined to produce a variety of therapeutic effects. Since ancient times, people have used plants for medical purposes. Both traditional Chinese medicine and India's Ayurvedic medical system are a huge number of plant-based treatments. Comparably, Hippocrates described using more than 300 different herbs in his medical treatments in ancient Greece (Fabricant et al., 2001).
There aren't any official guidelines for herbal preparations at the present time. Manufacturers who test their products have set their own standards, which are frequently basic and preliminary. Making sure all the chemicals listed in a formulation are actually present is still difficult. Hence, the main goal needs to be creating parameters that accurately determine every constituent (Kunle et al., 2012).

Moringa oleifera, a member of the Moringaceae family, is obtained from dried long, slender, triangular seed pods. The tropical tree Moringa oleifera has several uses. In addition to its many industrial, medical, and agricultural purposes, it is mostly utilized for food including the feeding of animals. nutrient-rich, quick-growing, and resistant to drought, since its rediscovery in the 1990s, this ancient plant's cultivation has grown in popularity throughout Asia and Africa, where it is one of the most valued crops in terms of the economy. The media has referred to it as the "tree of life" or the "miracle tree".

Moringa leaf extract has been used for a long time and is often utilized due to have their potent anti-inflammatory, immunomodulatory, anti-cancer, antibacterial, antidiabetic, and photoprotective properties. (Ariyanti et al., 2023).

Moringa leaves contain a variety of chemicals which are abundant in antioxidants, such as tannins, glucosinolates, alkaloids, phenolic acids, and isothiocyanates. (Susanty et al., 2019).

The secondary metabolites phenolic molecules and flavonoids serve as antioxidants, UV filters, and ant hyperproliferative responses to environmental stimuli. in order for moringa leaves to be utilized for regenerating the skin, skin brightening, skin protection against radiation and free radicals, skin damage prevention, skin moisturization, and skin protection (Yuniarsih et al., 2023). B2 is another ingredient found in moringa leaves, and it helps treat and preserve skin hydration.

Aloe vera barbadensis, a member of the Liliaceae family, is obtained from dried juice of aloe vera leaves. It is primarily found in dry areas of America, Europe, Asia, and Africa. It can be found in Tamil Nadu, Rajasthan, Andhra Pradesh, Gujarat, and Maharashtra in India. The plant contains tubular yellow flowers, many seeds inside the fruits and succulent leaves. Aloe vera leaves have three layers:

1) An inner clear gel consisting of 99% water and glucomannans, amino acids, lipids, sterols, and vitamins.

2) The middle part of latex, contains anthraquinones and glycosides.

3) The rind is a thick layer of 15–20 cells that produces proteins and carbohydrates while acting as a protective layer. (Tyler et al., 1993).

Aloe vera leaves has seventy-two essential components. there are eighteen different types of molecules such as amino acids, carbohydrates, lipids, water, vitamins, enzymes and drug substances antiseptic, antimicrobial etc. (Atikasari et al., 2021).

Biologically active substances found in aloe vera include anthraquinone, mannans acetate, polymannans, antioxidants, and different lectins (Murnalis et al., 2019).

Aloe Vera has several medicinal properties, including diuretic, stomachic, anthelmintic, cathartic, carminative and depurative. Juice is used to treat dyspepsia, amenorrhea, and sound skin medicine. For ulcerative colitis and weight ulcers, aloe vera gel is incredibly beneficial (Pegu et al., 2019).

In 1894, the word "Hydrogel" was first used to describe a colloidal gel. In 1960, Wichterle and Lim presented first study on hydrogels in the biomedical area. Hydrogels typically absorb a lot of water, which causes them to swell and take on their rubbery, squishy qualities. Hydrophilic groups including NH2, COOH, OH, CONH2, CONH, and SO3H are present in it. Hydrogels have found use in the food, biomedical, environmental, and other industries. Three generations can be distinguished from it (Thakur et al., 2018).

Chains of hydrophilic polymers combine to form three-dimensional network structures known as hydrogels. They can hold onto a lot of biological fluids or water while yet keeping their structural integrity. Hydrogels can have different structures based on their composition and intended use (Hoffman et al., 2012).

Hydrogel has use in the administration of drugs, bone regeneration, wound healing, biosensing, antimicrobial agents, hygiene products, and cancer therapy.

Hydrogel is divided on the basis of some factors including their source, origin, composition, crosslinking, network charge and reaction to outside stimuli.

Natural hydrogel derived from natural polymers like protein and polysaccharide. E.g. collagen, chitosan and dextran.

Synthetic hydrogel is made from synthetic monomers E.g. Polyvinyl alcohol (PVA) and n-isopropyl acrylamide.

Hybrid hydrogel is combination of natural and synthetic polymer like n- isopropyl acrylamide and alginate polyvinyl alcohol.

Stimuli sensitive hydrogel is responsible for external stimuli like ph, temperature, electricity, light and biological molecules.

Physical and chemical cross-linked hydrogel are classified based on the type of cross- linking, which can be either physical or chemical. Physical cross-linked hydrogel like poly vinyl alcohol based on cryogels via crystallization and protein- protein interaction. Chemical cross-linked hydrogel like covalent bonding between polymer chains using
cross linking agents such as glutaraldehyde (Ahmad et al., 2022).

Materials and methods

Collection of Moringa oleifera leaves

Moringa oleifera plants were collected from CSIR-National Botanical Research Institute in Lucknow. These was rinsed with water after collecting, the plant leaves were dried in the shade till constant weight of leaves was achieved. then blended until a fine powder was achieved.

Preparation of Moringa oleifera leaves Extracts

In cleaned and dried Conical flask, 100 gm Moringa leaf powder were filled and 800 ml of methanol was poured in to flask and shake very gently then left for overnight. Next day the extract was filtered by using filter paper. Now pour the filtrate in to a round bottom flask and evaporated by using rotavapor. The procedure was repeated 2 to 3 times with 800 ml methanol. The extract obtained by above procedure is known as Methanolic extract.

Collection of Aloe vera leaves

Aloe vera leaves were collected from CSIR-National Botanical Research Institute in Lucknow.

Preparation of Aloe vera leaves Extracts

These was rinsed with water after collecting. Then the yellow layer beneath the green rind was scraped off with a sharp knife while avoiding the vascular bundles, and the top rind was removed. In a similar manner, the bottom rind was cut off to get rid of the large amount of mucilage that adheres to it. Using a spoon, the transparent mucilaginous gel was removed. Blend the gel to obtain a smooth consistency.

Phytochemical screening

Extract obtained from the cold extraction was subjected to the preliminary phytochemical screening. Phytochemical screening provides an idea regarding nature of chemical constituent's presence in the extract. Phytochemical screening tests were carried out for alkaloids, glycosides, tannins, flavonoids, saponins, carbohydrate, quinones and triterpenoids etc.

Alkaloids Test

Dragendorff's Test (Potassium Bismuth Iodide Solution): The extract produced a reddish-brownish precipitate when it was treated with Dragendorff's reagent.

Wagner's Test (Iodine Solution in Potassium Iodide): The extract produced a red brownish precipitation with Wagner's reagent.

Mayer's Test (Potassium Mercuric Iodide): The extract produced whitish colour with Mayer's reagent.

Hager's Test (Picric Acids): The extract produced yellow colour with Hager's reagent.

Glycosides Test: In a test tube, 5 ml of extract was mixed with 25 ml of diluted sulfuric acid and boiled 15 minutes, cooled and treated with 10% NaoH to neutralize, after that, Fehling's solution (5 ml) was added. The presence of glycosides was indicated by the colour brick red.

Saponin Test

Foam Test: 5 ml of sodium bicarbonate and 5 ml of water were placed in a test tube, and 1 gram of extract was added after that, the substance was shaken vigorously. foam formation, indicating the presence of saponin.

Tannins Test

Ferric chloride test: One gram of extract 5 ml of methanol was warmed and subsequently filtered. A small amount of ferric chloride solution was added to the filtrate. Green colour formation indicates the presence of tannins.

Lead acetate Test:1 gm extract 5 ml methanol was taken and warmed then filtered. Few drops of lead acetate solution were added into filtrate. Formation of precipitate indicates presence of tannins.

Flavonoids Test

Lead acetate Test: A few drops of lead acetate solution were added into the extract. The appearance of yellow indicates the presence of flavonoids.

Terpenoids Test: A few drops of acetic anhydride and chloroform were mixed with two grams of extract. Then, slowly added concentrated sulfuric acid. Terpene presence can be observed by the formation of red violet.

Antioxidant activity of plant extract

Reagents

1. DPPH (1-diphynyl-2picrylhydrazyl) Solution: 3.94mg DPPH was dissolved in 100ml methanol, stored in dark placed for 1/2hr. before use.

2. Standard Gallic Acid Solution (1mg/ml): 1mg dissolved in 1ml methanol (always use freshly prepared solution).

3. Sample Solution (1mg/ml): 1mg extract dissolved in 1ml methanol.

Procedure

- Methanol was used to prepared a blank.
- 1ml methanol and 1ml DPPH was used to prepared a control.
- The antioxidant activity of aloe vera and moringa oleifera extract was determined by DPPH method with spectrophotometer.
- Suitable aliquots (20µl, 40µl, 60µl, 80µl, 100µl) of Gallic acid (standard solution 1mg/ml in methanol) was taken in test tubes. Then make up the volume with 1ml methanol and 1ml DPPH in every test tubes.
- Suitable aliquots (20µl, 40µl, 60µl, 80µl, 100µl) of extract solution (1mg/ml methanol) was taken in another test tubes. Then make up the volume with 1ml methanol and 1ml DPPH in every test tubes.
- Measured the absorbance at 517nm (Uddin et al., 2020).

Preparation of Hydrogel

For the preparation of hydrogel, weighing every component individually, we added the gelling agent to 50ml of distilled water and swirling constantly. The required quantity of preservative was added with 5ml of distilled water for create the preservative solution, and placed at water bath. When the preservative solution had cooled, glycerin was added. After that, it was incorporated with the initial outcome solution. A pre-measured amount of extract was added to this solution and carefully stirred. To produce a gel with the required viscosity and consistency, triethanolamine was added drop by drop while being constantly stirred. after careful stirring of the entire liquid and setting of the pH to skin (6.7–8).

Table 1: Preparation of Hydrogel

Ingredients	**F1**	**F2**	**F3**	**F4**
Moringa oleifera	0.5%	1%	1%	2%
Aloe vera	0.5%	1%	1%	2%
SCMC	1gm	2gm	1gm	2gm
Methyl Paraben	0.2ml	0.2ml	0.2ml	0.2ml
Propyl Paraben	5ml	5ml	5ml	5ml
Glycerine	2ml	2ml	2ml	2ml
Triethanolamine	Qs	Qs	Qs	Qs
Water	Qs	Qs	Qs	Qs

In Vitro Anti- Inflammatory Activity

Inhibition of Protein Denaturation

Reagent

1. Standard Drug: Ibuprofen drug was used as standard. Ibuprofen was crushed into fine powder. 10mg drug was dissolved in 10ml distilled water (1ml).

2. Egg Albumin: Egg albumin is a protein. These are used to observe denaturation process. Prepared a 1% solution of egg albumin in phosphate buffer (pH 6.3).

3. Buffer Solution (pH 6.3): Phosphate buffer saline (PBS) is used for maintain the pH.

4. Solvent: Solvent is used for anti-inflammatory activity to dissolve the test sample. Solvent like distilled water.

4. Test Sample: These are compound or extract that are evaluated for their potential anti-inflammatory effects.

Procedure

- Anti-inflammatory activity of formulation was determined by spectrophotometer.
- 1ml egg albumin and 1ml distilled water was used to prepared a control.
- Ibuprofen drug was used as a standard.
- Mixed 1ml of egg albumin solution with 1ml extract solution at different concentration (100µl, 200µl, 300µl, 400µl, 500µl).
- Mixed 1ml of egg albumin solution with 1ml ibuprofen drug solution at different concentration (100µl, 200µl, 300µl, 400µl, 500µl) for prepared a standard.
- Samples was incubated at 37´C. for 20 minutes.
- Then sample was heat at 57´C for 3 minutes to induce denaturation.
- Allow the sample cool at room temperature and measured the absorbance of all sample at 660nm by using a spectrophotometer.

Evaluation of Hydrogel Preparations

1. Organoleptic Test: Physical observations were made of the Aloe vera and moringa oleifera extract gel formulations, including their color, homogeneity, concentration, and Odor.

2. Determination of PH: A pH meter was used to measure the pH of each hydrogel. These are calibrated by buffer solution (pH value 4,7 and 10). At room temperature, the electrode was placed within the hydrogel sample to obtain a readout. Take note of the pH value.

3. Viscosity: The Brookfield Viscometer was used to determine the prepared gel's viscosity. 0.3, 0.6, 1.5, 3, 6, 12, 30, and 60 rpm was applied to the gel. At each speed, dial reading was recorded. The dial reading was multiplied by the factor listed in the Brookfield Viscometer catalogs to determine the gel's viscosity.

4. Spreadability: The spread ability of each hydrogel was determined by measuring the spreading area of 1.0 g of hydrogel between two glass slides. A standard weight of about 100.0 g was applied to the upper slide for 60 seconds in order to assess the spread ability. The following formula was used to calculate the outcome.

$S = M \times L / T$

Where,

S= Spreadibility,

M= Weight in the pan,

L= Length travelled by glass slide,
T= Time (in seconds).

5. Extrudability: For this test, a collapsible aluminium tube was used. Six aluminium tubes were taken, each containing 10 grams of gel, and were held between two fingers. Finger pressure was used to compress aluminium tubes, and the weight of the extruded gel was recorded.

6. Moisture content: Determine the empty Petridis' weight using an analytical balance, then fill it with a known volume of hydrogel sample. Weigh the Petridis with the hydrogel sample, then heat it for three hours at 105°C in the drying oven. Dry the sample until its weight stabilizes, then cool it in a desiccator before weighing it once again.

7. Stability Study: Both closed and open containers have been used in stability studies. In this case, the product was kept for a month at room temperature.

RESULTS AND DISCUSSION

Extractive yield of Herbal Extracts: A formula was used to determine the herbal extract's extractive yield.

Extractive yield of aloe vera was 50% whereas extractive yield of moringa oleifera was 5%.

Extractive yield (%) = Weight of extract/Weight of powered drug taken.

Table 2: Extractive Yield (%) of Herbal Extracts

Sr. No.	Plant	Solvent	Extractive yield
01	Aloe vera leaf	Methanol	50%
02	Moringa oleifera leaf	Methanol	5%

Phytochemical Screening of Extracts: The type of chemical component present in the extract can be identified through phytochemical screening. Tests for phytochemicals such as triterpenoids, alkaloids, glycosides, tannins, flavonoids, saponins, and carbohydrates were conducted.

Table 3: Phytochemical Screening of Extracts

Sr. No.	Test	Aloe vera	Moringa oleifera
1	Alkaloids	+	+
2	Glycosides	+	+
3	Tannins	+	+
4	Saponins	+	+
5	Flavonoids	+	+
6	Terpenoids	+	+

Note: (+) show present.

Table No. 3 showed the results of the phytochemical screening of Aloe vera and Moringa oleifera extract.

Antioxidant Activity of Plant Extracts:

Gallic acid was used as the standard in the DPPH method to measure the antioxidant activity. A calibration curve was used to determine the extracts' antioxidant ability, which was expressed as gallic acid equivalents. Moringa oleifera leaf extract showed better antioxidant activity then Aloe vera leaf extract.

Table 4: Antioxidant Activity of Aloe vera leave

Sr. NO.	Concentration (µg/ml)	Rate of inhibition (%)
1	20	4.032
2	40	6.25
3	60	9.677
4	80	17.74
5	100	22.37

Table 5: Antioxidant Activity of Moringa oleifera leave

Sr. NO.	Concentration (µg/ml)	Rate of inhibition (%)
1	20	27.79
2	40	39.85
3	60	50
4	80	69.11
5	100	71.76

This table no.5 showed antioxidant activity of Moringa oleifera and Aloe vera extract in which Moringa oleifera showed better antioxidant activity than Aloe vera extract.

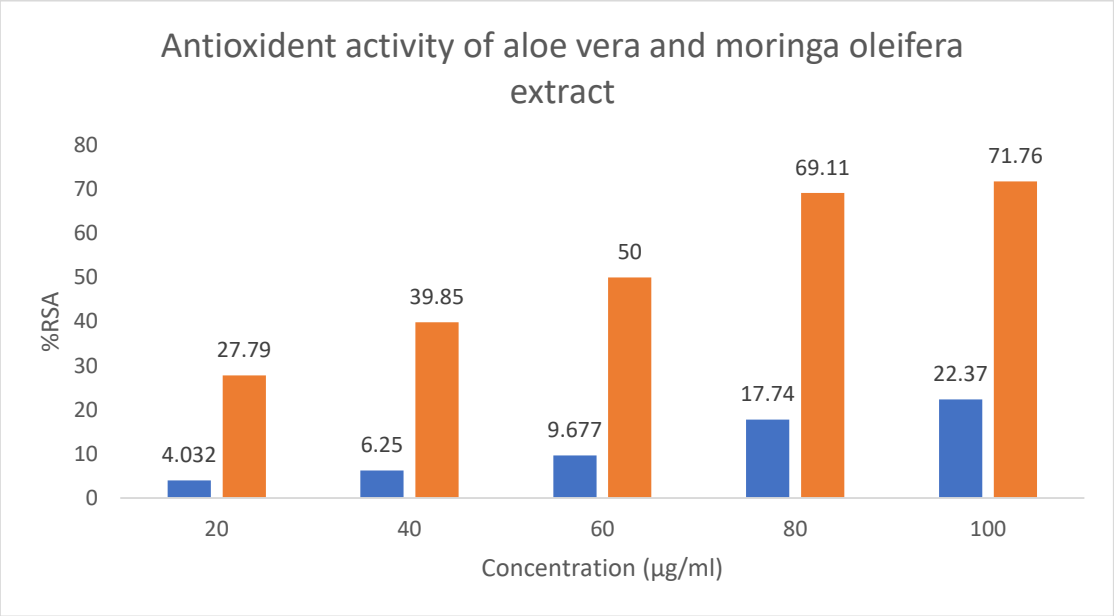

Figure 1 Antioxidant activity of Aloe vera and Moringa oleifera Extract

Evaluation of Hydrogel

Physicochemical Evaluation: Physical measurements were made to evaluate the formulation's physicochemical quality in terms of color, Odor, homogeneity, and consistency. The evaluation's observation was presented in the table.

Table 6: Physicochemical Evaluation of Formulations

Formulation	Colour	Odour	Consistency	Homogeneity
F1	Light green	Characteristic	Average	Homogeneous
F2	Green	Characteristic	Very Good	Homogeneous
F3	Green	Characteristic	Good	Homogeneous
F4	Dark green	Characteristic	Very Good	Homogeneous

This Table no. 6 dipcts the physicochemical evaluation of all four formulation and it was revealed that the color of formulations was Light green, Green and Dark green; the odour of formulations was characteristic; consistency of formulations was Average, very good and Good; and the homogeneity of all formulations was Good.

pH Determination: pH determination is an important criterion for topical application. it could irritate skin if its pH is different from that of normal skin. The formulation's pH was measured using a pH meter, which is similar to the pH of skin.

Table 7: pH Determination of Formulations

Sr. No.	Formulations	pH
1	F1	5.2±0.1
2	F2	5.3±0.1
3	F3	6.7±0.1
4	F4	6.8±0.1

This table no. 7 showed pH range of all formulations. Which is close to skin pH and not cause any irritation on the skin after application.

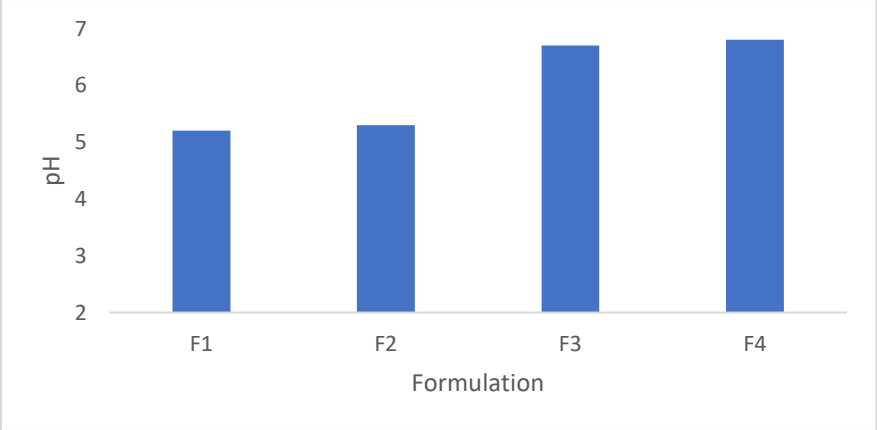

Figure 2 pH Measurement of Formulations

Viscosity: Viscosity is an important parameter for determining the formulations. Which affect physical and mechanical properties of formulations such as spreadibility. Viscosity of different formulation was determined by Brookfield viscometer.

Table 8: Viscosity of Formulations

Sr. No.	Formulations	Viscosity
1	F1	3025±1
2	F2	3402±2
3	F3	3165±1
4	F4	3561±2

This table no. 8 showed viscosity of all formulations. viscosity of F2 and F4 was found 3402 ±2 and 3561±2. Viscosity of F4 is better among all other formulations

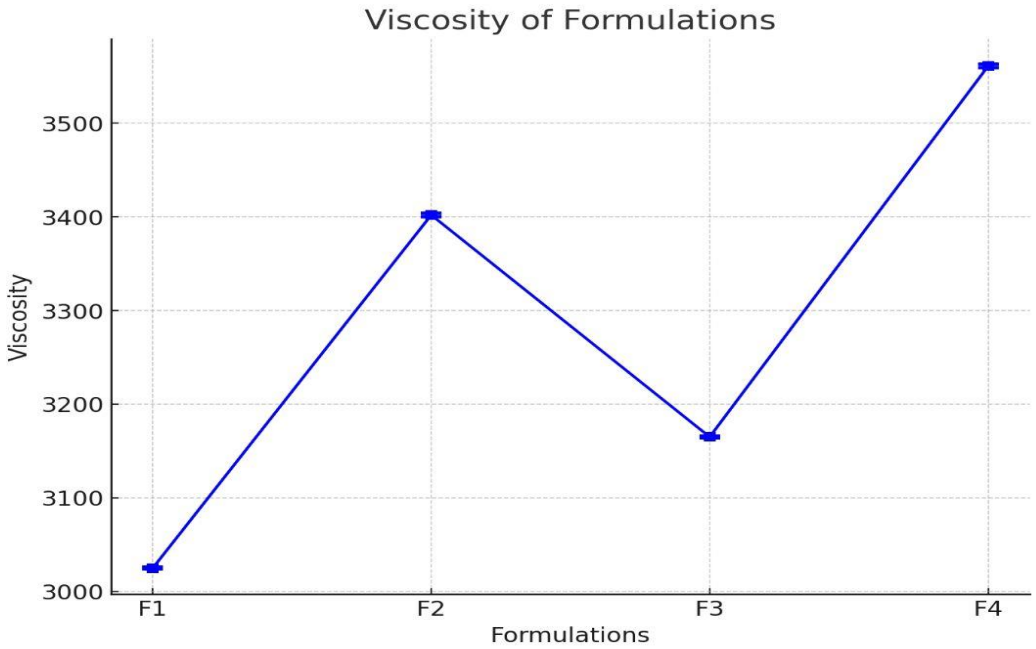

Figure 3 Viscosity Measurement of Formulation

Extrudability Study: Extrudability is an important parameter for the patient to accept the gel. If consistency of your formulation is high, it may not extrude from a tube, whereas formulation with low viscosity may extrude from a tube, due to their high flow rate.

Table 9: Extrudability of Formulations

Sr. No.	Formulations	Extrudability (%)
1	F1	69.65%
2	F2	70.35%
3	F3	84.81%
4	F4	52.76%

From table no. 9 it was showed extrudability of F4 formulations 70.35% have good extrudability among all other formulations.

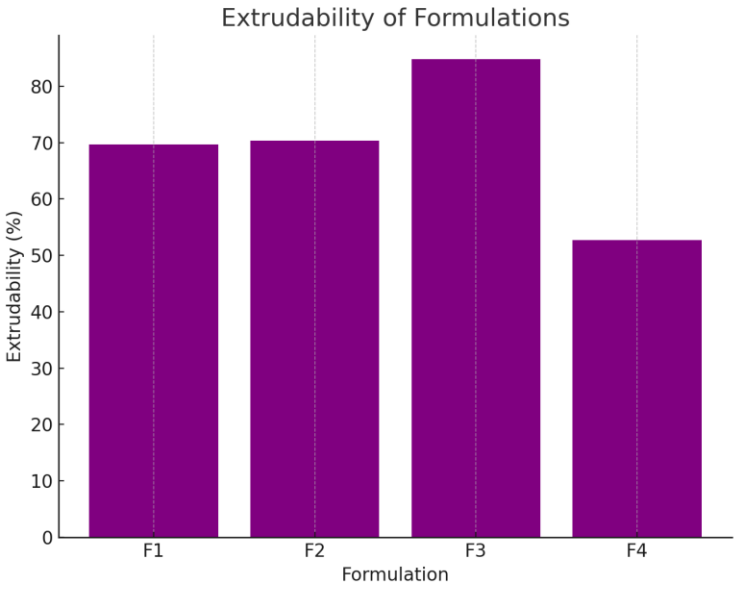

Figure 4 Extrudability Determination of Formulations

Spreadability Study: The spread ability of the gel is essential for its application. Poor spread ability can cause the gel to not adhere to the skin for sufficient time, resulting in diminished drug absorption and bioavailability.

Table 10: Spreadability of Formulations

Sr. No.	**Formulations**	**Spreadability (cm/sec)**
1	F1	6.32±0.14
2	F2	7.28±0.02
3	F3	8.34±0.09
4	F4	8.28±0.80

From table no. 10 it reflects that the spreadability of all four formulations were evaluated and the formulation F4 have high spreadability among all other formulations.

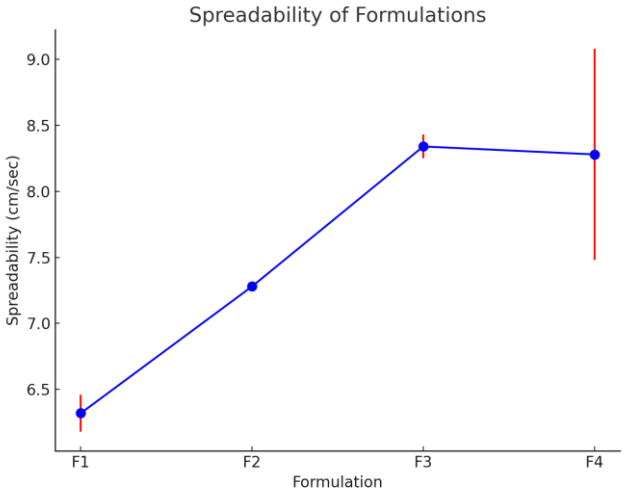

Figure 5 Spreadability Determination of Formulations

Moisture Content:

Moisture content is an important factor for determining of the formulation. Which can affect the stability, quality, and shelf life of formulation. Moisture content refers to the amount of water present in formulation.

Table 11: Moisture Content of Formulations

Sr. No.	Formulations	Moisture Content (%)
1	F1	95.68%
2	F2	99.68%
3	F3	99.73%
4	F4	99.22%

This table no.11 reveals the moisture content determination of all four formulations and the formulation F4 have good as compare to all other formulations.

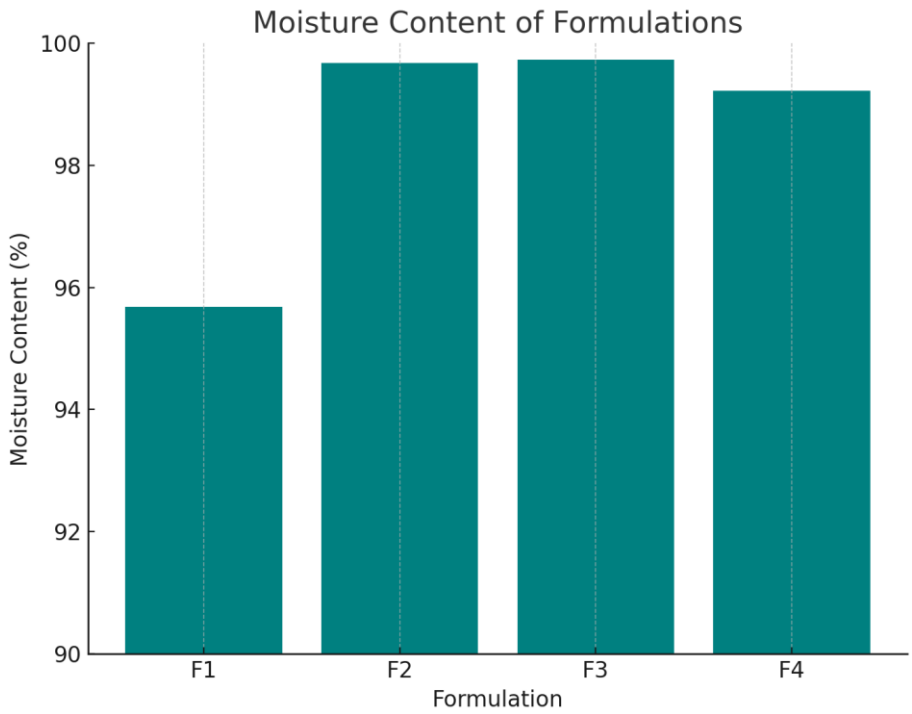

Figure 6 Moisture Determination of Formulations

Inhibition of Protein Denaturation Method: Anti-inflammatory activity of formulations was evaluated against denaturation of egg albumin method. In this study, ibuprofen drug was used as reference drugs. Ibuprofen is a nonselective NSAID. It is used as an analgesic, Anti-inflammatory and antipyretic agent.

Table 12: Inhibition of Protein Denaturation

Sr. No.	Concentration (µg/ml)	Rate of inhibition (%)	Rate of inhibition (%)			
		Standard	F1	F2	F3	F4
1	100	77.67	42.06	45.20	47.78	49.63
2	200	80.81	45.57	50.18	50.92	52.95
3	300	83.94	50.55	63.65	62.54	69.00
4	400	89.48	63.28	71.58	69.74	79.88
5	500	95.00	73.24	79.70	77.30	82.10

This table no.12 was showed Inhibition of protein denaturation of formulations. F4 formulation is better than other formulation.

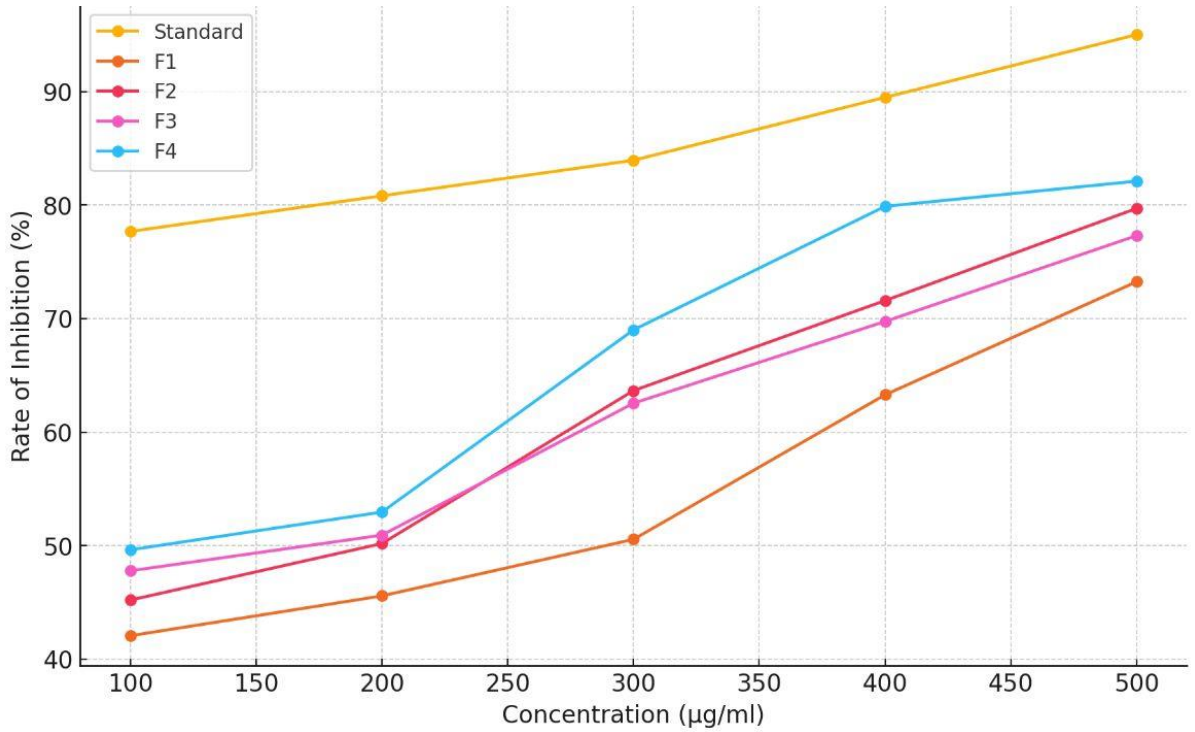

Figure 7 Protein Denaturation of Formulations

Conclusion

The formulation of hydrogel with Moringa oleifera and Aloe vera was successfully developed. The Moringa oleifera extract showed better antioxidant activity than Aloe vera extract. Evaluation of formulations' properties, including pH, viscosity, and extrudability, as well as their anti-inflammatory effectiveness, were done. The study's findings indicate that combining Moringa oleifera with Aloe vera can be beneficial for the development of medicinal and cosmetic applications.

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