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Design, Development and Characterization of *Moringa oleifera* Gel

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Abstract

Moringa oleifera (Family: Moringaceae) commonly called as Drumstick tree, Horseradish tree or Ben tree is an important medicinal herb referred as a miracle tree as all the parts of the plant possess nutritional and medicinal properties. The leaves of Moringa oleifera were air-dried for 12 days and then ground to a fine powder with a mortar and pestle.The ethanol extract gave a higher yield than the aqueous extract of *M.oleifera* leaves with 66.48% and39.5% respectively. The level of flavonoids, tannins, total phenols, saponin and alkaloids in aqueous extract of Moringa oleifera leaf meal were 3.63, 1.26, 2.04, 0.61 and 1.13% respectively. *In vitro* diffusion study of *M.oleifera* leaf extract hair gel of formulations F1 – F6 was found to be in 75.21 to 90.73% for 8 hrs. Of them the formulation F5 indicates the fastest diffusion with 90.73%.Out of all the models, Higuchi model showed the highest R^2 value for in-vitro release data for F5 (0.987) followed by Korsemeyer-Peppas model (0.985). The stability studies were directed for the optimized formulation, F5 for 3 months. No considerable changes were settled for the proven limits like appearance, pH, extrudability, spreadability, gel strength, viscosity, and antifungal action at both temperatures (room temperature and 40ºC) for 3 months.

Keywords: Moringa oleifera, Herbal Gel, Characterization of Gel

Introduction

Moringa oleifera (Family: Moringaceae) commonly called as Drumstick tree, Horseradish tree or Ben tree is an important medicinal herb referred as a miracle tree as all the parts of the plant possess nutritional and medicinal properties [1,2]. *M. oliefera* is an important medicinal plant and phytochemical investigations and isolated principles from them would be of use to achieve lead molecules in the search of novel herbal drugs [3,4].The Moringa family comprises 13 species *(M. oleifera, M. arborea, M. rivae, M. ruspoliana, M. drouhardii, M. hildebrandtii, M. concanensis, M. borziana, M. longituba, M. pygmaea, M. ovalifolia, M. peregrina, M. stenopetala*), of which M. oleifera has become well known for its use in nutrition, biogas production, fertilizer, etc., The main flavonoids found in its leaves are myricetin, quercetin, and kaempferol. Each part of the *Moringa oleifera* tree is used for a variety of nutritional and medicinal purposes [5]. The tree has anti-inflammatory, antimicrobial, antioxidant, anticancer, antihypertensive, hepatoprotective, anti-ulcer, antifertility, and diuretic properties.

Figure 1: Pictorial Representation of *Moringa oleifera*

Topical Gel of *Moring oleifera*

Gels are the semi-rigid systems in which the movement of the dispersing medium is restricted by interlacing of three-dimensional network of the particles or solvated macromolecules of the dispersed phase. Topical gel includes the following components:

1. Gel Forming Agent
2. Drug Substance
3. Penetration Enhancers

Materials and Methods

Materials

Fresh and healthy Moringa oleifera leaves were collected from Rohtak district. The plant was authenticated as *Moringa oleifera* Lam by Botanical Survey of India, Coimbatore. Carbopol 940, Polyethylene glycol, Povidone, Triethanolamine were purchased from CDH, New Delhi. All other reagents used were of analytical grade.

Methods

Collection of Plant Material (*Moringa oleifera*)

Fresh and healthy *Moringa oleifera* leaves were collected from Rohtak district. The plant was authenticated as *Moringa oleifera* Lam by Botanical Survey of India, Coimbatore. Stem and branches were cut from Moringa trees and spread out under the shade to dry at room temperature for 7 days. The leaves were removed manually and ground into fine powder and used for extraction.

Preparation of *Moringa oleifera*Leaf Extract

The leaves of *Moringa oleifera* were air-dried for 12 days and then ground to a fine powder with a mortar and pestle. The large particles were removed, while the powder obtained was stored in a polythene bag before analysis.

For the aqueous extract, 45 g of the powdered leaves was soaked in 380 mL of distilled water and left to stand for 72 hr to allow for maceration. The aqueous mixture was filtered using Whatman filter paper. For the ethanol extract, 45 g of the powdered leaves was soaked in 500 ml of ethanol and left to stand for 72 hr to allow for maceration. The ethanol mixture was filtered using Whatman filter paper. The dried extract was removed and stored in airtight container and used for further evaluation.

Qualitative and Quantitative Phytochemical Analysis of *M.oleifera*Leaf Extracts

Phytochemical screening for the class of carbohydrate, tannin, phenolics, flavonoids, glycosides and terpenoids was carried out using standard method.[6]

Detection of Flavonoids

To 2.0ml of aqueous and alcoholic extracts, few drops of sodium hydroxide solution were added. Formation of intense yellow color, which became colorless on addition of dilute HCl indicated the presence of flavonoids.

Detection of Tannins

To 2.0ml of aqueous and alcoholic extracts, 3 drops of 1% ferric chloride was added. Appearance of blue green color indicated the presence of tannins.

Detection of Total Phenols

2 ml of aqueous and alcoholic extracts were diluted with 2ml of 10% ferric chloride. Formation of bluish color indicated the presence of phenols.

Detection of Saponins

2 ml of aqueous and alcoholic extracts were diluted with 10ml of distilled water and mixed for 15min. Formation of layers of foam which remained for 10min indicated the presence of saponins.

Detection of Alkaloids

To 2.0ml of aqueous and alcoholic extracts, 2ml of picric acid (Hager's reagent) was added. Formation of an orange or yellow color precipitate indicated the presence of alkaloids.

Detection of Phlobatannin

To 2.0ml of aqueous and alcoholic extracts, 1ml of dilute HCl solution was added. Appearance of red precipitate indicated the presence of phlobatannins.

Detection of Hydrolysable Tannin

To 2.0ml of aqueous and alcoholic extracts, 2ml of ammonia solution was added. Formation of emulsion indicated the presence of hydrolysable tannin.

Detection of Terpenoids

To 2.0 ml of aqueous and alcoholic extracts, an equal amount of chloroform was added followed by addition of 2ml of concentrated H_2SO_4 along the sides of the test tube. Appearance of a brown color ring at the junction of two liquids indicated the presence of terpenoids.

Detection of Glycosides

To 2.0ml of aqueous and alcoholic extracts, 2ml of dilute H_2SO_4 was added and heated at 50˚C for 2 min. Then 1ml of 10% NaOH was added and 5ml each of Fehling's solution A and B were added. Appearance of brick red precipitate indicated the presence of glycosides.

Detection of Cardiac Glycosides

To 2.0 ml of aqueous and alcoholic extracts, an equal amount of glacial acetic acid was added. Then, one drop of 10% ferric chloride and 2ml of concentrated H_2SO_4 were added. Formation of three layers of colors like upper green, middle brown and lower violet indicated the presence of cardiac glycosides.

Detection of Volatile Oil

To 2.0ml of aqueous and alcoholic extracts, 0.1ml of NaOH and a small amount of dilute HCl were added. Formation of white precipitate indicated the presence of volatile oils.

Quantitative Phytochemical Analysis

This analysis was carried to determine the amount or concentration of the phytochemical constituents present in the aqueous extract of *Moringa oleifera* leaf.

Flavonoids

About 0.25 ml of extract (10mg/ml) was mixed with 0.75 ml of ethanol, 0.05 ml of 10 per cent aluminium chloride, 0.02 ml of 1M potassium acetate and 1.4 ml of distilled water. The reaction mixture was incubated at 37ºC for 30 minutes. The absorbance of the mixture was measured at 415 nm using UV- VIS spectrophotometer.

Tannins

About 1.0 gm of the sample was dispersed in 10 ml distilled water and agitated. This was left to stand for 30 min at room temperature and shaken every 5 min. After 30 min, it was centrifuged and the extract obtained. About 2.5 ml of the supernatant extract was dispensed into a separate 50 ml volumetric flask. Similarly, 2.5 ml of standard tannic acid was dispensed into a separate 50 ml flask.

Total Phenols

Total phenol was mixed with 0.5 ml of Folin-Ciocalteu phenol reagent (1:1 dilution with distilled water) and 2.5 ml of 20% sodium carbonate solution. The reaction mixture was allowed to stand for 40 min and the absorbance was measured at 725 nm using UV-VIS spectrophotometer.

Saponin

2 gm of dried plant sample was dissolved in 50 ml of petroleum ether. The suspension was heated over a hot water bath at 55ºC for 1hr with continuous stirring. The mixture was filtered and the residue was re-extracted in 50 ml of methanol. The combined filtrates were reduced to 10 ml by placing over water bath at about 90ºC. The concentrate was transferred into a 250 ml separating funnel and acetone was added slowly and shaken well. The aqueous layer was recovered and the purification process was repeated. The remaining solution which contains saponin was heated in a water bath and the sample was dried in oven and weighed.

Alkaloids

The quantity of alkaloids was estimated using about 1 gm of powered sample was mixed with 40 ml of 10% acetic acid and allowed to stand for 4 hrs. It was filtered and concentrated on water bath to one fourth of its original volume then concentrated ammonium hydroxide was added drop by drop to the extracts until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Formulation and Development of Herbal Gel

Measure the accurate amount of methyl paraben, glycerin and polyethylene glycol were dissolved in about 20ml of water in a beaker. Then it was stirred at high-speed using mechanical stirrer. Further, Carbopol 940 and PVP were added slowly to the solution prepared. Menthol was crushed and was added slowly in the above dispersion. The drug (moringa oleifera leaf extract) was added slowly. Then the formulation was neutralized by adding triethanolamine dropwise while stirring to attain gel structure. The prepared hair gel formulation was stored at room temperature.

Table 1: Formulation Table of *Moringa oleifera* Gel

Ingredients	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
***Moringa oleifera*Extract (%)**	4	4	4	4	4	4
Carbopol-940 (g)	0.5	0.75	1	1.5	2	3
PVP (mg)	5	5	5	5	5	5
Glycerin (ml)	3	3	3	3	3	3
PEG (g)	6	6	6	6.5	6.5	6.5
Triethanolamine	0.5	0.5	0.5	0.6	0.6	0.6
Methyl Paraben (g)	0.075	0.075	0.075	0.075	0.075	0.075
Flavouring agent (Menthol)	0.5	0.5	0.5	0.5	0.5	0.5

Evaluation of Gel

Appearance

The appearance of the formulations after and before gelling should be determined by visual inspection under light viewed against black and white backgrounds. It was observed for formation of turbidity or any unwanted particles dispersed in the solution.

Gelation Capacity

The gelling capacity of the formulation was determined by placing a drop of prepared formulation in a test tube containing 10 ml of freshly prepared STF and was visually observed for gelling time. Coding for the gelling capacity was described in Table 2.

Table 2: Coding for the Gelling System

Observation	**Coding**
No Gelation	-
Gelation occurred in few minutes, remained for few hour	+
Gelation Immediate, remained for few hour	++
Gelation Immediate, extended for long period	+++
Very Stiff Gel	++++

Viscosity Determination

To check the thixotropic behaviour for the prepared gel, rheological studies were performed. The viscosity measurements were carried out by using Brookfield viscometer model LVDV- II+P. The developed formulations were placed in the sampler tube using spindle no. 64 and viscosity of the prepared formulations was measured at various shear rates. The angular velocity was reversed (100 rpm to 0.5 rpm) with 1 min. of intervals. All measurements were performed in triplicate.

Washability

The prepared hair gel formulation is applied on the skin and then ease and extent of washing with water is checked normally.

Homogeneity

After the gel formulations have been set in the container, all developed gels were tested for homogeneity by visual inspection. They were tested for their appearance and presence of any lumps, flocculates or aggregates.[7]

pH Determination

The pH of all hair gel formulations were determined by using the digital pH meter. One gram of gel was dissolved in 100 ml distilled water and stored for two hours. Electrodes were completely dipped into the hair gel formulations and pH was noted. The

measurement of pH of each formulation was done in triplicate and average values were calculated.

Extrudability Determination

The hair gel formulations were filled into collapsible metal tubes. The tubes were pressed into extrude the material and extrudability of the formulations was checked. The extrudability of the formulations was determined in terms of weight in grams required to extrude a 0.5 cm ribbon of gel in 10 seconds.

Spreadability

Spreadabilitywas determined by the apparatus which consists of a wooden block, which was provided by a pulley at one end. By this method spread-ability was measured on the basis of slip and drag characteristics of gels. An excess of gel (about 2 gm) under study was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. Weight of 1 kg was placed on the top of the slide for 5 min to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 50 gm. With the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 6.5 cm be noted. A shorter interval indicates better spreadability.

Spreadability was calculated using the followingformula:

$$\mathbf{S = M \times L / T}$$

Where, S = Spreadability, M = Weight in the Pan (tied to the upper slide), L = Length moved by the glass slide and T = Time (in sec.) taken to separate the slide completely each other.

Diffusion Study

The diffusion study was important to determine the drug release of prepared *M.oleifera* leaf extract herbal hair gel formulation. It carried out such way taken Franz tube in which 1gm of herbal hair gel was taken packed on the bottom with cellophane membrane. The tube surface is dipped in to the solution. Phosphate buffer solution use to absorption media of drug and maintain the pH 7.4 of solution. Remove the 5 ml sample from the media time to time half hours 0.5,1, 2,3,4,5,6,7,8 hours determine drug release of herbal gel.

Release Kinetic Study

The data of dissolution profile, obtained with each drug's nanoparticles i.e., drug was fitted in release kinetic models.

1) Zero-order Release Kinetics

$$\mathbf{Q_{(t)} = k_0\, t}$$

As a function of time 't' (in minutes), Q (t) represents the percentage of the drug that has been dissolved, and k_0 stands for the dissolving rate constant for zero-order release. If the release follows zero-order release kinetics, a plot of the percentage of drug released against time will be linear. The slope of the percent medication released versus time plot was used in each case to determine the values of the release rate constant k_0.

2) First-order Release Kinetics

$$\mathbf{\log Q_t = \log Q_0 + \frac{K_1\, t}{2.303}}$$

The first-order equation describes the release from systems where release rate is concentration dependent. Where Q_0 is the initial amount of the drug, 't' is in minutes and k_1 describes the dissolution rate constant for first-order release kinetics. A plot of the logarithm of the percent drug remained against time will be linear if the drug obeys first-order release kinetics. Values of release rate constant kt were obtained in each case from the slope of the log percent drug remained versus time plots.

3) The Simplified Higuchi Model

$$\mathbf{Q\,(t) = kHt1/2}$$

Where Q (t) is the percent of drug dissolved, time 't' in minutes and kH is a dissolution rate constant for square root of time kinetics in percent dissolved $min^{-½}$. A plot of the fraction of drug released against square root of time will be linear if the release obeys Higuchi equation. Values of release rate constant kH were obtained in each case from the slope of the percent drug released versus square root of time plots.

4) The Fickian and Non-Fickian Drug Release Model

To evaluate the contribution of the release mechanisms, other than diffusion, other models of the release kinetics were employed. Since erosion of the matrix will contribute to the release a model describing general solute release from hydrophilic polymers as employed by the Korsmeyer et al (1983) may be used. Applied to the hydrophilic polymers, it has been simplified to an empirical form.

$$\mathbf{\frac{Mt}{M\infty} = kt^n}$$

Where k is the release rate and 'n' is the release exponent. Values of the release exponent (n) and the kinetic constant (k) were obtained in each case from the slope and y-

intercept of a logarithmic plot of percent released versus time respectively. Peppas used this n value in order to characterize different release mechanisms.

Stability Studies

All the formulations were equally good with respect to appearance, homogeneity, pH, viscosity and extrudability. The stability studies was carried out for all the prepared gel formulations at room temperature and 40ºC at 75% RH. The stability study was conducted for the period of 3 months. The parameters like appearance, pH, extrudability, colour were tested every month.

Qualitative Analysis of *Moringa oleifera*Leaf Extract

The ethanol extract gave a higher yield than the aqueous extract of *M.oleifera* leaves with 66.48% and 39.5% respectively. Therefore, ethanol is better a better extracting solvent. Qualitative phytochemical constituents of aqueous and alcoholic extracts of *Moringa oleifera* leaf meal are shown in the Table 4.

Qualitative analysis showed that flavonoids, tannin, total phenols, saponin, alkaloids and phlobatannin were present in both aqueous and alcoholic extracts of *Moringa oleifera* leaf meal. However hydrolysable tannin and terpenoid were present in aqueous extracts of *Moringa oleifera* leaf meal.

Table 3: % Yield of Aqueous and Ethanol Extracts of *Moringa oleifera*Leaf

Extracts	**Powdered Leaf Material (gm)**	**Yield (gm)**	**Yield (%)**	**Colour**
Aqueous	45	17.98	39.95	Black
Ethanol	45	29.92	66.48	Light Green

Table4: Qualitative Phytochemical Constituents of Alcoholic and Aqueous Extract of *Moringa oleifera* leaf

Phytochemical Constituents	**Alcoholic Extract**	**Aqueous Extract**
Flavanoids	++	+
Tannins	+	+
Total Phenols	++	++
Saponin	+	+

Alkanoids	++	++
Terpenoids	-	+
Phylobatannin	+	+
Hydrolysable Tannin	+	-
Volatile Oil	-	-
Glycosides	-	-
Cardiac Glycosides	-	-

Quantitative Analysis of *Moringa oleifera*Leaf

Quantitative phytochemical constituents of aqueous extracts of *Moringa oleifera* leaf meal are present in the Table 5. The level of flavonoids, tannins, total phenols, saponin and alkaloids in aqueous extract of *Moringa oleifera* leaf meal were 3.63, 1.26, 2.04, 0.61 and 1.13% respectively.

Table 5: Qualitative Phytochemical Constituents of Aqueous Extracts of *Moringa oleifera*Leaf

Phytochemical Constituents	Composition
Flavonoids	3.63
Tannins	1.26
Total Phenols	2.04
Saponins	0.61
Alkaloids	1.13

Evaluation of *Moringa oleifera* Hair Gel

Physical Appearance

The physical appearance was checked visually for the texture of hair gel formulation as shown in Table 6.

Gelation Capacity

The gelation capacity was checked by placing a drop of formulation in to the vial filled and interpretation can be done by using codes for gelling capacity as shown in Table 6. Formulation F5 shows immediate gelation and remains for few hours and formulations F1, F2, F3, F4 and F6 were showing immediate gelation and remains for extended period of time.

Table 6: Results of Physical Appearance and Gelation Capacity

Formulation	Gelation	Appearance
F1	+++	Opaque, Light Yellowish Green, Smooth on Application
F2	+++	Opaque, Light Yellowish Green, Smooth on Application
F3	+++	Opaque, Light Yellowish Green, Smooth on Application
F4	+++	Opaque, Light Yellowish Green, Smooth on Application
F5	++	Opaque, Light Yellowish Green, Smooth on Application
F6	+++	Opaque, Light Yellowish Green, Smooth on Application

Viscosity Determination

The viscosity was dependent on the polymeric content used in the formulation. The viscosity of formulations for F1, F2, F3, F4, F5, F6 before gelation and after gelation is given below in Table 7.

Table 7: Rheological Studies of *Moringa Oleifera* Leaf Extract Loaded Gel Before Gelation

S.No.	Shear Rate (RPM)	Viscosity of Formulations (cps)					
		F1	F2	F3	F4	F5	F6
1.	0.5	1257 ±0.16	1362.71 ±0.18	1581.65 ±0.12	2348.2 ±0.11	3158.32 ±0.16	4074.34 ± 0.17
2.	1	849.23 ±0.12	958.27 ±0.19	840.24 ±0.51	1157.54 ±0.23	2073.67 ±0.73	2847.24 ± 0.44
3.	2.5	643.21 ±0.29	738.57 ±0.76	578.62 ±0.54	853.77 ±0.88	1007.84 ±0.27	1873.98 ± 0.29
4.	5	431.20 ±0.62	534.65 ±0.51	538.14 ±0.92	586.53 ±0.30	784.85 ±0.52	1224.63 ±0.39

5.	10	326.84 ±0.77	208.14 ±0.71	248.37 ±0.17	413.72 ±0.83	563.89 ±0.76	833.78 ± 0.57
6.	20	118.12 ±0.18	121.86 ±0.71	147.65 ±0.16	217.92 ±0.24	257.86 ±0.46	547.72 ± 0.74
7.	50	98.76 ±0.31	109.98 ±0.15	135.18 ±0.77	192.42 ±0.39	168.78 ±0.66	320.68 ± 0.52
8.	100	87.15 ±0.11	124.03 ±0.36	106.48 ±0.27	185.94 ±0.72	118.86 ±0.44	217.18 ± 0.66

Table 8: Rheological Studies of *Moringa Oleifera* Leaf Extract Loaded Gel After Gelation

S.No.	**Shear Rate (RPM)**	**Viscosity of Formulations (cps)**					
		F1	**F2**	**F3**	**F4**	**F5**	**F6**
1.	0.5	4991.35 ± 0.73	6132.7 ± 0.18	6032.6 ± 0.43	7018.1 ±0.31	7234.6 ± 0.12	8435.1 ±0.54
2.	1	2964.34 ±0.22	3692.5 ±0.26	3762.5 ±0.23	4469.7 ±0.47	4722.7 ±0.98	5197.5 ±0.77
3.	2.5	2196.67 ± 0.73	2458.1 ±0.87	2850.9 ±0.18	3456.2 ±0.97	3521.7 ±0.85	3818.2 ±0.56
4.	5	752.17 ±0.92	862.31 ±0.13	883.77 ±0.18	2507.4 ±0.54	2413.62 ±0.76	2977.4 ±0.42
5.	10	404.53 ±0.87	353.22 ±0.87	396.87 ±0.08	607.87 ±0.93	621.87 ±0.32	792.82 ±0.14
6.	20	233.03 ±0.54	248.32 ± 0.64	275.33 ±0.51	296.31 ±0.45	313.4 ±0.43	380.81 ±0.87
7.	50	221.31 ±0.62	236.92 ± 0.21	255.96 ±0.78	269.7 ±0.23	296.63 ±0.82	341.67 ±0.31
8.	100	212.45 ±0.84	233.05 ±0.26	237.98 ±0.92	262.63 ±0.33	276.85 ±0.87	317.95 ±0.92

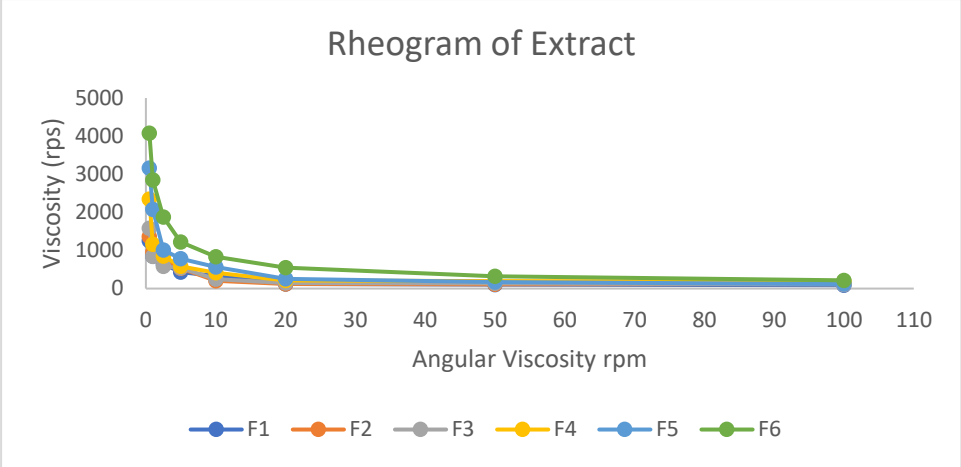

Figure 2: Rheogram of *Moringa oleifera*Leaf Extract Solution Before Gelation

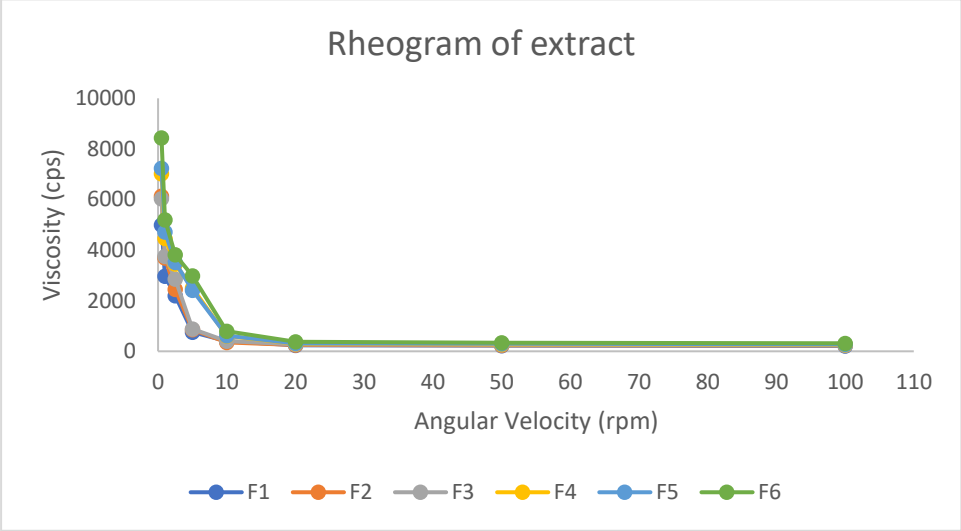

Figure 3: Rheogram of *Moringa oleifera*Leaf Extract Solution After Gelation

Extrudability

All the formulations shows extrudability when extruded from the metallic collapsible tube as shown in table below.

Table 9: Extrudability of Formulated Gel

Formulation	Extrudability
F1	84.23±0.002
F2	82.44±0.003
F3	86.02±0.001
F4	84.04±0.002
F5	87.32±0.004
F6	83.01±0.002

Skin Irritation

The prepared herbal hair gel was applied on skin of hand and exposed to sunlight for 4-5 min. It was found skin compatible and non-irritant.

Spreadability

A good gel takes less time to spread and shows excellent spreadability as shown in Table 10 below.

pH Determination

The pH value of herbal hair gel is determining by the pH meter. The measurement was performed detect pH of the prepared formulations.

Homogeneity

The consistency was found good for all formulations.

Table 10: Compiled Results of Homogeneity, pH Determination and Spreadability

Formulation	**pH**	**Spreadability (gcm/sec)**	**Homogeneity**
F1	6.7	10.94±0.04	Good
F2	6.8	13.87±0.03	Good
F3	6.0	12.98±0.01	Good
F4	7.2	11.05±0.02	Good
F5	6.9	14.62±0.02	Good
F6	7.1	9.85±0.02	Good

Drug Content

This parameter shows the uniformly dispersion of drug in the formulation as shown in Figure 4 and Table 11.

Table 11: Drug Content Uniformity of the Formulation

Formulation	**% Drug Content**
F1	89.9
F2	91.3
F3	92.7
F4	93.6
F5	95.2
F6	90.6

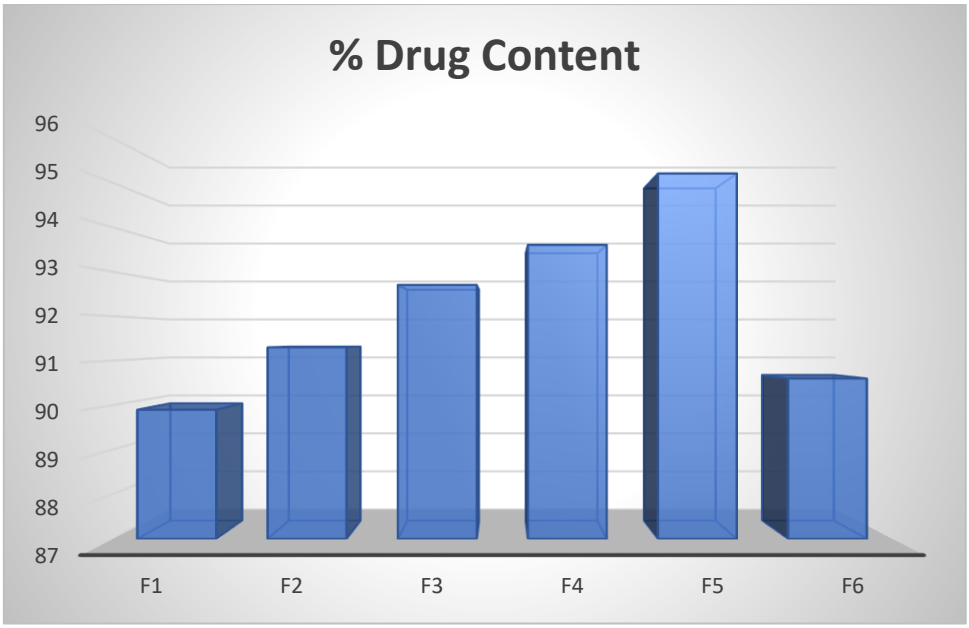

Figure4: Graphical Illustration of % Drug Content of *M.oleifera*Leaf Extract Loaded Gel

***In-vitro*Diffusion Studies**

In vitro diffusion study of *M.oleifera* leaf extract hair gel of formulations F1 – F6 was found to be in 75.21 to 90.73% for 8 hrs. Of them the formulation F5 indicates the fastest diffusion with 90.73%. Formulation F5 shows 44.89% cumulative drug release after 2hr, 68.89% after 4hr, 87.89% after 6hr and 90.73% after 8hr. Figure 5shows the graphical representation of *in-vitro* diffusion study.

Table 12: *In-vitro* Studies of the *M.oleifera*Leaf Extract Loaded Gel

S. No.	Time(hr)	F1	F2	F3	F4	F5	F6
1.	0	0	0	0	0	0	0
2.	0.5	12.32 ± 0.17	11.52 ± 0.12	10.78 ± 0.88	15.02 ± 0.18	18.43 ± 0.92	21.75 ± 0.10
3.	1	17.17 ± 0.19	14.75 ± 0.14	22.10 ± 0.90	26.86 ± 0.89	25.43 ± 0.28	27.43 ± 0.20
4.	1.5	30.59 ± 0.09	15.57 ± 0.19	30.99 ± 0.77	31.94 ± 0.80	35.92 ± 0.13	35.44 ± 0.37
5.	2	40.99 ± 0.30	21.96 ± 0.16	36.13 ± 0.60	36.08 ± 0.71	44.89 ± 0.26	43.33 ± 0.40
6.	2.5	47.97 ± 0.25	27.75 ± 0.20	42.98 ± 0.50	42.94 ± 0.55	52.97 ± 0.17	48.75 ± 0.49

7.	3	52.21 ± 0.94	32.75 ± 0.18	48.22 ± 0.56	50.59 ± 0.86	60.27 ± 0.09	54.77 ± 0.40
8.	4	59.98 ± 0.66	43.87 ± 0.13	56.90 ± 0.64	58.41 ± 0.78	68.89 ± 0.27	61.89 ± 0.18
9.	5	66.98 ± 0.50	51.86 ± 0.10	63.49 ± 0.32	68.89 ± 0.33	74.23 ± 0.80	74.97 ± 0.37
10.	6	77.22 ± 0.30	61.54 ± 0.12	75.99 ± 0.10	79.21 ± 0.90	87.89 ± 0.23	78.55 ± 0.60
11.	8	84.87 ± 0.50	75.21 ± 0.11	79.29 ± 0.33	82.21 ± 0.20	90.73 ± 0.93	81.96 ± 0.82

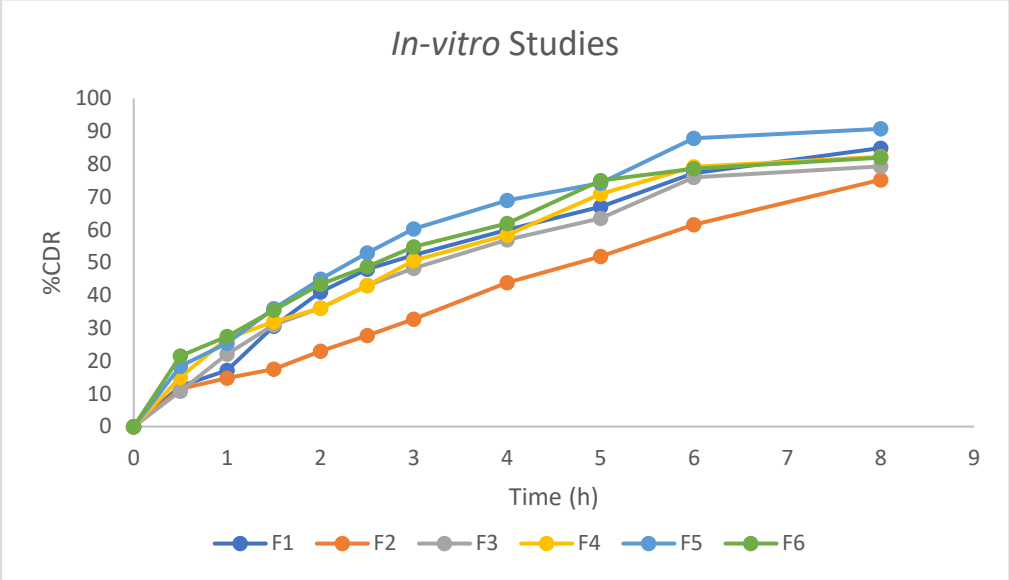

Figure 5: *In-vitro* Studies of *M.oleifera*Leaf Extract Loaded Gel

Release Kinetics

Data collected from *in-vitro* release study was fitted into various kinetic models to assess the mechanism of drug release.Out of all the models, Higuchi model showed the highest R^2 value for *in-vitro* release data for F5 (0.987) followed by Korsemeyer-Peppas model (0.985).

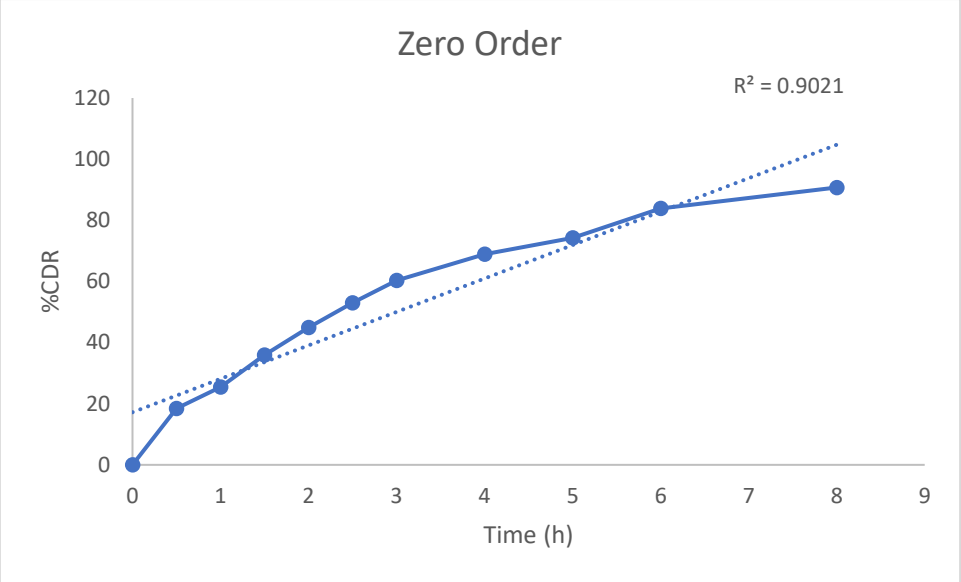

Figure 6: Zero Order Drug Release Profile of Optimized Formulation

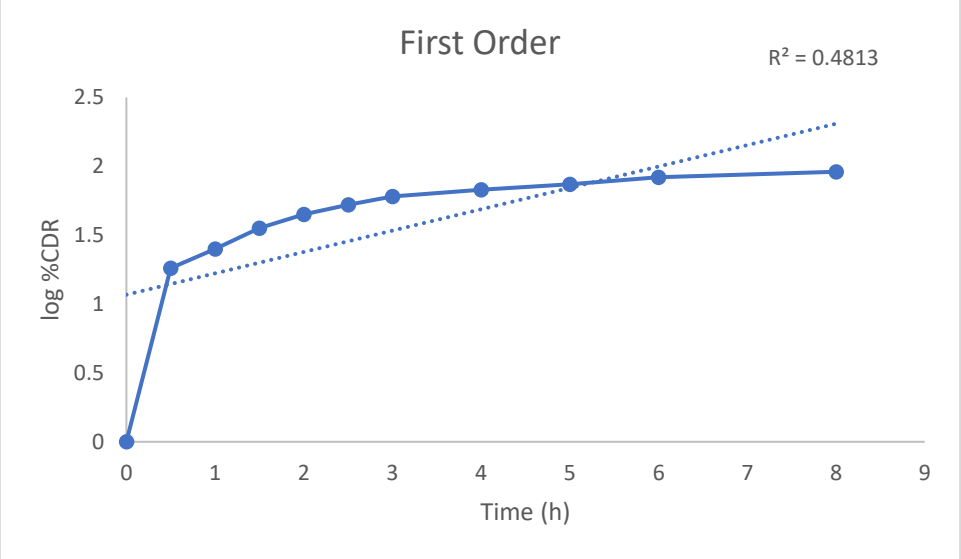

Figure7: First Order Drug Release Profile of Optimized Formulation

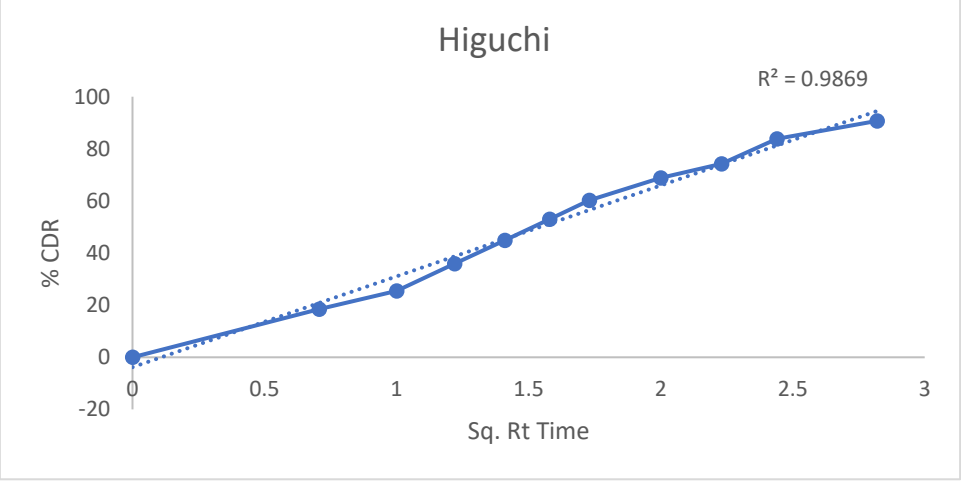

Figure 8: Higuchi Drug Release Profile of Optimized Formulation

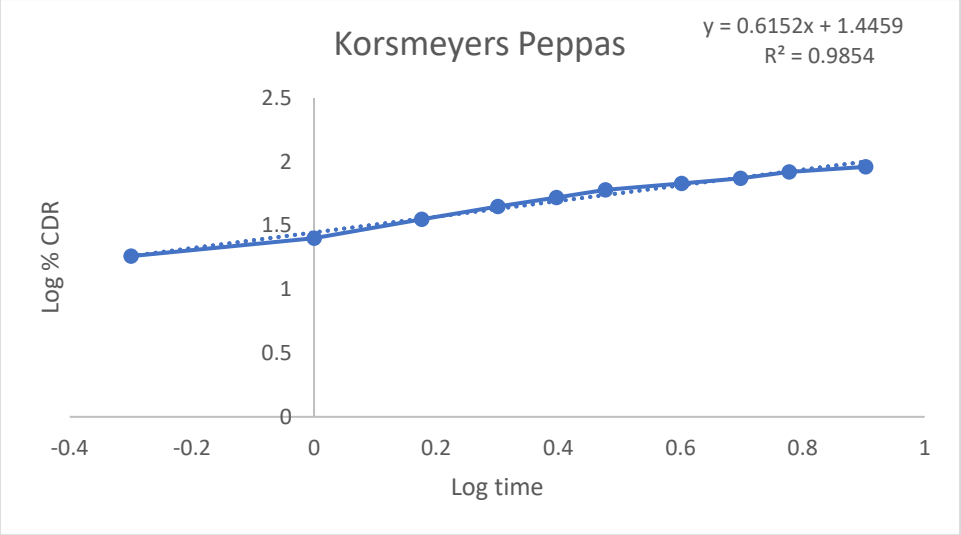

Figure 9: Korsmeyers Peppas Drug Release Profile of Optimized Formulation

Table13: Drug Release Kinetics of Optimized Formulation (F5)

Release Model	**Correlation Coefficient (R^2)**
Zero Order	0.902
First Order	0.481
Higuchi Release Model	0.987
Korsemeyer-Peppas Model	0.985

Stability Studies

The stability studies were directed for the optimized formulation, F5 for 3 months. No considerable changes were settled for the proven limits like appearance, pH, extrudability, spreadability, gel strength, viscosity, and antifungal action at both temperatures (room temperature and 40ºC) for 3 months.

Table 14: Stability Studies of Optimized Formulation

S.No.	**Parameters**	**Observations**		
		First Month	**Second Month**	**Third Month**
1.	Appearance	Opaque and smooth	Opaque and smooth	Opaque and smooth
2.	pH	6.9	6.87	6.72
3.	Extrudability	Excellent	Excellent	Excellent
4.	Viscosity	7233	7233	7233
5.	Spreadability	14.62±0.02	14.05±0.01	14.05±0.01

Conclusion

The study revealed that *Moringaoleifera* leaves are rich in phytochemicals and antimicrobials. Also, this study revealed that ethanol extract was a better extracting solvent and showed better inhibitory activity against the bacterial isolates.The *in-vitro* drug release data of the optimized formulation were fitted to various kinetic models mentioned above to understand the mechanism of drug release and kinetics of drug release from tablets. The correlation coefficient (R^2) values were obtained for determining the mechanism and kinetics of drug release. Out of all the models, Higuchi model showed the highest R^2 value for in-vitro release data for F5 (0.987) followed by Korsemeyer-Peppas model (0.985). The stability studies were directed for the optimized formulation, F5 for 3 months. No considerable changes were settled for the proven limits like appearance, pH, extrudability, spreadability, gel strength, viscosity, and antifungal action at both temperatures (room temperature and 40ºC) for 3 months.

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