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Formulation and Evaluation of Herbal (*Moringa oleifera*) Based Hydrogel for Treatment of Acne

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ABSTRACT

Acne vulgaris is an inflammatory skin condition that affects virtually everyone at some point. Papules, comedones, pustules, scarring, and nodules are standard features of the disease and can have a detrimental social and psychological impact on an individual. Although allopathic acne treatments are available, they have adverse side effects, are expensive, and are prone to cause antibiotic resistance. The present study is aimed at formulating and evaluating topical hydrogel gels containing Moringa oleifera extracts as potential antiacne drugs. Nine formulations containing the herbal extracts were prepared using 1%, 1.5% and 2% with Carbopol 934, HPMC K 100 M and Gaur gum. The phytochemical composition of the plant extracts was determined. The extracts and gels' minimum inhibitory concentration (MIC) was assessed using the microbroth dilution method. The physicochemical properties of the formulated gels, such as homogeneity, colour, texture, odour, spreadability, extrudability, viscosity, pH, and drug content, were evaluated. All the plant extracts contained alkaloids, flavonoids, tannins, triterpenoids, and coumarins. The gel formulations showed varying activity against Staphylococcus epidermidis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Candida albicans at various concentrations. The phytochemical components of the plant extracts are probably responsible for the antimicrobial activity of the gel formulations. The 1.5% HPMC formulation (MFG2) gel formulation showed excellent activity against Staphylococcus epidermidis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Candida albicans, with MICs of 12.50, 6.25, 3.13, 25.00, 12.50 mg/mL, respectively. The gels generally had good physicochemical and antimicrobial properties and could be used as antiacne remedies.

KEYWORDS: Acne vulgaris, Hydrogel, Moringa Oleifera

INTRODUCTION

Acne can cause by bacterial activity such as Staphylococcus epidermidis [1]. Currently acne treatment is antibiotic therapy which has skin irritation side effects and resistance in long-term use [16]. Moringa is a shrub that used widely as a vegetable or animal feed. Moringa leaves are empirically known has an antibacterial activity, because Moringa leaves contain secondary metabolites such as flavonoids, alkaloids, and phenols [2]. Previous research that has been carried out on ointment preparations of Moringa leaf extract showed an antibacterial activity against Propionibacterium acne [3]. Ethanolic extract of Moringa leaves with concentrations 5, 10, and 15% in ointment preparations has strong inhibitory activity against Staphylococcus aureus [4].

Medicinal plants have the advantages of patient tolerance and wide acceptability [5]. For example, Moringa oleifera have been folklorically used as antiacne agents [6–8]. They are additionally known to have nutrients and minerals to improve the general strength of the skin. Acne accounted for 5.3% of all skin diagnoses reported, and acne vulgaris was the second most common by gender [1, 9, 10]. This research was carried out by made a formulation of anti-acne gel using Hydroxy propyl methyl cellulose (HPMC), Carbopol 934 and Guar Gum as polymer and ethanol extract of Moringa leaf for acne treatment. Gel has better potential topical drug facilities than ointments, because gel is not sticky, requires less energy for formulation, more stable, and has good aesthetic value. [11, 12] Another advantage of gel preparation is quickly absorbed, so it is more effective to help absorption of active ingredient in acne area. Ethanolic extract of Moringa leaves gel with HPMC as a polymer has activity to inhibit different microorganism [13, 14]. This study was carried out using a variation concentration 1% of ethanolic extract of Moringa oleifera leaves and formulated in to anti-acne gel with HPMC polymers, Carbopol 934 and guar gum. Moringa oleifera leaves gel preparation were determine their antimicrobial activity.

MATERIALS AND METHODS

Collection and Authentication: The fresh leaves of Moringa oleifera were collected at the flowering stage in January from side of Local region of Gwalior, India. Identification number of moringa oleifera and specimen were submitted to the herbarium of Ram Nath Singh Mahavidyalaya (Pharmacy), Gormi, Bhind for further reference.

Physicochemical Characters: After botanical evaluation, the shade-dried plant material was subjected to size reduction to get coarse powder and then passed through sieve no. 43 to get uniform powder. Then, the uniform powder was subjected to standardization with different parameters Extractive values, ash value foreign matter and loss on drying as per literature.

Preparation of Ethanolic Extract: In the present study, the leaves were carefully selected washed to remove impurities and shade dried. The dried material was reduced to fine powder in the mechanical grinder. The fine powder was passed through sieve no. 43 and stored in an airtight container for further use. About 100 gm of powdered material was extracted with ethanol as a solvent by hot extraction method using Soxhlet apparatus. The extraction continued until the solvent in the thimble became clear then few drops of solvent were collected in the test tube during the completion of the cycle and chemical test of the solvent was performed. After each extraction, the extract evaporated to dryness in rotary vacuum evaporator. Moreover, some parts of the extract was preserved for preliminary phytochemical screening for the detection of various plant constituents and rest extract was used for formulation of gel batches.[82]

Formulation And Development: During formulation three gelling agents used at two different concentrations, resulting in six different batches of gels for leaves extract and six batches for root extract, total twelve batches prepared. In this case HPMC K100M, Carbopol 934, and Gaur gum, these three types of gelling agents were taken. [85, 86] Three gelling agents were used as follows:

- HPMC K 100 M (at concentration 1% 1.5% and 2%)
- Carbopol 934 (at concentration 1%, 1.5% and 2%)
- Xanthan gum (at concentration 1%, 1.5 % and 2%)

Gel composition was finalized after making many trial and errors. And the composition finalized is described here. The same experimental design was applied for both types of extract which results in total twelve batches of gel formulations. All the batches were prepared according to the experimental design.

PHYSICOCHEMICAL EVALUATIONS

Physical appearance: The prepared gel formulations containing *M. oliefera* were inspected visually for their color, homogeneity, consistency and phase separation.[87]

Measurement of pH: The pH of developed gel formulations was determined using digital pH meter. 1 gm of gel was dissolved in 100 ml distilled water and kept aside for two hours. The measurement of pH of each formulation was done in triplicate and average values are calculated.

Spreadability: Spreadability was determined by the apparatus which consists of a wooden block, which was provided by a pulley at one end. Spreadability was calculated using the following formula:

$$S = M \times L / T$$

Where, S= Spreadability,

M= weight in the pan (tied to upper slide), L= Length moved by the slide,

T= Time (in sec.)

Rheological Study: The viscosity of the developed gel formulations was determined by using Brookfield viscometer (Brookfield viscometer RVT) with spindle No. 7.

Extrudability: The gel formulations were filled in standard capped collapsible aluminum tubes and sealed by crimping to the end. Weights of the tubes were recorded. The tubes were placed between two glass slides and were clamped. 500 gm was placed over the slides and then the cap was removed. The amount of the extruded gel was collected and weighed. The percentage of the extruded gel was calculated (>90% extrudability: excellent, >80% extrudability: good, >70% extrudability: fair). [88, 89]

***In-vitro* Drug Diffusion Study:** The diffusion study of the gel were carried out in Franz diffusion cell. Gel sample (0.5 g) was taken on cellulose nitrate membrane diffusion studies were carried out at 37 ± 1 °C using 15 ml phosphate buffer (pH 6.8) as the dissolution medium. At time intervals of 0.5, 1, 2, 3, 4, 5, 6, 12 and 24 hours and replaced by fresh medium 2 mL sample were withdrawn and drug content was determined by UV spectrophotometer. [90]

Stability Study: Stability testing of new drug substances and products was carried out using international conference on harmonization (ICH) Q1A (R^2). The guideline recommends following in period and condition was used during stability evaluation. The present study involves investigation of the stability of the formulated gels under influence of 25°C ±2°C/60% RH ± 5% RH and 4°C + 2°C storage conditions for a period of 3 months. The study was carried out to evaluate the effect of storage conditions on essential attributes of gels such as appearance, viscosity and spreadability after specified time intervals.

Antimicrobial Activity

Subculture of Microorganisms: Staphylococcus epidermidis and Staphylococcus aureus (gram-positive), Escherichia coli and Pseudomonas aeruginosa (gram-negative), and Candida albicans (fungi) are the organisms used. The Mueller-Hinton broth test tube was sterilized before use by flaming the mouth with a Bunsen burner under a laminar flow cabinet. Then, 1mL of the pure isolate was introduced into the broth, flamed again, and capped with a cork to be incubated at 37°C for 24 hours.[94]

Streaking of the Sub-cultured Organisms to Obtain Pure Isolates: The agar was prepared, transferred into test tubes, and sterilized in an autoclave for 30 minutes at 121°C. It was poured onto a petri dish and cooled in the safety cabinet. The sub-cultured organisms were streaked over the surface of the agar and incubated for 24 hours with a sterile inoculum loop.

Estimation of Minimum Inhibitory Concentrations (MIC) of Gel: The microbroth dilution technique was employed to estimate the MIC. The microtiter plate was filled with appropriate and calculated amounts of the Mueller-Hinton nutritious broth, sterile water, distinct gel concentrations, and microorganisms (as compared to the McFarland standard) and incubated at 37°C for 24 hours. The MIC was determined using test formulations with the lowest dilution concentration and no apparent growth.[96]

RESULTS AND DISCUSSION

Organoleptic Characterization for Leaves: The feathery leaves of the tripinnate complex have green curved leaflets that are 1–4 cm long. Because of its leaves, the tree is frequently mistaken for a leguminous plant. The alternate twice or thrice pinnate leaves appear at the branch tips in most cases. They have a long petiole with 8–10 pairs of pinnae, each bearing two sets of inverse elliptic leaflets and one at the apex and are 20–70 cm long when young. The leaves are bitter and have an indistinct odour.

Physiochemical Evaluation: The ash value and foreign contents gave an idea of the inorganic content or other impurities present along with the crude drug. Besides the extractive values provided the additional information which might be useful in the determination of adulterated drug powder. During last few decades, there has been an increasing urge in the study of medicinal plants and their traditional uses in different parts of the world. Herbal remedies are considered as the oldest form of health care known to mankind on this earth. The traditional system of medicine that have evolved over the centuries within various communities, are still maintained as a great traditional knowledge base in herbal medicine. Physicochemical study taken up with *M. oleifera* suggested that the drug powder has high water soluble extractive values which might be due to its high content of water soluble bioactive components.

Table 1: Extractive values

S. No.	Parameters	Observed value in %	Standard value
1	Extractive Value		
	Alcoholic	4.11	Not more than 6%
	Water	9.89	Not more than 12%
2	Ash Value		
	Total Ash	7.87	Not more than 10%
	Acid Insoluble ash	0.78	Not more than 1%
3	Foreign organic matter	1.15	Not more than 2%
4	Loss on drying	6.78	Not more than 9%

Extraction: The ethanol extract obtained was light to dark green in colour, greasy with an indistinct odour and astringent in texture. The extracts were soluble in an organic solvent and were considered in acid to be insoluble.

Preliminary Phytochemical Study: The phytochemical screening results revealed that the after which it was observed whether the alkaloids were absent by no indication of turbidity and/or precipitate formation. The no colour changed from violet to blue or green in some samples indicated the absence of steroids. An interface with a reddish brown coloration was not formed in the absence of terpenoids, as positive result. Red coloration identifies the presence of flavonoids (Shinado's test). Below two observation indicated presence of Saponins Formation of stable foam confirmed the test the formation of a soluble emulsion confirmed the test. The formation of blue colour in acetic acid layer confirmed the test. The Formation of red color confirmed the test. The above two observations indicated presence of glycosides.

Table 2: Phyto-chemical Screening of extract

(A) Saponin

Test	Observation
Foam test	+

(B) Alkaloids

Test	Observation
Dragendroff's reagent	+
Meyer's reagent	-
Wagner's test	-
Muroxide test	-

(C) Carbohydrate

Test	Observation
Fehling	+
Benedict	+

(D) Test For Flavonoids

Test	Observation
Ferric chloride test	+
Shinoda test	+
Zinc-hydrochloric acid-reduction test	-

(E) Test For Proteins

Test	Observation
Biuret test	+
Million's test	+

Xanthoprotein test	-
Ninhydrine test	-

(F) Test For Glycosides

Test	Observation
Baljet' s Test	+
Keller-killiani test	-
Bromine water test	+
Legal's test	+

(G) Test For Amino Acids

Test	Observation
Ninhydrine test	-
Test for Tyrosine	+
Test for cysteine	-

(H) Test For Steroids

Test	Observation
Salkowski reaction	-
Liebermann's reaction	-

+ mean present, -mean absent

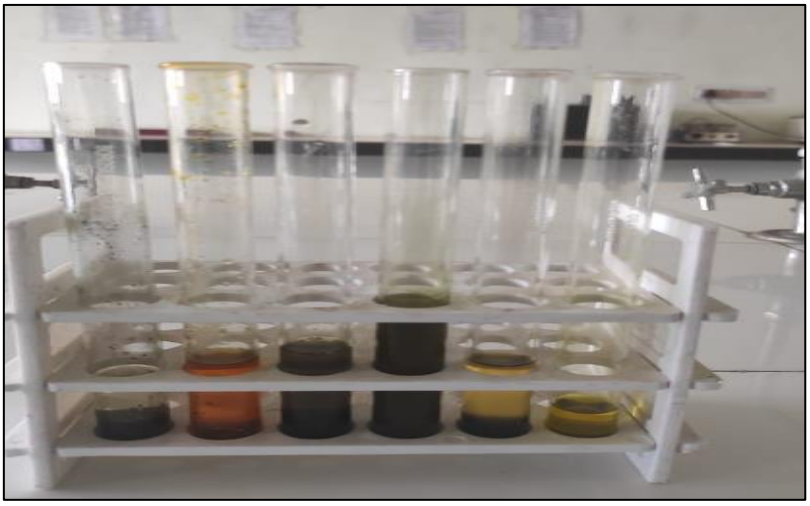

Figure 1: Preliminary phytochemical investigation of extract

Formulation And Development: During formulation three gelling agents used at three different concentrations, resulting in nine different batches of gels for leaves extract, total nine batches prepared. In this case Carbopol 934, HPMC K 100 M and Xanthan gum, these three types of gelling agents were taken. Three gelling agents were used as follows: a. Carbopol 934 (at concentration 1% 1.5% and 2.0%) b. HPMC K 100 M (at concentration 1% 1.5% and 2.0%) c. gaur gum (at concentration 1% 1.5% and 2.0%) All the batches were prepared according to the experimental design. For leaves extract there is same experimental design used which results in total 9 gel batches into which 9 batches for leaves extract.

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Table 3: Formulation chart

Comp.	Formulation								
	MFG1	MFG2	MFG3	MFG4	MFG5	MFG6	MFG7	MFG8	MFG9
Extract	1%	1%	1%	1%	1%	1%	1%	1%	1%
HPMCK100M	1%	1.5%	2%	-	-	-	-	-	-
Carbopol 934	-	-	-	1%	1.5%	2%	-	-	-
Gaur Gum	-	-	-	-	-	-	1%	1.5%	2%
Propylene glycol	5%	5%	5%	5%	5%	5%	5%	5%	5%
Methyl paraben	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%
Propylene paraben	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%
Triethanolamine	2%	2%	2%	2%	2%	2%	2%	2%	2%
Purified water	QS	QS	QS	QS	QS	QS	QS	QS	QS

Characterization and Physiochemical Evaluation of Gel

Appearance: The appearance was checked visually. After gelling the clarity of the formulations colour was determined by visual examination under light, alternatively against white and black background. The colour of gel was observed to be about yellowish green with translucent appearance.

Determination of pH: The pH of formulations was in the range of 5.12 to 6.45 which considered acceptable to avoid the risk of skin irritation upon application to skin. The Result are shown in Figure 1. The optimized formulation (MFG2) pH was found to be 6.45. There was no significant change in pH values as a function of time for all formulation.

Spreadability study: Hydrogels agent exhibited spreadability values ranging from 12.64-19.45 g.cm/s. The spreadability of various gel formulations are given below in table 2. HPMC K-100M based formulation showed better spreadability than the Carbopol 934 and gaur gum formulations. All formulation shows good spreadability after compare with marketed gel formulation.

Determination of Viscosity: The measurement of viscosity of the prepared gel was done with Brookfield viscometer of (Brookfield Engineering Laboratories). The average viscosity of formulations lies in the range from 1937.49 to 3650.63 cps. The Viscosities of all gel formulations are shown in Table 3 and was found to be decreased on increasing the shear rate i.e., pseudo plastic behaviour was noted.

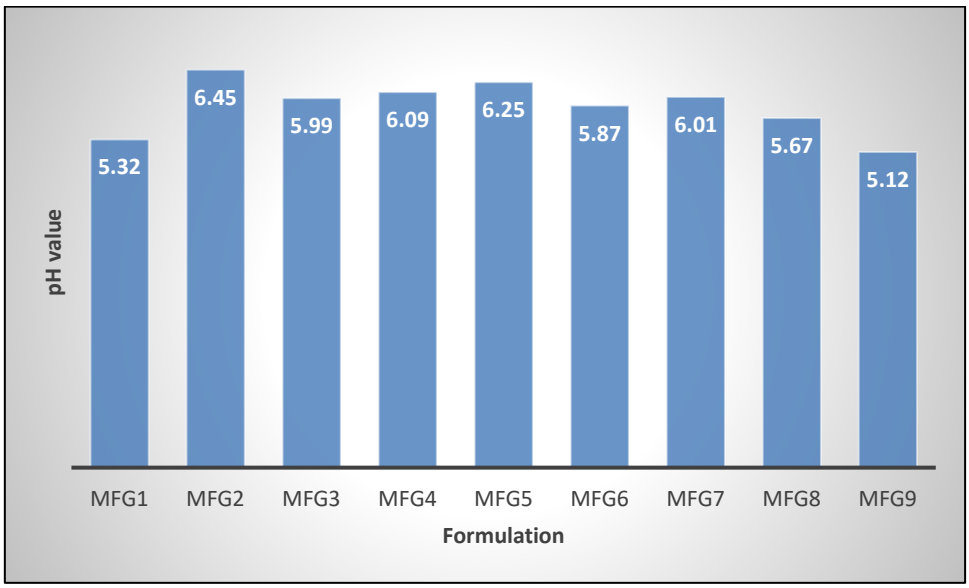

Figure 2: pH of formulated hydrogel (MFG1-MFG9)

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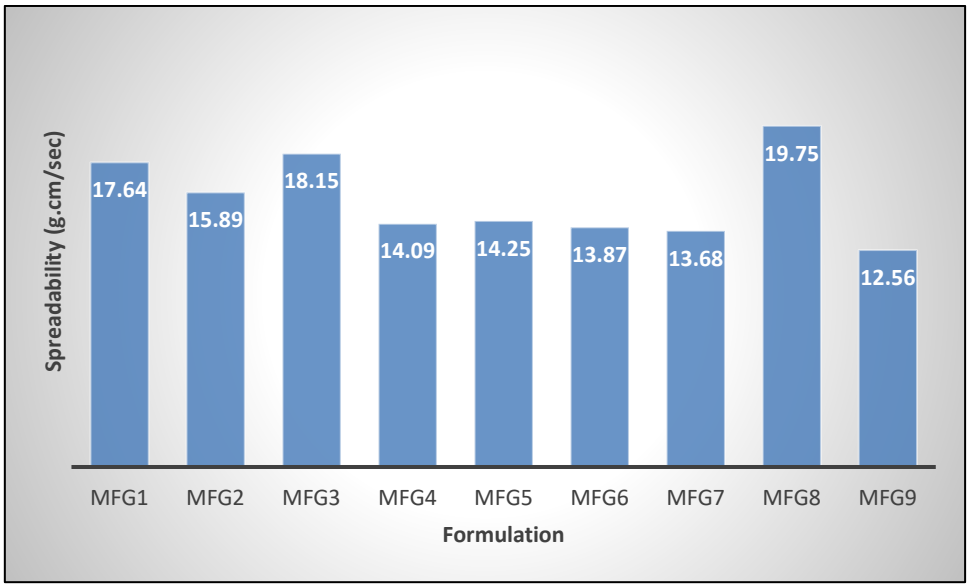

Figure 3: Spreadability of MO loaded hydrogel (MFG1-MFG9)

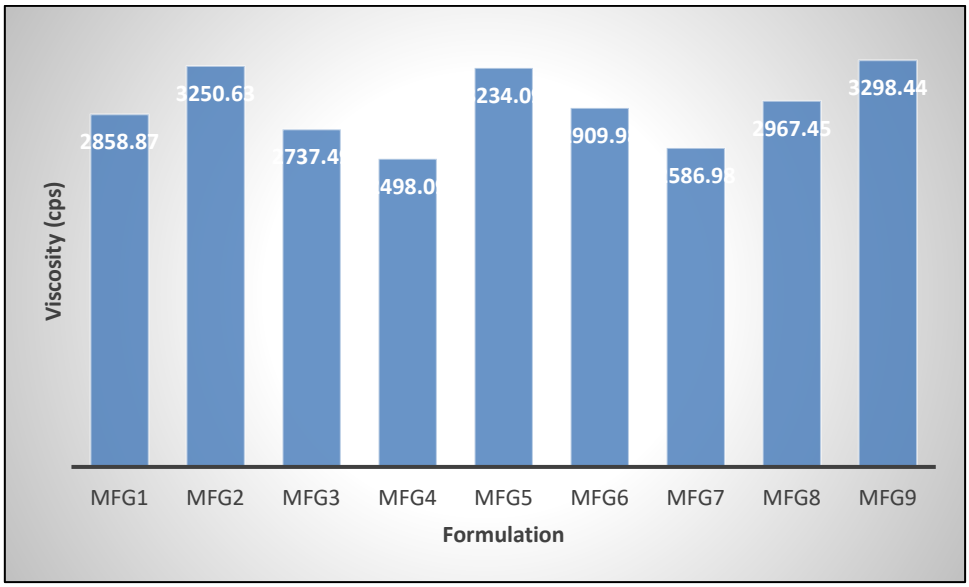

Figure 4: Viscosity of formulated hydrogel (MFG1-MFG9)

Extrudability: The Extrusion of the gel from the tube is an important during its application and in patient acceptance. Gels with high consistency may not extrude from tube whereas, low viscous gels may flow quickly and hence suitable consistency is required in order to extrude the gel from the tube. Extrudability of HPMC K-100M gel formulations was found to be good. Whereas Extrudability of Carbopol 934 and gaur gum gels were satisfactory.

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Table 4: Extrudability study of various gel formulations

Formulation	Weight of formulation	Weight of gel extruded	Extradibility amount (%)	Grade
MFG1	15.97	13.25	86.43	Good
MFG2	15.78	13.86	86.31	Good
MFG3	15.64	13.11	86.65	Good
MFG4	15.20	12.99	82.86	Good
MFG5	15.23	13.02	83.75	Good
MFG6	15.67	13.75	84.54	Good
MFG7	15.65	13.34	85.64	Good
MFG8	15.11	12.66	85.54	Good
MFG9	15.45	13.24	82.75	Good

***In-vitro* drug release study:** The in vitro drug release studies were carried out across cell membrane. The results of in-vitro release of gel are shown in table 4.7, 4.8 4.9. The cumulative percentage drug release for 8 hrs was highest for formulation MFG2 using HPMC K-100M.

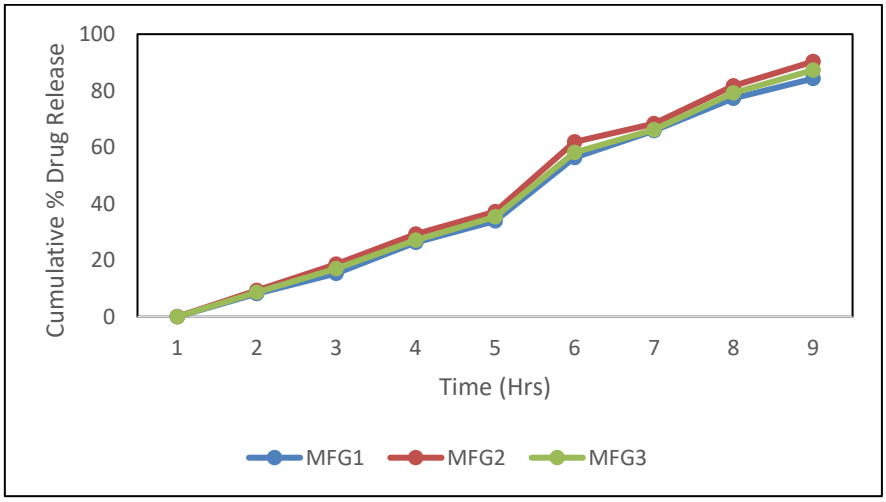

Figure 5: In-vitro drug release study of (MFG1-MFG3)

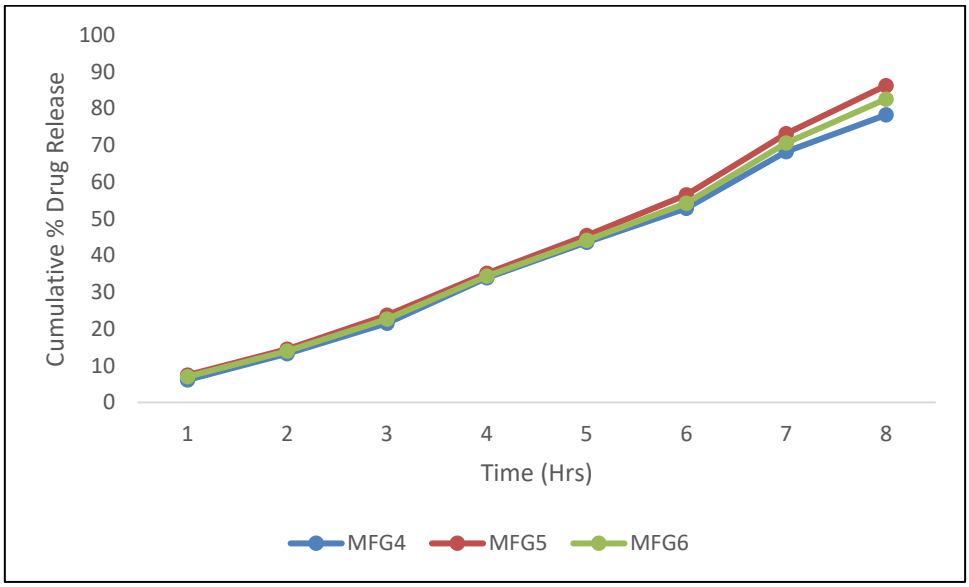

Figure 6: In-vitro drug release study of (MFG4-MFG6)

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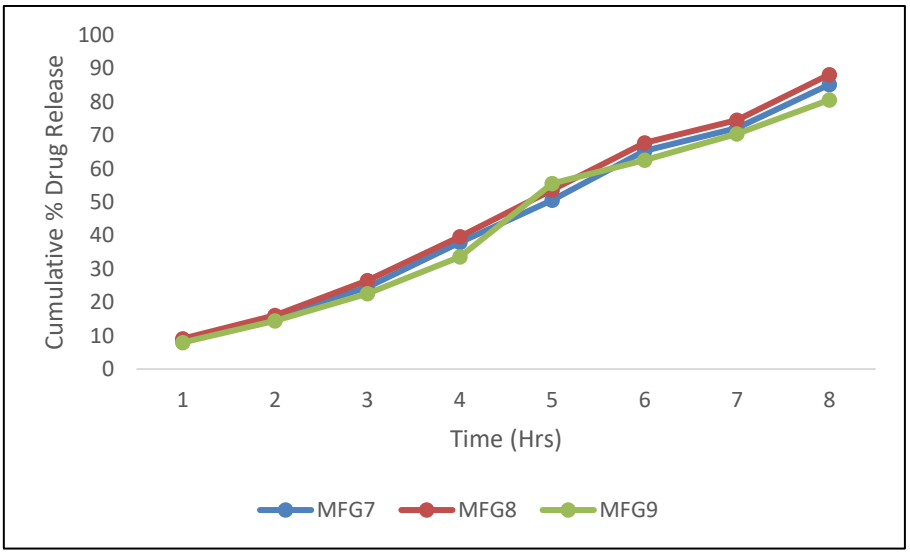

Figure 7: In-vitro drug release study of (MFG7-MFG9)

Stability Studies: The pH, viscosity, spreadability were analysed and there was a marginal difference between the formulations stored at different temperatures as shown in table 4.10. Hydrogel formulations retained good stability throughout the study.

Table 5: Stability studies

Parameters	1 month	2 months	3 months
pH	6.45	6.50	6.55
Viscosity (cps)	3250.63	3233.34	3211.56
Spreadability (gm.cm/sec)	15.89	15.11	14.34

Microbiological Evaluation Of Extracts And Formulated Gels: The MIC of the extracts against the tested organisms is required to calculate the dose in the formula. This MIC was then increased by ten to generate the dose, which was then utilized to calculate the amount of extract used in the gel formulations. The MIC results are shown in Tables 6. The antibacterial properties of the plant extracts were tested using the microbroth dilution technique. The findings of this study showed that the plant extracts were active against the test organisms. The different gel formulations showed inhibitory effects against Staphylococcus epidermidis and Staphylococcus aureus at 6.25, 12.5, and 25 mg/mL (Table 6). Only formulations containing HPMCK-100M only and gaur gum exhibited the most antibacterial effect against Staph epidermidis. Table 6 further revealed that some formulations were bacteriostatic while others were bactericidal on the test organisms. The current research reveals the formulations' therapeutic potential in antibacterial properties. According to the results of the tests mentioned above, the extracts have a significant growth inhibitory action on the organisms. The efficacy of these formulations based on MIC values supports their use in the prevention and treatment of bacterial infections caused by a variety of pathogenic bacteria with antibiotic resistance, most importantly, acne.

Table 6: Mean MICs (mg/mL) of the different gel formulation against test organisms

Gel formulations	Staphylococcus epidermidis	Staphylococcus aureus	Escherichia coli	Pseudomonas aeruginosa	Candida albicans
MFG1	6.25±0.02	25.00±0.05	12.50±0 05	-	12.50±0 05
MFG2	12.50±0.07	6.25±0.31	3.13±0.15	25.00±0 22	12.50±0.22
MFG3	12.50±0.10	25.00±0.22	6.25±0.21	25.00±0.10	12.50±0.08
MFG4	6 25±0.06	25.00±0.20	-	25.00±0.13	12.50 ± 0.21
MFG5	25.00 ± 0.13	25.00 ± 0.08	25.00±0.31	-	6.25 ± 0.43
MFG6	6.25±0.06	25.00 ± 0.20	25.00±0.13	12.50±0.21	-
MFG7	12.50±0.10	25.00±0.22	6.25±0.21	25.00±0.10	12.50±0.08
MFG8	25.00±0.13	25.00±0.08	25.00±0.31	-	6.25±0.43
MFG9	6.25±0 06	25.00±0.20	25.00± 0.13	-	12.50 ± 0.21

Key: (-) no inhibition.

CONCLUSION

It can be concluded from the present investigation that proper selection of polymers and drug is a prerequisite for designing and developing a transdermal drug delivery. The physical compatibility studies suggest that polymers selected i.e. HPMC K-100M, Carbopol 934, and gaur gum were found to be compatible with drug Moringa oleifera. The varying concentration of the three polymers was found to affect the gel parameters like viscosity and spreadability. Gel formulations prepared with Carbopol 934, HPMC K 100 M and gaur gum showed good homogeneity, no skin irritation, good stability and anti-inflammatory activity. However, the HPMC-K100M based gel proved to the formula of choice, since it showed the highest percentage of extrudability, good spreadability and rheological properties. Formulation MFG2 with 1.5% leaves extract of *Moringa oleifera* showed the best formulation with significant antimicrobial activity.

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