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FORMULATION, CHARACTERIZATION AND OPTIMIZATION OF TOPICAL HERBAL HYDROGEL CONTAINING *ALOE VERA, TURMERIC* AND *MARIGOLD*

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Keywords:

Herbal hydrogel, Topical formulation, Optimization technique, *Aloe vera*, *Turmeric*, *Marigold*

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ABSTRACT: The investigation of herbal components in topical formulations is a response to the growing desire for natural and effective remedies. The development, modification, and characterization of a topical herbal hydrogel intended for use in dermatological applications are the main objectives of this work. During the formulation procedure, plant extracts of *Turmeric*, *Aloe vera* and *Marigold* were chosen for well-known medicinal properties. Viscosity and spreadability of herbal hydrogel were optimized. Spreadability and viscosity were the response variables that were employed in the design of formulation. The viscosity and spreadability of the optimized formulation were 176000 m Pa. S and 8.25 g.cm/sec respectively. Phytochemical screening confirmed that the active phytoconstituents present in the herbal hydrogel. According to the antioxidant study, the herbal formulation exhibited good radical scavenging activity, reaching up to 78.02%. Attenuated total reflectance infrared (ATR-IR) spectroscopy revealed that there were no major interactions between the excipients and the herbal drugs. The microstructure of formulated herbal hydrogel was observed using field emission scanning electron microscopy (FE-SEM) imaging, which also showed that the herbal constituents were homogeneously distributed throughout the hydrogel. The hydrogel was also analysed using X-ray diffraction (XRD) and average particle size was found to be 16.660 nm. The qualitative and quantitative HPTLC confirmed that herbal ingredients were sufficiently incorporated in the formulation. The antimicrobial study showed considerable effectiveness with zone of inhibition of 1.57 cm against *Staphylococcus aureus*.

INTRODUCTION: The development of safe and efficient topical formulations is a crucial aspect of modern pharmaceutical and cosmetic exploration. In recent years, there has been an increased interest in using the therapeutic potential of herbal extracts for skincare applications.

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Herbal hydrogels are becoming popular choices for the topical delivery of bioactive compounds derived from medicinal plants because of their unique properties.

This thesis investigates the formulation, development, and evaluation of a topical herbal hydrogel with a focus on the possible uses in medicine and cosmetics. The medical benefits of herbal extracts, such as their ability to reduce inflammation, heal wounds, function as antioxidants, and fight bacteria, have long been recognized. These herbal compounds have

improved bioavailability, longer release, and improved epidermal penetration when added to hydrogel formulations. The natural source of herbal extracts aligns with customers' growing desire for products seen to be safer and more environmentally friendly. The formulation of herbal hydrogel requires a careful balancing act between ensuring the durability and efficacy of the final product and utilizing the right herbal extracts and gel base optimization. This procedure calls for a multidisciplinary approach that integrates knowledge from the fields of chemistry, dermatology, and pharmaceutical sciences. Optimizing the formulation parameters to achieve the desired rheological, physicochemical, and medicinal properties is the aim of the optimization phase. Approaches to experimental design often help this process [1].

Herbal hydrogels are evaluated using *in-vitro* tests, physicochemical characterization, thorough investigation, and technological considerations. To ensure the product is dermatologically friendly, it is important to consider safety considerations such as microbiological testing. This seeks to progress the field of herbal skincare formulations by providing insights into the intricate processes involved in the production of herbal hydrogel. The results of this study may further our understanding of herbal formulas and have implications for the broader pharmaceutical and cosmetic industries. This might result in the creation of innovative topical treatments with potential applications in the beauty and therapeutic fields that are inspired by nature [2].

MATERIALS AND METHODS:

Plant Materials: The fresh leaves of the *Aloe vera*, *Turmeric* rhizomes and *Marigold* leaves were collected from the medicinal plant garden, Bengal School of Technology, Hooghly, West Bengal, an eastern region in India. Medicinal plant gardens are primarily focused on the conservation, cultivation, research and educational activities related to authenticated herbal plant species known for medicinal purposes.

The plants specimens were identified from Central National Herbarium (Botanical Survey of India, Kolkata, certificate No. CNH/Tech. II/2023/173 issued on 08-11-2023). The plants were identified are as following **Table 1.**

TABLE 1: SPECIMEN NUMBER CORRESPONDING TO IDENTIFIED PLANT SPECIMEN

Sl. no.	Specimen No.	Scientific Name	Family
1	BST/AB-01	*Curcuma longa L.*	Zingiberaceae
2	BST/AB-02	*Aloe vera (L.) Burm.f*	Asphodelaceae
3	BST/AB-03	*TageteserectaL.*	Asteraceae

Chemicals: Carbopol 940 was purchased from Loba Chem Pvt Ltd, Mumbai, India and Carboxy Methyl Cellulose (CMC) was purchased from Simson Chem, Mumbai, India. Glycerine was purchased from Qualikems Laboratory Reagents, Delhi, India and Triethanolamine was purchased from Nice Chemicals Pvt Ltd. Kerala, India. Acetone, chloroform, and methanol were purchased from Loba Chem Pvt Ltd, Mumbai, India. All the chemicals used, including the solvents, were of analytical grade.

Extraction of *Turmeric* Rhizome, *Aloe vera* Gel and *Marigold* Leaf: The *Turmeric* rhizomes were cleaned properly and cut into pieces. Then it was sun dried for 7-10 days and kept in hot air oven at 60ºC for 2-3 days. Then dried mass was milled to powder. 200 g of powder was taken in a glass beaker and extracted with 500 ml of methanol as a solvent with frequent stirring for 6 hours for 5 days. Beaker was kept closed with aluminium foil. After that the soaked sample-solvent mixture was filtered and the filtrate was evaporated by vacuum rotary evaporator to concentrate the residue 40-60 ºC and 50 revolutions per minute RPM). The concentrated extract was weighed, around 15 g. After that it was freeze dried at -70ºC at 35 PSIA (Pounds per square inch absolute) pressure for 7 days. The dried powder extract was stored at below 20ºC until use [3].

The fresh leaves of *Aloe vera* were taken, washed with tap water, then transversely cut into pieces. The thick epidermis was removed with the help of a sharp knife and the inner gel-like pulp in the centre of the leaf was scooped with a spoon. Fresh *Aloe vera* gel was collected in a beaker. 50g of *Aloe vera* gel homogenized by adding 30 ml of

water within it. Then the homogenized gel was freeze dried at -70ºC under 35 PSIA pressure until completely dried. The dried powder extract was stored at below 20ºC until use [4]. Then dried leaves of *Marigold* were powdered. 200 g of powdered leaves were extracted with 500 ml methanol as a solvent with frequent stirring for 6 hours for 5 days. Beaker was kept closed with aluminium foil. After that the soaked sample-solvent mixture was filtered and the filtrate was evaporated by vacuum rotary evaporator to concentrate the residue at 40-60 ºC 50 revolutions per minute (RPM). The semi solid extract was weighed, it was around 13 g. After that it was freeze dried at -70ºC at 35 PSIA for 7 days. The dried powder extract was stored at below 20ºC until use [5, 6].

Optimization of Formulation using Design of Experiment (DoE): Formulation development is a challenging endeavour that often involves choosing variables or parameters to get the optimum outcome in a situation with a lot of uncertainty or unpredictability. For this reason, effective optimization techniques may be highly helpful in producing superior formulations [7]. Optimization studies for the given procedure were performed by using full factorial design (Design Expert Software Trial version13.0.5). 2^2 factorial design (two-factor two-level factorial design) was used for the formulation design and optimization of the herbal hydrogel. It is a type of experimental design used in statistical experimentation to investigate the effects of two factors, each at two levels, on a response variable [8]. The experimental design involves systematically combining the levels of the two factors to create a set of experimental runs. For a 2^2 factorial design, there would be a total of $2^2 = 4$ experimental runs. Carbopol-940 and sodium carboxy methyl cellulose (CMC) were selected as two factors for the formulation of herbal hydrogel for the optimization ((Design Expert Software Trial version13.0.5). Viscosity and spreadibility were the response variables selected for this research work [9].

Preparation of Herbal Hydrogel: Carbopol 940 and carboxy methyl cellulose (CMC) were used in the formulation of herbal hydrogel. Carbopol 940 was added slowly into the water and stirred continuously. Once the carbopol 940 was fully dispersed, CMC was added to the mixture and stirred continuously to prevent clumping. The required quantity of methyl paraben and propyl paraben were added. Then the required quantity of *Aloe vera* gel, *Turmeric* and *Marigold* were added to the above mixture. The herbal mixture was stirred continuously with the required volume of double distilled (DD) water until it becomes homogeneous. After that, required amount of methyl paraben and propyl paraben were added to the mixture. Triethanolamine was added dropwise to the formulation for adjustment of the required pH and to obtain gel in the required consistency [10, 11]. **Table 2** provides the composition of all ingredients used for the preparation of the formulations.

TABLE 2: FORMULATION INGREDIENTS FOR HERBAL HYDROGEL

Ingredients	Formulation code			
	F1	F2	F3	F4
Aloe Vera Gel (gm)	2	2	2	2
Marigold Leaf Extract (gm)	2	2	2	2
Turmeric Rhizome Extract (gm)	2	2	2	2
Carbopol 940 (gm)	0.75	1.25	0.75	1.25
Sodium CMC (gm)	0.1	0.1	0.2	0.2
Glycerin (gm)	5	5	5	5
Methyl Paraben (gm)	0.02	0.02	0.02	0.02
Propyl Paraben (gm)	0.002	0.002	0.002	0.002
Triethanolamine (gm)	1.2	1.2	1.2	1.2
Double Distilled Water (gm)	q.s.	q.s.	q.s.	q.s.

Evaluation of Prepared Herbal Hydrogel:

Organoleptic Properties: A small amount of each hydrogel formulation was observed for colour, odour, and appearance. Texture was also observed after applying on bare skin [12].

pH: 1 g of each formulation was accurately weighed and solubilized in 100 ml purified water. The pH of the solution was measured using pH meter, which was calibrated before use with standard buffer solution at 4.0, 7.0 and 9.0.

The measurements of pH were done in triplicate and average values were calculated. The procedure was repeated 3 times for each formulation [13].

Viscosity: The viscosity of the formulations was determined by using a viscometer (Brookfield viscometer, Brookfield Corp., Canada). For this study, spindle number 64 was used at 3 RPM where spindle factor 2000 was used for the calculation (where Dial reading X Factor=Viscosity in centipoise, mPa.s).

Hydrogels were taken in a beaker and the spindle was dipped in about 2 min and then reading was taken. The procedure was repeated 3 times for each formulation [14].

Spreadability: To determine spreadability, 2 g of each gel formulation was placed within a circle of 1 cm diameter pre-marked on a glass slide, over which a second glass slide was placed for 5 min to expel air and distribute uniformly between two slides. The excess gel was scrapped off from the edges. The top slide was subjected to a pull of 3 g with the help of string attached to the hook and the time (second) required by the top slide to cover a distance (cm) was noted [15]. The procedure was repeated 3 times for each formulation.

Spreadability was calculated using the following formula:

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

Where, S = Spreadability, M = Weight in the pan (tied with the upper slide), L= Length moved by the glass slide, T = Time in seconds needed to separate the top slide from the bottom slide.

Homogeneity: The formulations were tested for homogeneity by visual inspection after gel had been filled in the container. They were tested for their appearance and presence of any aggregates [16].

Phase Separation Study: Centrifugation method was used to induce phase separation in hydrogels by separating components based on their density. The higher density components may migrate towards the bottom, creating distinct phases. Each sample was centrifuged for 30 minutes at 3000 RPM. Centrifuged formulations were observed visually to look for any visible separation into distinct layers or phases [7].

Swelling Test: Hydrogels are polymeric materials which exhibit the ability of swelling in water and retaining a significant fraction of water within their structure without dissolving in water. Tea bag method was used to measure the swelling index. Each formulation was lyophilized at -60ºC until completely dried. Then it was powdered. The powdered sample was transferred to a petri dish and kept in hot air oven for 5 days at 120ºC to remove moisture completely. 1.2 g of the powdered hydrogel was taken. The empty tea bag was measured. The hydrogel powder was placed in an empty tea bag and the bag was dipped in an excessive amount of water. Then the tea bag was placed on a dry tissue paper and gently wiped with another dry tissue paper to remove excess water and weakly bound water. Then the tea bag was weighed [17].

$$\text{Swelling Index} = (W_2 - W_1) / W_1 \times 100$$

Where, W_2= Weight of hydrogel after swelling, W_1= Weight of pre-dried gel before immersion.

Washability: Small amount of each formulation was applied on proper clear skin and washed with water to check ease of wash ability [18].

Extrudability: To determine extrudability, a closed collapsible tube was taken and 5 gm of prepared gel was added pressing thoroughly and folding the crimped end to restrain its rolling back. When the cap was removed, formulation extruded until the pressure dissipated. The weight of the empty tube was recorded. The amount of extrudate gel was collected and weighed. The percentage of the extrudate gel was calculated (>90% extrudability: excellent, >80% extrudability: good, >70% extrudability: fair) [19].

Microscopic Study: Little amount of each formulation (pre-dried) was placed on slides, covered and examined under a Digital Binocular Microscope. Image analysis aided by software that allows for the studies such as uniform mixing, surface morphology of the formulation [8, 19].

Phytochemical Screening: Phytochemical screening was performed by following Mayer's test [20], Hager's test [21] Dragendorff test, Foam test[22], Salkowski test [23], Molisch's test, Benedict's test [24], Modified Brontrager's test, Keller Kiliani test,

Xanthoproteic test [24], Modified Brontrager's test and Keller Kiliani test [25].

Antioxidant Activity Study: The DPPH (1,1 diphenyl-2-picrylhydrazyl) assay is a method for evaluating the antioxidant activity of a substance. Specified weight of herbal hydrogel was dissolved in 25 ml of 99% methanol which was taken in a conical flask. The flask was sealed using aluminum foil and sonicated in a bath sonicator for 30 minutes. The sample was then centrifuged for 30 minutes at 5000 RPM. The sample was filtered using a whatman filter paper [20, 26]. The DPPH solution was prepared in methanol and added the reagent to the samples for the evaluation. Absorbance of samples were measured at 517nm by spectrophotometric method using methanol as blank. % Radical Scavenging Activity (%RSA) and 50% Inhibitory concentration (IC_{50}) values were calculated as the following formula.

%RSA= (Absorbance of Control-Absorbance of Sample) / (Absorbance of Control) × 100%

IC_{50} = (Concentration of Sample - (y - intercept) of %RSA standard curve) / (Slope of % RSA standard curve)

Antimicrobial Study: Hydrogels can exhibit antimicrobial properties due to the presence of bioactive compounds in the herbal extracts. Antimicrobial activity of optimum hydrogel was performed using Agar Well Diffusion Method against *Staphylococcus aureus* [27, 28]. Marketed formulation of Clindamycin Gel was purchased and was used as the positive control for this experiment.

Drug Excipients Interaction Study: Attenuated total reflectance infrared (ATR-IR) (Bruker Alpha II) spectroscopy was used as an effective analytical method for evaluation of interactions between drugs and excipients if any [29, 30].

Field emission scanning electron microscopy (FE-SEM): Field emission scanning electron microscopy (FE-SEM) (Zeiss, Sigma300) was used to study the surface morphology and microstructure of the prepared herbal hydrogel [31, 32].

X-ray Diffraction (XRD): Powder X-ray diffraction (XRD) (Bruker D8 Advance) was used for determination of the crystal structure of the herbal hydrogel.

High-Performance Thin-Layer Chromatography: High-Performance Thin-Layer Chromatography (HPTLC) is a chromatographic method for separating, identifying, and quantifying constituents in mixtures [33]. HPTLC (Camag, Linomat 5) was performed for qualification and quantification of the active constituents present in the herbal formulations (ISF Analytical Laboratory, ISF College of Pharmacy, Moga, Punjab, India). For the analysis, CAMAG Linomat 5 linked with winCATS software was used as sample application device. Silica gel 60, F254, 10 x 10 cm HPTLC plates were used for this experiment. Ethyl acetate: methanol: water (100:13.5:10) was used as mobile phase for the detection of *Aloe vera.* Chloroform: methanol (97:3) was used as mobile phase for the detection of *Turmeric* and hexane: acetone (8:2) was used as mobile phase for the detection of *Marigold* [34].

RESULTS AND DISCUSSION: After investigation, it was noticed that the formulation colour was remained constant at bright yellow. The smell was nice, subtle, and not overbearing. It has a smooth, non-sticky texture that was easier to apply to the skin. It was immediately absorbed by the skin and left no sticky or residue behind, giving way to a soothing, non-greasy, and cooling experience.

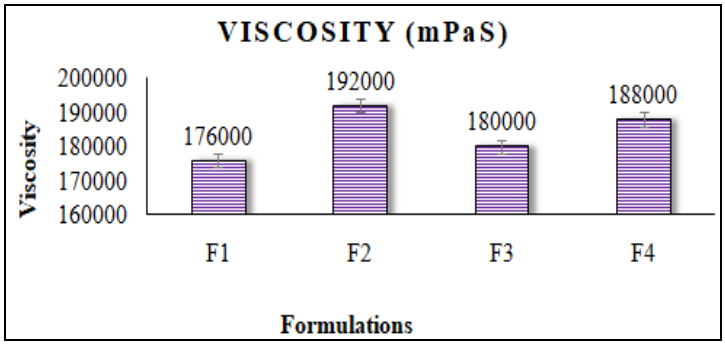

FIG. 1: VISCOSITY OF EACH FORMULATION (DATA GIVEN IN MEAN ±SD, N=3)

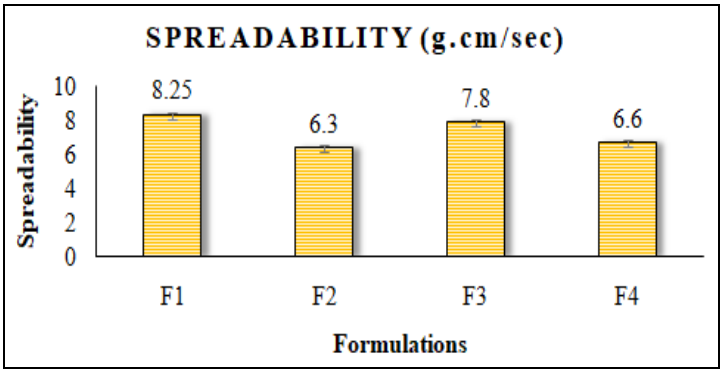

FIG. 2: SPREADABILITY OF EACH FORMULATION (DATA GIVEN IN MEAN ±SD, N=3)

After physico-chemical investigations herbal formulation was optimized in terms of pH, spreadability, viscosity, extrudability, swelling index, and homogeneity **Fig. 1-3**. Phase separation study ensures that there were no indications of sedimentation, phase separation, or any other unfavourable alterations. Each hydrogel formulation in this study was easily removed with water after application. The laboratory test revealed the presence of alkaloids, saponins, flavonoids, phenols, phytosterols, carbohydrates, glycosides, amino acids, and tannins in herbal crude drugs (*Turmeric*, *Aloe vera* and *Marigold*). No steroidal compounds were present.

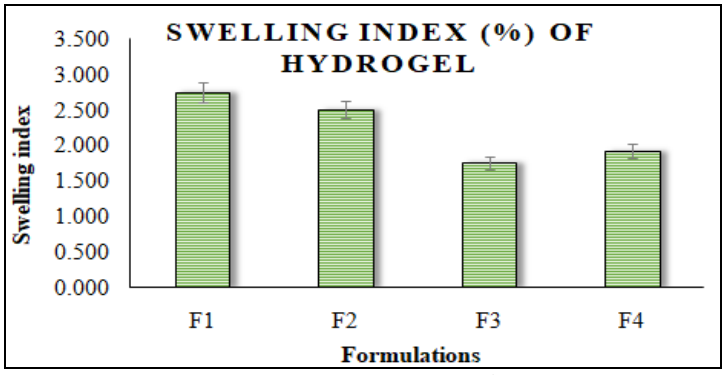

FIG. 3: SWELLING INDEX OF EACH FORMULATION (DATA GIVEN IN MEAN ±SD, N=3)

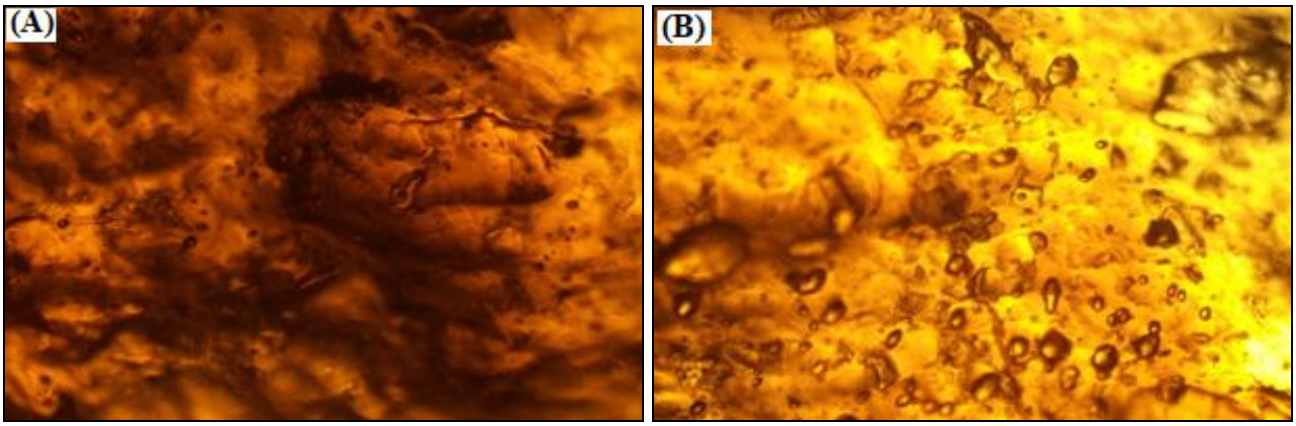

FIG. 4: MICROSCOPIC IMAGES OF HYDROGEL (DRIED) UNDER 100X MAGNIFICATION

Each hydrogel formulation (pre-dried) was examined under a Digital Binocular Microscope. Image analysis aided by software that showed uniform mixing of herbal drugs and excipients **Fig. 4**. 2^2 factorial design (two-factor two-level factorial design) was used for the formulation design optimization of the herbal hydrogel (Design Expert Software Trial version 13.0.5). Optimization of herbal hydrogel with respect to spreadability and viscosity as response variables were designed.

3D response curves were plotted against the CMC, carbopol 940 (independent variables). This response curve denoted that the amount of the

CMC and the amount of the carbopol 940 were directly proportional to the viscosity of the herbal hydrogel **Fig. 5**. Response surface methodology has been reported to be an effective tool for the optimization of a process when the independent variables have a combined effect on the desired response.

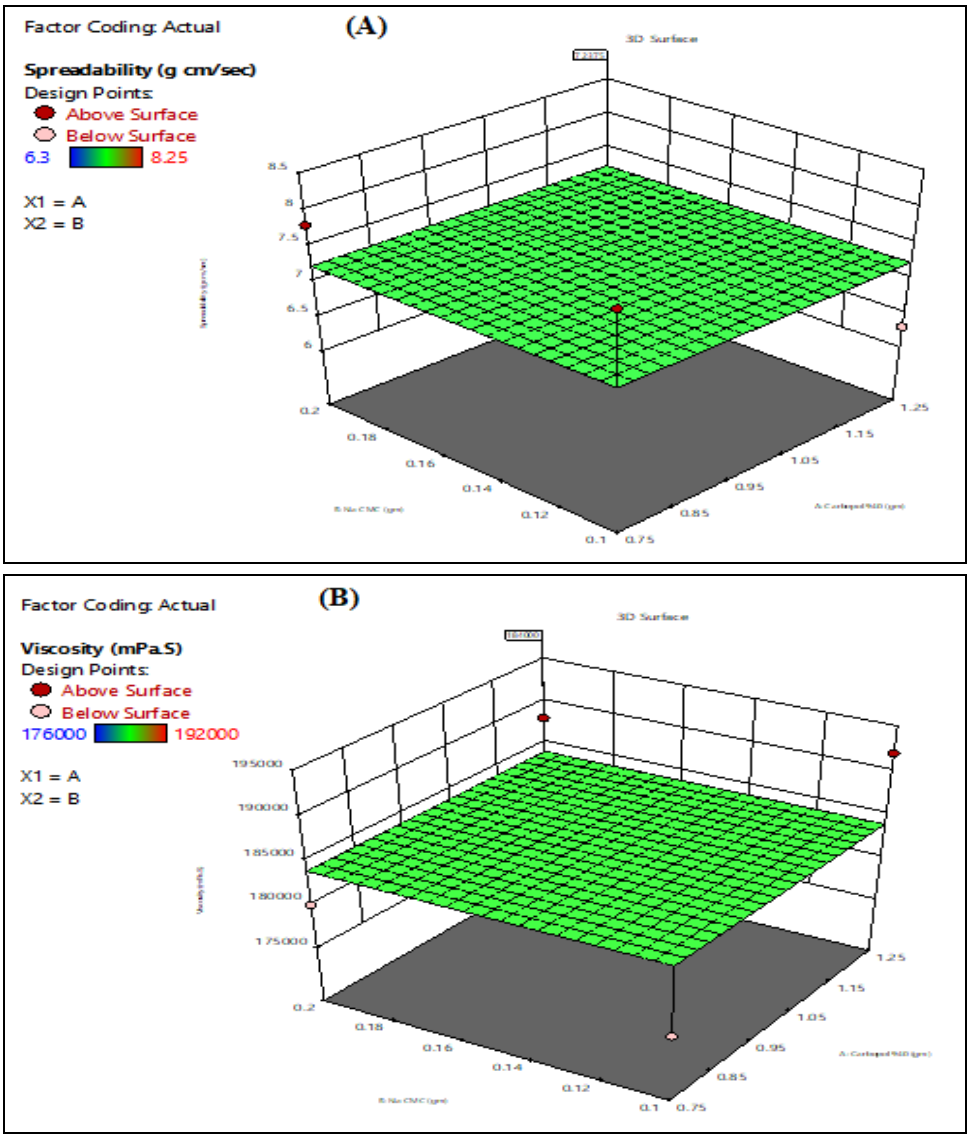

FIG. 5: 3D RESPONSE SURFACE PLOT FOR (A) SPREADABILITY AND (B) VISCOSITY

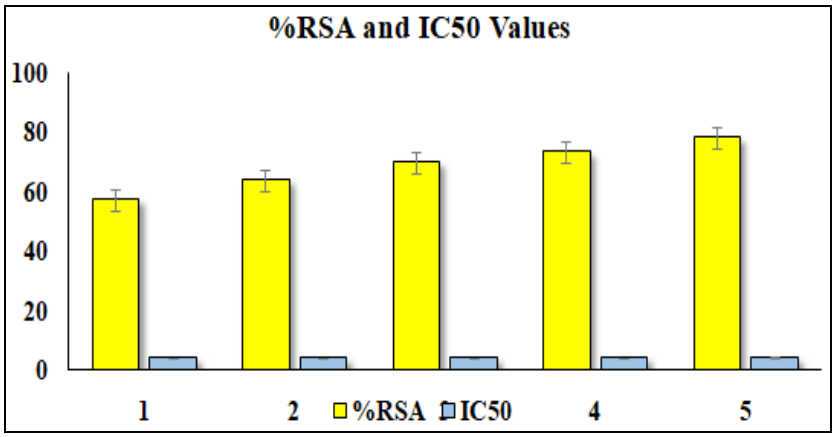

FIG. 6: %RADICAL SCAVENGING ACTIVITY AND IC50 VALUES OF HERBAL HYDROGEL (DATA GIVEN IN MEAN ±SD, N=3)

This study results showed that the formulations exhibited good radical scavenging activity using DPPH against the free radicals, reaching up to 78.02%. The phytoconstituents that exhibit antioxidant activity include, *Turmeric* contains curcumin (phenol), *Aloe vera* contains phenolic compounds such as flavonoids and polysaccharides. *Marigold* contains flavonoids such as quercetin and kaempferol derivatives, carotenoids such as lutein **Fig. 6**.

Antimicrobial Study:

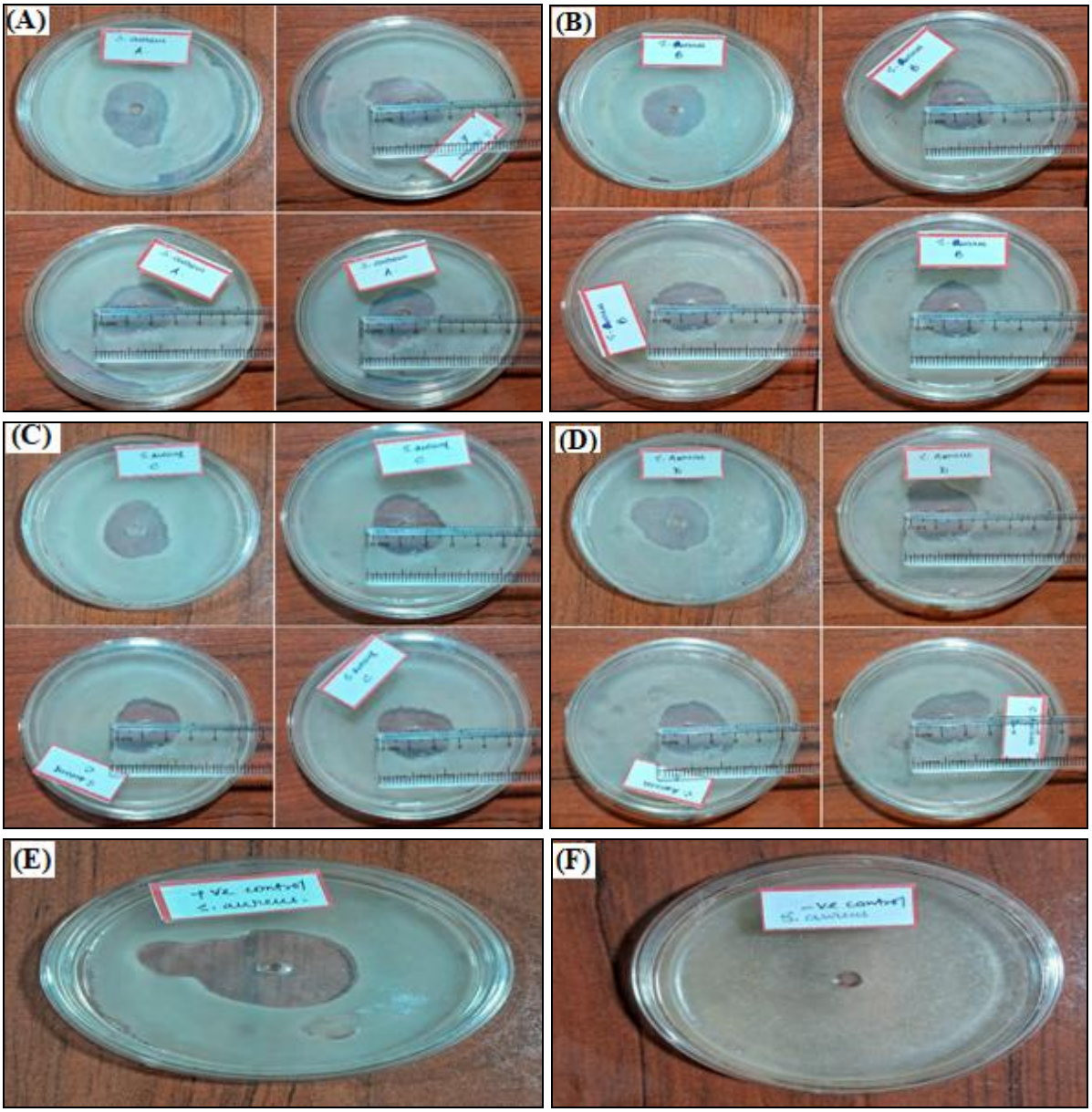

FIG. 7: ANTIMICROBIAL ACTIVITY (ZONE OF INHIBITION) OF HERBAL HYDROGEL AT VARIOUS DILUTIONS (A-D), (E) POSITIVE CONTROL (F) NEGATIVE CONTROL

TABLE 3: CALCULATION OF ZONE OF INHIBITION (ZOI)

Sample	Diameter (1) (cm)	Diameter (2) (cm)	Diameter (3) (cm)	Avg. Diameter (cm)	Avg. Radius (cm)	Zone of Inhibition (cm^2)
Negative control	0	0	0	0.00	0.00	0.00
Sample A	2.6	2.7	2.7	2.67	1.33	5.58
Sample B	2.8	2.8	2.6	2.73	1.37	5.87
Sample C	2.8	2.8	3	2.87	1.43	6.45
Sample D	2.9	3.2	3.3	3.13	1.57	7.71
Positive control	3.5	3.4	3.3	3.40	1.70	9.08

Antimicrobial activity of formulated hydrogel was performed against *Staphylococcus aureus.* Hydrogel showed zone of inhibition (ZOI) of 7.71 cm^2 which was compared with ZOI exhibited by positive control **Table 3 & Fig. 7**. *Turmeric* exhibits antimicrobial activity primarily due to presence of curcumin. Additionally, turmerone, a compound found in the turmeric oil, also contributes to its antimicrobial property. *Aloe vera* contains various phytoconstituents that exhibit antimicrobial activity including anthraquinone such as aloin and emodin, polysaccharides, flavonoids, saponin and phenolic compound including catechins. *Marigold* contains phytoconstituents which shows antimicrobial activity includes flavonoids such as quercetin and kaempferol derivatives, carotenoids particularly lutein and zeaxanthin and phenols.

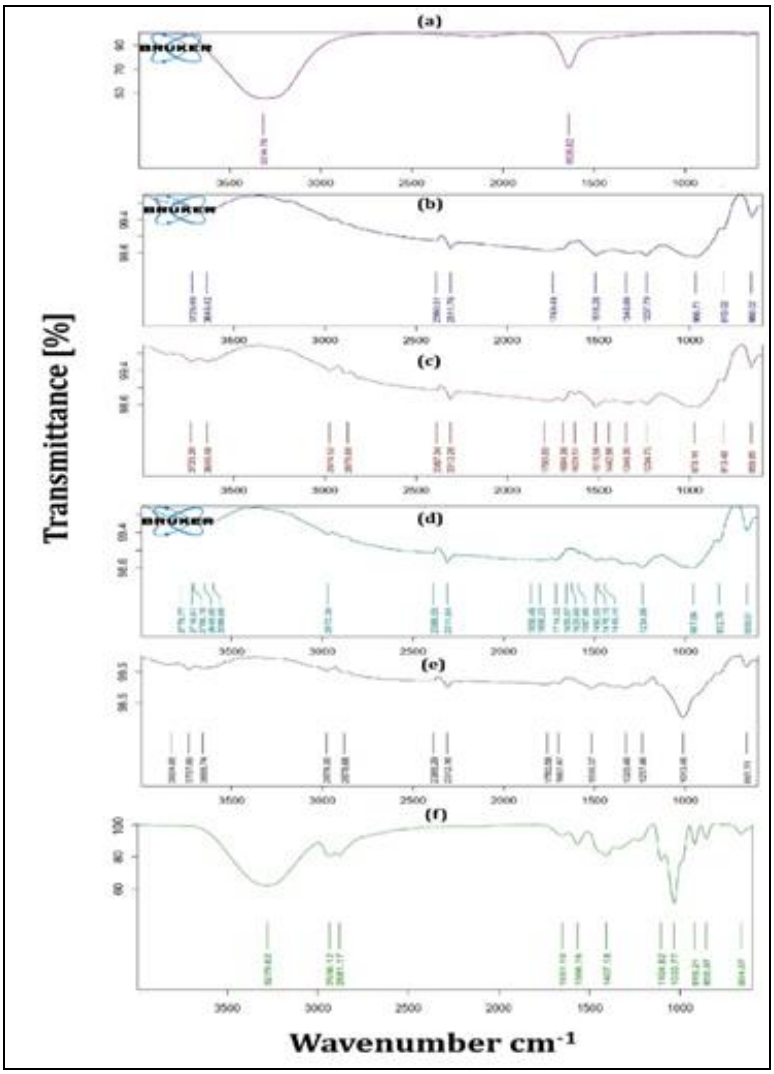

FIG. 8: ATR-IR SPECTRA OF (A) *ALOE VERA* GEL, (B)*MARIGOLD* LEAF EXTRACT, (C) *TURMERIC* EHIZOME EXTRACT, (D) CARBOPOL 940 (E) SODIUM CMC (F) HERBAL HYDROGEL

In the ATR-IR analysis of the samples, the characteristic functional groups were shown. In the spectra for *Aloe vera* gel, O-H hydroxyl stretching and N-H amino bending were observed. In the spectra of *Marigold*, O-H (alcohol) stretching, C-H (alkane) weak stretching, C=O (α, β- unsaturated aldehydes, ketones) stretching, C-O (carboxylic acid) stretching, C=C alkane (bending), aromatic C-H Bend in them. In the spectra for *Trmeric*, O-H alcohol stretching, C-H (alkane) stretching, C=O (acid halide) stretching, C=C (cyclic alkene) stretching, C-O (alkyl aryl ether) stretching and C=C bending were observed. The spectra of carbopol 940 and sodium CMC were recorded. Finally, the spectra of prepared herbal hydrogel was also recorded. After performing the ATR study of *Aloe vera* gel, *Turmeric*, *Marigold*, the excipients and formulation; the study report

revealed that there were no major changes in the stretching or bending of major functional groups of the herbal drugs with all excipients. So, incompatibility between herbal drugs and excipients was not observed **Fig. 8**.

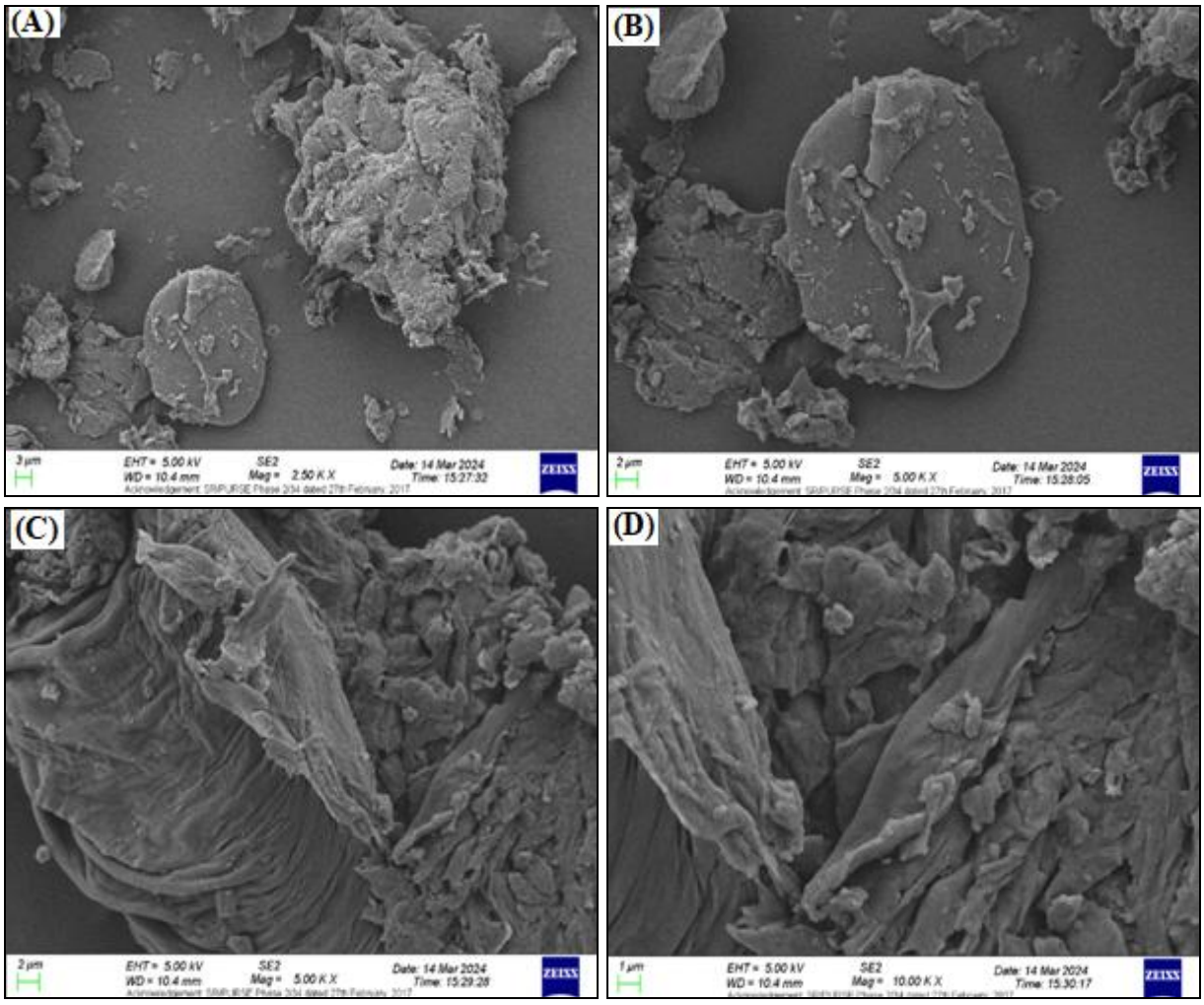

FIG. 9: FIELD EMISSION-SCANNING ELECTRON MICROSCOPY (FE-SEM) IMAGE OF HERBAL HYDROGEL

In the image of scanning electron microscopy (FE-SEM) crude drug particles were observed throughout the herbal hydrogel. Surface morphology of the formulation also revealed that the formulation could be porous **Fig. 9**.

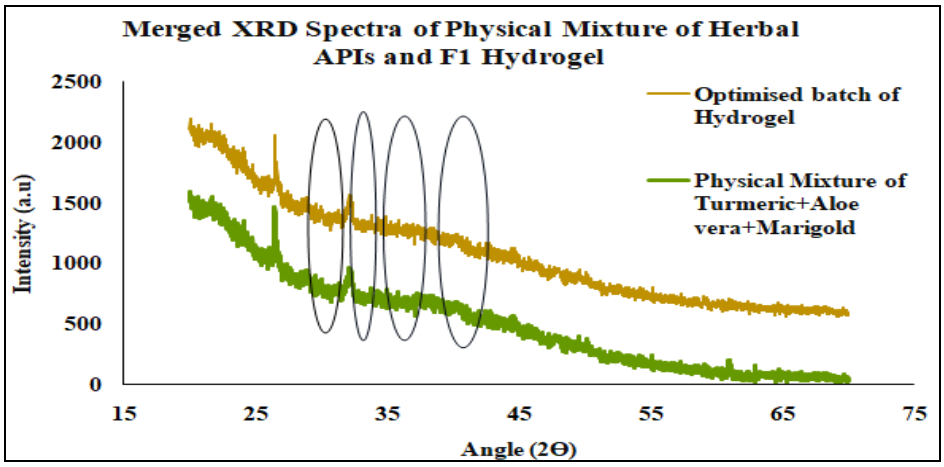

FIG. 10: X-RAY DIFFRACTION (XRD) SPECTRA OF THE MIXTURE OF HERBAL DRUGS AND HERBAL HYDROGEL

TABLE 4: AVERAGE PARTICLE SIZE FROM XRD SPECTRA OF HYDROGEL

2Θ	Θ	Θ (in radians)	CosΘ	β (FWHM in Degree)	β (FWHM in Radian)	βcosΘ	βcosΘ /λ	kλ	D=kλ/βcosΘ	Avg D
26.477	13.2385	0.231055413	0.973425	0.4886	0.008527679	0.008301	0.053882	0.138654	16.7031726	16.66026658
28.847	14.4235	0.251737565	0.968481	0.5391	0.00940907	0.009113	0.059149	0.138654	15.21579201	
32.218	16.109	0.281155089	0.960736	0.4468	0.007798131	0.007492	0.04863	0.138654	18.50708397	
36.979	18.4895	0.322702652	0.948382	0.5166	0.009016371	0.008551	0.055504	0.138654	16.21501773	

From X-ray diffraction (XRD) analysis, it was observed that **t**he two different spectra does not have distinct sharp peaks, which revealed that the powders are amorphous in nature **Table 4 & Fig. 10.** From the overlayed spectra of the physical mixture of herbal drugs and herbal hydrogel formulation, the average particle size was determined using Scherrer Equation. Average particle size of particles was found to be 16.660 nm.

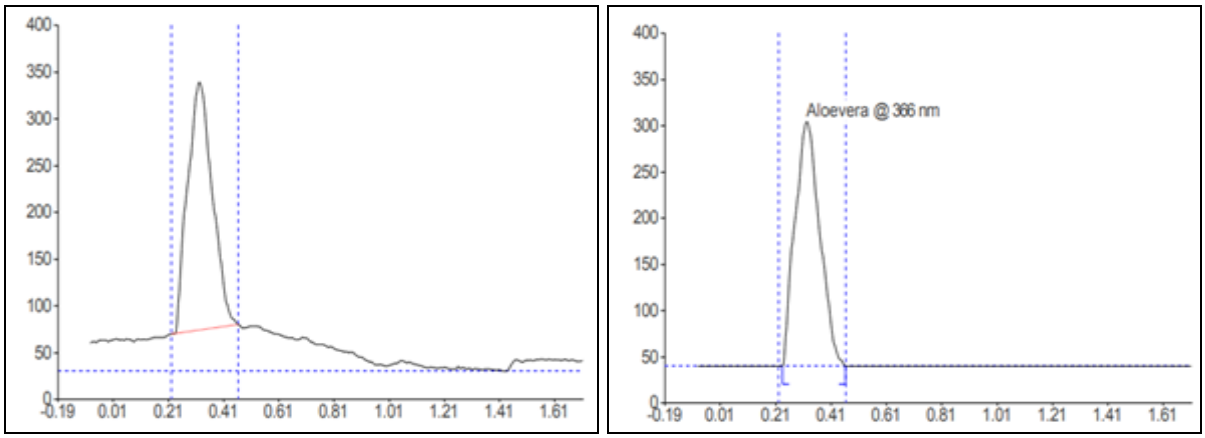

FIG. 11: PEAK FOR RETENTION FACTOR AND AREA UNDER CURVE OF *ALOE VERA*

TABLE 5: RETENTION FACTOR AND AREA UNDER CURVE FOR EACH PEAK OF *ALOE VERA*

Track	Peak	Vial	Start Rf	Start height	Max. Rf	Max. height	End Rf	End height	Area	Assigned substance
1	1	1	0.21	0.7	0.33	307.39	0.42	2.2	5311.02	Aloe vera
2	1	1	0.28	0.5	0.30	321.66	0.50	0.9	5432.04	Aloe vera
3	1	1	0.22	0.3	0.31	346.54	0.41	0.6	5240.02	Aloe vera
4	1	1	0.27	0.8	0.34	323.13	0.55	1.3	5348.06	Aloe vera
5	1	1	0.25	0.4	0.33	330.37	0.45	0.7	5210.29	Aloe vera
6	1	2	0.22	0.1	0.32	316.78	0.43	2.4	6184.34	Aloe vera
7	1	2	0.24	0.3	0.31	356.93	0.40	2.7	4119.76	Aloe vera

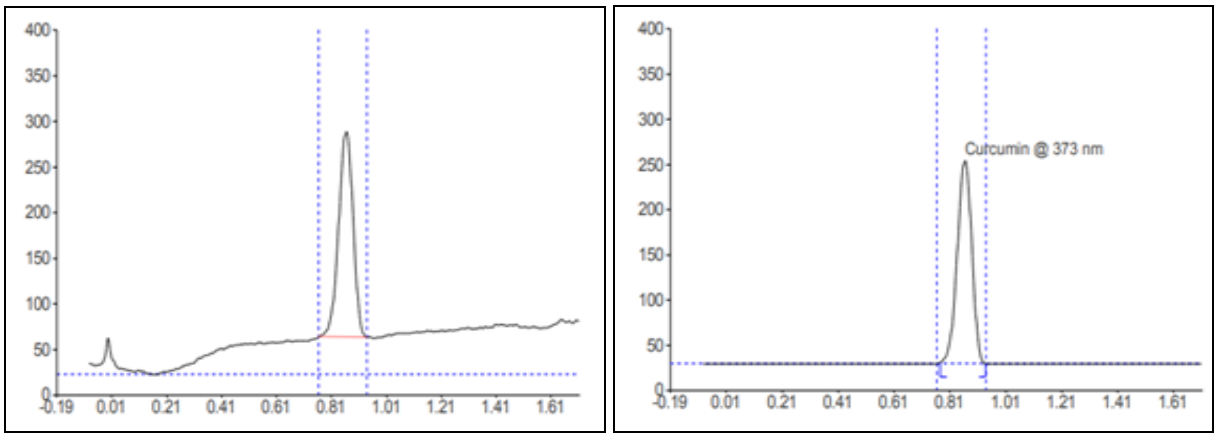

FIG. 12: PEAK FOR RETENTION FACTOR AND AREA UNDER CURVE OF *TURMERIC*

TABLE 6: RETENTION FACTOR AND AREA UNDER CURVE FOR EACH PEAK OF *TURMERIC*

Track	Peak	Vial	Start Rf	Start height	Max. Rf	Max. height	End Rf	End height	Area	Assigned substance
1	1	1	0.78	1.6	0.87	325.2	0.94	0.0	8100.3	Turmeric
2	1	1	0.77	3.1	0.84	431.4	0.93	0.0	8557.9	Turmeric
3	1	1	0.77	4.4	0.83	463.2	0.91	0.0	8786.9	Turmeric
4	1	1	0.77	7.9	0.83	399.8	0.90	0.2	8235.3	Turmeric
5	1	2	0.77	7.2	0.85	388.8	0.93	7.8	8120.6	Turmeric
6	1	2	0.77	9.3	0.86	253.4	0.91	0.5	8050.0	Turmeric

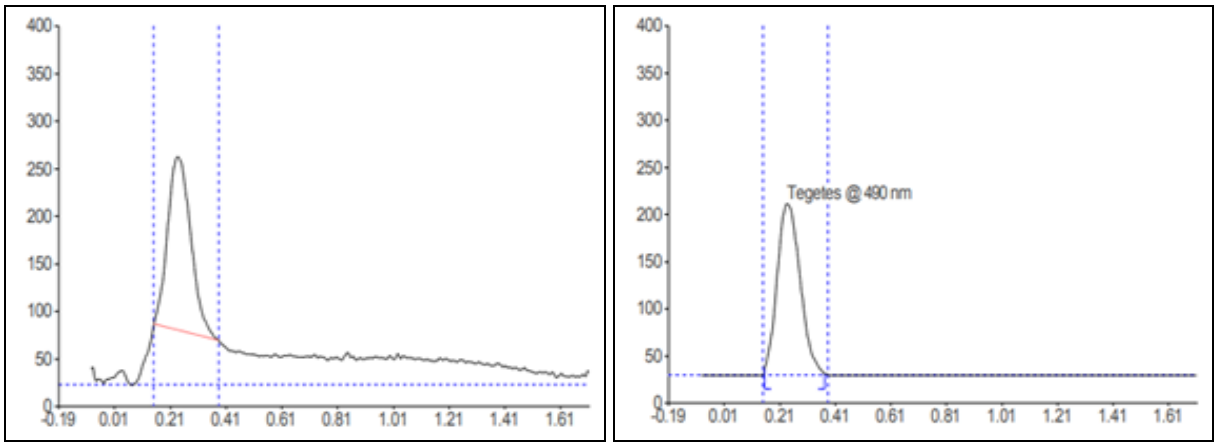

FIG. 13: PEAK FOR RETENTION FACTOR AND AREA UNDER CURVE OF *MARIGOLD*

TABLE 7: RETENTION FACTOR AND AREA UNDER CURVE FOR EACH PEAK OF *MARIGOLD*

Track	Peak	Vial	Start Rf	Start height	Max. Rf	Max. height	End Rf	End height	Area	Assigned substance
1	1	1	0.15	0.9	0.27	179.8	0.38	0.6	50942.2	Marigold
2	1	1	0.16	6.9	0.26	202.5	0.37	1.8	51009.2	Marigold
3	1	1	0.16	12.9	0.25	257.2	0.38	0.5	51058.5	Marigold
4	1	1	0.16	2.3	0.26	315.9	0.37	6.9	51158.5	Marigold
5	1	2	0.15	1.2	0.27	320.4	0.38	2.5	69265.0	Marigold
6	1	2	0.16	3.0	0.26	361.2	0.38	0.5	63691.3	Marigold

For the qualitative analysis, in the chromatogram for the High-Performance Thin-Layer Chromatography (HPTLC) of *Aloe vera*, the range of retention factor (Rf) spanned between start Rf 0.21 to end Rf 0.55 for the two extremes **Table 5 & Fig. 11**. This indicates the diversity of compounds present within the sample. Similarly, for herbal hydrogel, the start Rf and end Rf were recorded as 0.22 and 0.43 respectively, which revealed comparable range of compounds. The Rf value 0.21 corresponds to the flavonoids. The range between 0.28-0.34 Rf marks the presence of various polar compounds such as phenolic acid and polysaccharides. Additionally 0.36-0.43 corresponds to the presence of alkaloids, while 0.45-0.55 Rf indicates the presence of anthraquinones, specifically aloin. Similarly, in the chromatogram for T*urmeric*, the range of retention factor extended from start Rf 0.77 to end Rf 0.94 **Table 6 & Fig. 12**. The starting Rf 0.77 refers to the presence of curcumin. Rf 0.80-0.85 corresponds to other curcuminoids such as demethoxycurcumin. 0.87 Rf confirms the presence of turmerones. 0.90-0.94 possibly related to certain polysaccharides and other minor compounds such as proteins and amino acids. Consequently, for the presence of *Marigold*, the start and end Rf were recorded as 0.15 and 0.38 respectively **Table 7 & Fig. 13**. The Rf 0.15-0.21 corresponds to flavonoids and glycosides. Rf 0.28 refers to polyphenols, 0.32-0.35 refers to terpenoids and carotenoids. 0.36-0.38 possibly related to polyacetylenes and additional terpenoids [33].

In this research work, we embarked on the formulation, optimization, and characterization of a topical herbal hydrogel. Through careful selection of herbal extracts, polymers and other excipients, we aimed to develop a hydrogel that could effectively deliver active herbal compounds to the skin. During the optimization process, independent

variables such as the amount of carbopol 940 and CMC were systematically adjusted to achieve the desired rheological properties and stability. Through this process, we identified an optimal formulation (F1) that exhibited ideal pH, viscosity, spreadability, extrudability, homogeneity which ensure ease of application and uniform distribution of active ingredients. The integration of herbal extracts into the gel matrix was confirmed by ATR-IR spectroscopy, which also showed the existence of distinctive functional groups. Crystalline characteristics and microstructure were revealed by powder X-ray diffraction (XRD) and Scanning electron microscopy (SEM) respectively.

Phytochemical screening confirms the active bio constituents present in the formulation. The presence and quantity of certain phytochemicals in the hydrogel formulation were confirmed by HPTLC analysis, which made it easier to identify and quantify them within the herbal extracts. The qualitative HPTLC confirmed that herbal ingredients were sufficiently incorporated in the formulation. The antimicrobial study showed considerable effectiveness with zone of inhibition of 1.57 cm against *Staphylococcus aureus* [30, 34].

CONCLUSION: The comprehensive characterization of the topical herbal hydrogel underscores its suitability for dermatological applications. Its favourable physical properties, structural integrity, chemical composition, and biological activities collectively support its potential as a promising formulation for skincare or wound healing purposes. However, further research work through *in-vivo* studies is warranted to confirm its safety, efficacy, and clinical relevance, thus facilitating its translation from laboratory research to practical therapeutic use.

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