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Design and Evaluation of Anti-Microbial Hydrogel of ClitoriaTernatea Leaves

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ABSTRACT
The current study was performed to formulate a hydrogel of Clitoria Ternate leavesusing different polymers such as Carbapol 934, Carbapol 940 and HPMC**.** Various parameters were performed to evaluate the stability, spreadability, viscosity, drug content and in vitro drug release of the drug in the controlled environment. The results showed that these critical parameters were definitely pronounced the positive approach in the making of the herbal hydrogel. The viscosity and spreadability of the Hydrogel was excellent, the drug content and in vitro release was appeared excellent for all the formulations. The focus of the present study was to develop the herbal hydrogel of ClitoriaTernateaLeaves.

I. INTRODUCTION

The microbe of people' bodies may be influenced by the diversity of microbe found in their built surroundings. Humans who spend a lot of time outside or who live in homes with more exposure to open space has a lot of unfiltered or mildly filtered airflow with the surroundings have more diversified microbe than those who live in homes with more closed designs[1].

However, it is not clear to what extent the indoor microbe contributes to this diversity or lack of it.In addition, research suggests that exposure to reduced microbial (particularly bacterial) diversity may not be primarily a result of the microbial content of buildings, but rather a result of our modern diet, which is less diverse than that of our ancestors[2]. It varies little with the seasons; may be affected by antibiotic use; and may select for a limited number of human microbial species, particular in the gut[3]. There may be a benefit to human health as microbe may be important for immune development and the processing of nutrients in the gut, which may not work as well when gut microbial communities change[3]. Those in economically disadvantaged and less developed societies who spend more time outdoors may be at higher risk for infectious diseases and infant mortality[4].

Hydrophilic gels, also known as hydrogels, are polymer chain networks that are occasionally found as colloidal gels and have water as the dispersion medium[5].

Gels have been characterized in a variety of ways by scientists throughout the years. The most prevalent is hydrogel, which is a water-swollen, cross-linked polymeric network made by a chemical interaction of one or more monomers[6]. Another description is a polymeric substance that can expand and hold a substantial amount of water inside its structure but will not soluble in water. Due to their remarkable promise in a wide variety of applications, hydrogels have gotten a lot of attention in the last 50 years because of their high water content; they have a degree of elasticity that is extremely close to real tissue[7].

The hydrophilic functional groups connected to the polymeric backbone provide hydrogels their capacity to absorb water; while cross-links connecting network chains give them resistance to disintegration[8]. Hydrogels are made up of a variety of components, both natural and manmade. Natural hydrogels have gradually been superseded by synthetic hydrogels, which have a longer service life, a higher water absorption capacity, and higher gel strength, over the previous two decades. Synthetic polymers, on the other hand, generally have well-defined patterns that may be tweaked to provide custom degradability and usefulness. Hydrogels can be made entirely of synthetic materials. It is also stable in the presence of both sharp and significant temperature variations[9].

II. MATERIALS AND METHODS

2.1. Collection of the Leaves [10]

The leaves of the ClitoriaTernatea were collected nearby nursery of ACME Research Solutions laboratory, Delhi. Fresh leaves of ClitoriaTernatea were collected and stored. Then leaves were properly washed to remove the dust debris and foreign particles. The leaves of the ClitoriaTernatea were dried for at least 30 days under the sun in the closed container. The leaves

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were crushed and hammered in the small pieces so that it could easily grinded. The grinded part was again passed through the sieve. The remaining material was collected in the air tight container. The leaves powder were again undergone through the certain parameters i.e. color, odor and physical part of the leaves,

2.2. Extraction[11]

The extraction of the leaves was done after making its powder. The powder was filled in the upper part of the Soxhlet apparatus. The powder weighed 250g and extractors were taken as Methanol, ethanol and water. The each extraction process with the specific extractor was done for the specific time and temperature. The extract was air dried at room temperature and stored. The stored extract was subjected to undergone parameters such as %yield, phytochemical screening and extractive values to select the final extractor. These values were determined and mentioned in the table-1&2.

2.3. Chemicals and Instruments[12]

Carbapol 934, Carbapol 940, HPMC, Triethanolamine, Ethanol, Methanol, Methyl Paraben, Buffer Solutions etc. were obtained from the Loba chemicals, BRM chemicals, and CDH chemicals. The entire chemicals that were used in the experiment were the extra pure and laboratory grade. The equipment were involved in the study were Viscometer, Magnetic stirrer, UV spectroscopy, electronic balance, pH meter, Franz diffusion apparatus and pipette.

2.4. Phytochemicals Screening[13]

The phytochemical screening of the extract was done to evaluate and to find out the available phytochemical constituents in the leaves of the plant. Tests were performed to observe the presence of the Alkaloids, saponins, flavonoids, and steroids etc mentioned in the table. The phytochemical screening procedure was followed as described by the Nathan Harris et al, (2005) and SampatNavale et al, (2018)Table-3.

2.5. Formulation of the Hydrogel[14]

The Carbapol934, 940 and HPMC were used as they have the good dispersion rate and forms gel very quickly. All three gelling agents were dissolved in 50mL of water and stirrer continuously until it gets dissolved at the 40°C. The 1%w/w drug was dissolved in the water and added gently to the gel. The combination of the methyl paraben and propyl paraben were dissolved in the water and mixed thoroughly at the hot temperature and mixed in the master solution. The glycerin was added as the quantity sufficient. The master formulations were kept stirring at the constant temperature 30°C and RPM of 700. The mixing procedure was kept going on until the clear and homogenous gel phase achieved. The final volume was made up to 100mL. At the end, the Triethanolamine was added to the final mixture to adjust the pH in accordance of the skin pH (6.8–7). Each formulation was prepared in triplicates. The formulae are mentioned in the Table-4.

2.6. Characterization of the Hydrogel

The characterizations of the hydrogel were assessed using the following parameters:

2.7. Physical Evaluation[15]

Physical parameters of hydrogel were assessed by inspecting it visually i.e. color and physical appearance (Table-5).

2.8. pH determination[16]

The determination of the formulated Hydrogel was evaluated by using the pH meter. Small quantity of the hydrogel was taken in the volumetric flask and dissolved in the 100mL of the water and mixed it properly. Each formulation was mixed with the water and pH was determined by taking the at least three readings for the average pH (table-6&7).

2.9. Viscosity[17]

Viscosity of hydrogel was evaluated by the viscometer at different RPM (1&2) by using spindle no. 52 and the results were taken in the triplicates. To perform the viscosity the gel was taken in the beaker and temperature was maintained. The spindle was cleaned using 70% alcohol and balancing bubble was set in middle by moving the apparatus. The spindle was moving until a constant reading obtained. During this process reading was take three times to obtain an average value (table-8).

2.10. Centrifuge Test[18]

The centrifuge test was done to evaluate the phase separation of the Hydrogel. The gel was filled in the test tube (15mL) and fixed in the rotator. The RPM was set at 6000 for 30 min. After the set time over, the sample were taken out and observed for the phase separation (table-9).

2.11. Spreadability[19]

Spreadability was measured using the two glass watch glasses. The 500mg of the Hydrogel was taken in the watch glass and left stable. The other watch glass was dropped on the hydrogel at height of 5cm and measured the spreadability. The spreadability was measured by the force applied and how much spread has happened over the glass. It is measured in cm/sec (table-10).

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2.12. Drug content[20]

To obtain the drug content the formulation (1g) was dissolved in the 20mL of the phosphate buffered saline for minimum 30 min. The resultant mixture was then filtered through a Whatman filter paper. The filtrate was again diluted with the 10mL of buffer; absorbance of the mixture was taken at 215nm buy the UV-VIS spectrophotometer. The record values were taken in the triplicates, average values was determined and noted in table-11.

2.13. In vitro drug release[21]

In vitro drug release studies were carried out with Franz diffusion cell apparatus. The herbal hydrogel was taken in the small amount and kept on the membrane which is fixed with receiver. Each of the receivers was filled with the 7.4pH phosphate buffer and fixed with the magnetic stirrer to make it warm at the physiological temperature. The drug then comes in the contact of the buffer and getting dissolved in the buffer. The 1 mL of the buffer taken and took the absorbance for the same at 215nm by using UV-VIS spectrophotometer for 8h. Every hour the drug was introduced in the buffer and same amount of the buffer was replaced by the new one. The in vitro drug release studies done for observe the concentration of the drug releases through the membrane. The absorbance of the drug was taken in triplicates and took the average; results are mentioned in table-12.

III. RESULTS AND DISCUSSION

The herbal hydrogel of ClitoriaTernatea was prepared using the three different polymers Carbapol 940, 934 and HPMC. The leaves of the ClitoriaTernatea was collected and stored at the normal temperature to get dry and after drying stored in the air tight container so that the air interaction with the hydrogel would be less. The extracts were prepared using the Soxhlet apparatus. Three kinds of solvents were used in the study methanol, ethanol and water. These solvents were selected in accordance of their polarity nature and solubility of the crude drug. The crude dug then was subjected to phytochemical screening (table-3). The extractive values were also evaluated to know the stability and strength of the crude drug. The extractability values were came in the limit. Hydrogel were prepared using the formulae prepared initially to make ratio of the drug content, polymers, and preservatives. The formulae of the hydrogel were based on the standard refrences (table-4). After preparing the herbal hydrogel the characterization was done by using the different parameters such as physical examination, pH determination, viscosity, drug content and in vitro drug release. The formulation was gone through the organoleptic properties inspecting by visualization of the hydrogel. The properties of the appearance like physical appearance (smoothness and clarity) and color of hydrogel was inspected. The Formulation was found smooth and color was brownish (table-5). The pH determination was done to identify whether drug formulation is suitable for skin or not, the results showed all the prepared formulation were near at the skin pH (table-). Rheological study was evaluated to identify the viscosity of the hydrogel, the viscosity was found be in the range. All the formulations were significantly close to each other (table-). The drug content was identified by using the UV-VIS spectrophotometer, the drug content appeared to be above for all the formulations. The formulation 1 was significant higher among all the formulations (table-11). In vitro drug release studies also revealed that the drug release from the formulation was excellent and was found significant in terms of progressive release of the drug at the different time intervals. The highest drug release was observed in the formulation 1 (table-12). Hence, it could be concluded that in accordance with obtained results the Hydrogel from the Clitoriaternatea could be formulated.

IV. CONCLUSION

The hydrogel of Clitoriaternatea was prepared using different polymers. Various parameters were performed to see the activity of the Hydrogel. The drug content and in vitro drug release study suggested that formulation made by using Carbapol 932 pronounced the stability and release of the drug at the defined time period.

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V. TABLES AND GRAPHS

Table-1: Extractive Values

Sn.	Extractive Values	Results
1	Water Soluble Extractive	2.10%
2	Alcohol Soluble Extractive	1.98%
3	Ash Value	1%
4	Acid Insoluble Ash	1%
5	Water soluble Ash	2%

Table-2: Percentage yield

Sn.	% Yield	Result
1	Ethanol	10.01%
2	Methanol	8.21%
3	Water	9.05%

Table-3: Phytochemical Screening

Sn.	Test	Solvents		
		Methanol	Ethanol	Water
1	Alkaloids	+	+	+++
2	Carbohydrates	++	-	++
3	Saponins	+	+	++
4	Glycosides	+	-	-
5	Steroids	+	+	-
6	Phenolics	+	-	-
7	Flavonoids	+	-	-

Table-4: Formulae of Hydrogel

Sn.	Ingredients	Formulations		
		F1	F2	F3
1	Extract	1	1	1
2	Carbopol 934	1	NA	NA
3	Carbopol 940	NA	1	NA
4	HPMC	NA	NA	1
5	Propylene glycol	5	5	5
6	Methyl Parabene	0.03	0.03	0.03
7	Propyl Parabene	0.03	0.03	0.03
8	Water	qs	qs	Qs

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Table-5: Physical Evaluation of Hydrogel

Sn.	Formulations	Gel	Color	Physical Appearance
1	F1	1	Brownish	Smooth
2		2	Brownish	Smooth
3		3	Brownish	Smooth
4	F2	4	Brownish	Smooth
5		5	Brownish	Smooth
6		6	Brownish	Smooth
7	F3	7	Yellow-Brown	Smooth
8		8	Yellow-Brown	Smooth
9		9	Yellow-Brown	Smooth

Table-6: pH of Hydrogel (Individual data)

Sn.	Formulations	Triplicates	pH
1	F1	1	6.2
2		2	6.4
3		3	6.1
4	F2	4	6.2
5		5	6.1
6		6	6.3
7	F3	7	5.9
8		8	6.2
9		9	6.1

Table-7: pH of Hydrogel (Mean and SD)

Sn.	Formulations	Triplicates	pH
1	F1	1	6.23±0.15
2		2	
3		3	
4	F2	4	6.20±0.10
5		5	
6		6	
7	F3	7	6.07±0.15
8		8	
9		9	

Table-8: Viscosity (Mean and SD)

Sn.	Formulations	Triplicates	Viscosity (cps)	
			1 RPM	2 RPM
1	F1	1	8926.33±31.56	7448.67±49.90
2		2		

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3		3		
4	F2	4	8666.00±98.01	7443.67±47.82
5		5		
6		6		
7	F3	7	8701.33±2.08	7445.00±38.16
8		8		
9		9		

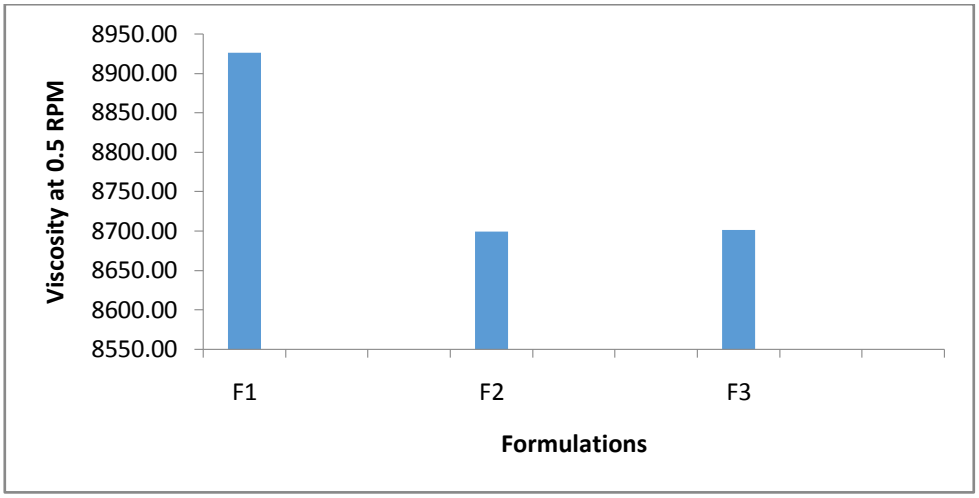

Fig.1: Viscosity at 1RPM

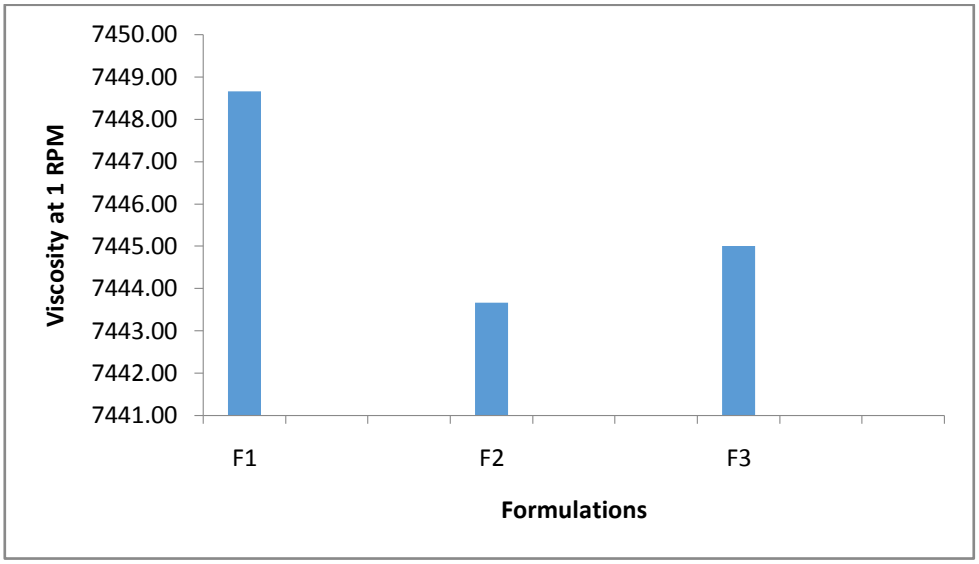

Fig.2: Viscosity at 2RPM

Table-9: Centrifuge Test

Sn.	Formulations	Gel	Centrifuge	Physical Appearance
1	F1	1	5000	Not Separated
2		2	5000	Not Separated

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3		3	5000	Not Separated
4	F2	4	5000	Not Separated
5		5	5000	Not Separated
6		6	5000	Not Separated
7	F3	7	5000	Not Separated
8		8	5000	Not Separated
9		9	5000	Not Separated

Table-10: Spreadability (Mean and SD)

Sn.	Formulations	Triplicates	Spreadability
1	F1	1	35.20±1.01
2		2	
3		3	
4	F2	4	32.49±1.37
5		5	
6		6	
7	F3	7	35.07±0.06
8		8	
9		9	

Table-11:Drug Content

Sn.	Formulations	Triplicates	Drug Content at 248nm
1	F1	1	94.47±0.15
2		2	
3		3	
4	F2	4	94.27±0.21
5		5	
6		6	
7	F3	7	93.50±0.10
8		8	
9		9	

Table-12:In vitro Drug Release

Sn.	Time	% Drug Release		
		F1	F2	F3
1	0	0	0	0
2	1	9.90	9.55	11.89
3	2	29.76	29.41	26.90
4	3	38.90	38.55	29.93
5	4	59.47	59.12	49.78

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6	5	60.99	60.64	60.54
7	6	72.63	72.28	67.97
8	7	84.90	84.55	77.97
9	8	95.31	94.96	89.34

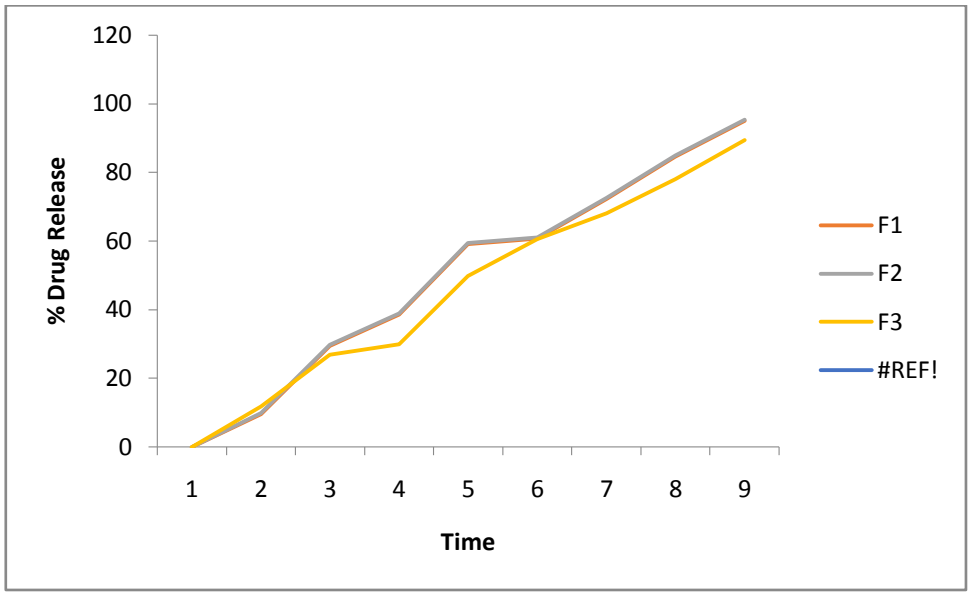

Fig.3: In vitro Drug Release

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