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RESEARCH ARTICLE

DESIGN AND ANALYSIS OF *CISSUS QUADRANGULARIS L.* BY DESIGN OF EXPERIMENTS AS A NOVEL HERBAL GEL

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Abstract

Cissus quadrangularis has a common Bengali name as 'HARJORA' has the property of BONE-HEALING. Using DESIGN OF EXPERIMENTS, it was formulated a novel Gel; with Propylene Glycol and Carbopol 940 as Independent-Variable; and Viscosity, Spreadibility Factor and Endurability as Dependable-Variable. Thus, a Factorial Design by DOE as 3 to the power 2, or 3^2; or 9 nine Formulations. An optimum formulation was obtained as F-1; from a series of F-1 to F-9. Out of these nine formulations tested for Quality Control; F-1 depicts the gem of nine. In case of a crack or fracture F-1 can be used to heal the bone by applying locally. In DOE 'factorial design', -1,0, & +1 Level for Propylene Glycol [PG] and Carbopol 940 were taken for experimentation purpose. With 1:1 EtOH:H_2O as a solvent, the green CQ Plant modified stem was dissolved 3 times of the macerant. After filtration and centrifugation; the dose was adjusted in 5ml of the extract. An elaborate study by varying the ingredients formulations were prepared. These formulations were undergone analysis by Macro-Physico established protocols. Viscosity, Spreadibility, Extrudability, Conductivity and pH homogeneity were among the parameters. In future, stability indicating protocols to be developed that will assure the shelf-life of this Harjora-Herbal-Gel.

Introduction:-

Herbal remedies have been used for centuries to treat a wide range of ailments, and in recent years, there has been a growing interest in natural therapies as a complement to modern medicine. *Cissus quadrangularis* is a plant that has been used in traditional medicine for its medicinal properties, particularly in treating bone fractures and joint pain [1]. Bone healing is a complex physiological process that involves the repair and regeneration of damaged bone tissue. When a bone is fractured, a series of events are initiated to stimulate the formation of new bone tissue, including inflammation, proliferation of new cells, and the deposition of mineralized bone matrix. The process of bone healing is regulated by various factors, including genetics, age, nutrition, and the presence of any underlying health conditions. There are several treatment options available to promote bone healing, including immobilization, physical therapy, and the use of pharmacological agents such as bisphosphonates and growth factors. The

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development of natural remedies, such as *Cissus quadrangularis* herbal gel, has also shown potential in promoting bone healing and reducing the time needed for recovery [2-8].

In recent years, there has been a growing interest in the use of *Cissus quadrangularis* as a topical gel for the treatment of musculoskeletal injuries. The aim of this research paper is to evaluate the efficacy of *Cissus quadrangularis* herbal gel in the treatment of musculoskeletal injuries. We will explore the chemical composition of the plant and its pharmacological properties, and review existing studies on the use of *Cissus quadrangularis* in the treatment of musculoskeletal injuries. We will also conduct a clinical trial to assess the effectiveness of the herbal gel in reducing pain and inflammation in patients with musculoskeletal injuries. This research has the potential to provide valuable insights into the use of natural therapies for the treatment of musculoskeletal injuries and to identify new options for patients seeking safe and effective alternatives to traditional medicine.

Cissus quadrangularis or the cissus succulent has habitat as a tropical perennial annual herb is reported since Ayurvedic era. *Cissus quadrangularis* plant is native to the regions of Asia and Africa where traditional herbal medicine is still practiced. It is used as a traditional herbal medicament for the treatment of bone fracturing or breakage and it is also used in the pain management. It orients itself above the soil as a twining, twisting pattern of modified stem to climb up as an herbal climber. Other than other reported pharmacological properties of the CQ plant, as diabetes controller, antimicrobial, antiulcer, anti-inflammatory, tonic to muscle, analgesic, antipyretic, gastro intestinal rejuvenator, female hormonal regulatory disorders; to say a few; CQ is a BONE-SETTER. To set a mechanically injured bone or its part, CQ-Gel is an authentic one to spread topically over the injured site [9-12].

Herbal gels are formulations that consist of plant-based extracts and other natural ingredients mixed with a base material such as water, glycerin, or aloe vera. They are designed to be applied topically to the skin, where the active ingredients can penetrate the epidermis and enter the bloodstream to provide therapeutic benefits. Herbal gels have gained popularity as an alternative to conventional medicines because they are perceived to be safer and more natural. They have been used to treat a wide range of conditions, including skin disorders, musculoskeletal injuries, and pain relief. *Cissus quadrangularis* herbal gel, for example, is a topical formulation that contains extracts from the *Cissus quadrangularis* plant. This plant has been traditionally used for its anti-inflammatory and analgesic properties and has been shown to have potential in promoting bone healing and reducing pain and inflammation in patients with musculoskeletal injuries. Herbal gels are generally considered safe and well-tolerated, but it is important to note that not all natural remedies are safe for everyone. Some people may be allergic to certain plant extracts or have other health conditions that could be affected by the use of herbal gels. It is always recommended to consult with a healthcare professional before using any natural remedies or alternative treatments [13-19].

The management of musculoskeletal injuries is a challenging task, and traditional treatments such as non-steroidal anti-inflammatory drugs (NSAIDs) and physical therapy may not always provide satisfactory relief. Natural remedies, on the other hand, have gained attention in recent years for their potential to alleviate symptoms without the side effects associated with conventional medicine. *Cissus quadrangularis* is a herb that has been traditionally used in Ayurvedic medicine for the treatment of bone fractures and joint pain. Recent research has shown that it may have anti-inflammatory and analgesic properties, making it a potential candidate for the management of musculoskeletal injuries. According to Indian Pharmacopeial Commission “Topical preparations should be evaluated for clarity, homogeneity, pH, suspendability (for lotions), consistency, viscosity, particle size distribution (for suspensions, when feasible), level of microbial contamination/sterility and weight loss (when appropriate)”. [20-25,42]

Materials & Methods:-

Materials:-

Propylene Glycol (Ravi Marketing; Ahmedabad), Carbopol-940 (Bo International Wazirpur, Delhi), Glycerin (Niram Chemicals, Vile Parle West, Mumbai), Triethanolamine (Sigma aldrich), Methyl Paraben (Unicorn Petroleum Industries Private Limited Chembur East, Mumbai), Menthol (K. K. Enterprise Udhna, Surat), Rose water (Local Market Purchase) are purchased from the parties mentioned within the parenthesis to formulate the gel.

Plant materials:
The stem part of the *CISSUS QUADRANGULARIS L.* has been collected from the Medicinal Garden of Bengal School of Technology, Sugandha, Hooghly, 712102 (Lat: 22.908716°; Long: 88.338777° on 26.10.2021) in the month of July. The herbarium of the plant has been submitted to Pharmacognosy department.

Extraction:
Modified-Stem of the climber CQ plant is taken and the dirt & debris are removed. Subsequently, longer pieces are cut, then chopped and dissolved in 300 % Water – Ethanol (50:50) as Macerating agent, for seven days; for the organized drug to leach optimally from the cell wall. Filtered in cloth. Centrifuged and send for Gel Formulation. In all the nine Formulation prototype base 5 ml of the CQ-Extract was added and stirred in one direction [3].

Table 1:- Pharmaceutical Aids and their activity to form the Gel.
To achieve at the Gel of the CQ – Plant, the following Inactive Ingredients were used:

Sl. No.	EXCIPIENTS	USED AS
1	Propylene Glycol	Moisturizer
2	Carbopol-940	Thickener
3	CQ – Extract	Crude Drug Extract
4	Glycerin	Plasticizer& Humectants
5	Triethanolamine	pH Modifier, Gel Forming Agent
6	Methyl Paraben	Preservative
7	Menthol	Cooling sensation
8	Rose water	Aromatiser

Macro-physico parameters' Analysis:
Organoleptic properties:
Organoleptic compounds, which include additives for color, flavor, sweetening, and texturing, play a significant role in pharmaceutical excipients and medicines. However, their physical and chemical properties must be assessed before use to prevent deterioration. In this experiment the gel formulation has been introduced for organoleptic evaluation[26-31].

Viscosity:
The viscosity, reverse to flow, or Fluidity; works as a Rotational Meter, with Spindle dipped into the 'Harjora Gel', measures its dynamic viscosity, with a constant speed; measured with a "Brookfield Viscometer". The Torque to counteract the flow resistance by the Gel to propagate the cure time for a gel is fixed with a 'Spindle Number' 64, at an RPM of 3. With the dial number 9.5 the viscosity in Centipoise is calculated as = **Dial Reading X Factor = 9.5 x 2000**;

Where, 2000 is the factor for this instrument [32-38].

Spreadibility:
Spreadibility was performed by using the standard procedure mentioned by Nurman et al. [36]. The gel was placed on glass-coated graph paper after being weighed up to 0.5 g. We then positioned a second glass on top of the gel pile. By measuring the length of multiple sides' diameters, the gel's diameter was estimated. The gel's diameter was then measured after 150 g of extra load had been introduced and 1 minute had passed. This test was performed on aIn - house Spreadibility Tester prepared by Mr. Hironmoy Malik.
Spreadability was calculated by using the formula,
S= M.L/T
Where, S = Spreadibility, M = Weight tied to upper slide, L = Length of glass slide; T = Time taken to separate the slides completely from each other.

Extrudability:
To determine the force necessary to extrude the material from the tube, this empirical test is typically performed. The technique used to determine the amount of applied shear in the area of the rheogram where the shear rate is greater than the yield value and plug flow is evident. As mentioned by Bhalke et al. Standard collapsible aluminium tubes with caps were filled with the gel compositions, and the ends were crimped shut to seal. The tubes' weights

were documented. The tubes were clamped after being positioned between two glass slides. The slides were covered with 500 g, and the cap was then taken off. The extruded gel's volume was gathered and weighed [39,40].

Effect of pH:
pH determination was performed according to Basha et al.[37]. Each gel formulation was weighed at 50 gm, transferred into a 10 ml beaker, and its pH was then determined using a digital pH meter. To treat skin infections, the pH of the topical gel formulation should be between 3 and 9. It has been experimentally found that the pH has varying effects on the barrier function in the epidermis.[38]

Homogeneity:
Homogeneity measurements were carried out on gel preparations that had been made before and after being given storage conditions. Homogeneity test is carried out by means of the gel preparation applied to a piece of glass or other suitable transparent material, then the homogeneity of the preparation is observed. [41]

Statistics:
Formulation method – development:
Following the DOE; a three square (3^2) – Factorial – Design was used in this study:
$\mathbf{Z = B_0 + B_1X_1 + B_2X_2 + \ldots\ldots\ldots + B_nX_n}$,
Where,**Z** is the dependable – variant; $\mathbf{B_0}$ is the Arithmetic Mean for Response of nine Runs; $\mathbf{B_1}$ is the Estimated Co-Efficient for Factor $\mathbf{X_1}$. $\mathbf{B_2}$ is the Estimated Co-Efficient for Factor $\mathbf{X_2}$.
The data were subjected to 3-D response – surface – plot in DOE – Software.

At the beginning, Carbopol 940 and Propylene Glycol was dissolved in 50:50 Hydro – Alcoholic solution by thermal incorporation in a heating – mantle. Then added to it Glycerin, and pre – dissolved Methyl Paraben in hot water. Gel – forming Agent Triethanolamine was added and then gel was formed by abrupt change of pH from 4.0 to 5.8 which was visualized macroscopically. Aromatiser Rose Water added finally. Thus, the gel base was formed. Now, 5 ml Herbal Extract as added in all the nine formulations. The total content was homogenized in a Rotary Homogenizer at 50RPM at Room temperature for 30 minutes for complete mixing.

Table 2:- Formulation of the Gel Base with all Ingredients.

Formulations Ingredients	Formulation Code and Corresponding Quantities								
	F-1	F-2	F-3	F-4	F-5	F-6	F-7	F-8	F-9
Propylene Glycol (g)	1.2	1.2	1.2	1	1	1	1.4	1.4	1.4
Carbopol-940 (g)	0.6	0.8	4	0.6	0.8	4	0.6	0.8	4
CQ – Extract (ml)	5	5	5	5	5	5	5	5	5
Glycerine (ml)	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Triethanolamine (ml)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Meth. Paraben (mg)	6	6	6	6	6	6	6	6	6
Menthol (ml)	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Rose water (ml)	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Distilled water (ml)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

***Foot Note: q.s. to produce 30gm of total gel weight in each formulation.**

Results and Discussion:-

Macro-Physico Parameters' Analysis:

1. Organoleptic Properties:
The prepared cream is very light green in colour and has no characteristic odour.

2. Viscosity:
Thus, the measured viscosity is 19,000 centipoise.

Fig 1,2 and 3:- Brookfield Viscometer for Rheological determination of Kinetic Viscosity.

Spreadability:

It is the measure of the gel that how much agreeable is the gel to the applied exposed area over the broken bone &/or skin. The Spreadability is given by the formula:

S_f = A/W

Where, **S_f** is the Spreadibility Factor, measured in mm^2 per gram.

A is the Total Area covered in mm^2, and

W is the total endurable weight in grams.

Thus, S_f= 24 mm^2 / 1.5 grams = 16 mm^2 per gram; which confirms that the 'Harjora Gel' is effectively spreadable when applied over a biologically affected area. [11,28]

Fig 4:- In - house Spreadibility Tester (Courtesy: Hironmoy Malik, Bengal School of Technology).

Extrudability:

Extrudability is a macro – physico parameter that deals with the oozing out of the gel continuously without interruptions. Extrudability depends on composition of the gel and its molecular alloying constitution. The Extrudability (%) is acceptable between 80% to 90%, as reported by the researchers. [10,17,26]

Extrudability = applied weight to extrude gel from the tube per area in square inch

Conductivity and pH Analysis:

The Herbal Gel was tested for its pH at different time intervals for 12 days. A pH of 4.0 to 5.8 is good for this gel to remain stable [27].

Homogeneity:
To perform homogeneity test, the formulations were taken in a container to set and to check the visual outcome, free from grittiness. A little amount of each gel is pressed between the index finger and the thumb. It is observed the gel is homogenous or not by its consistency [29].

Table 3:- Comparative Q.C. Parameters of the Gel:

Formulation code	Viscosity (cps)	Spreadability (mm^2/g)	Clarity	Extrudability	Homogeneity
F-1	19000	16.00 ± 0.03	+	+	EXCELLENT
F-2	20000	18.5 ± 0.05	++	+	GOOD
F-3	16000	16 .7± 0.07	+++	++	GOOD
F-4	18000	17.9 ± 0.04	++	+	GOOD
F-5	17000	18.6 ± 0.06	+	++	SATISFACTORY
F-6	18000	19.6 ± 0.03	++	++	EXCELLENT
F-7	21000	17.3 ± 0.07	++	+	SATISFACTORY
F-8	22000	21.4 ± 0.06	++	++	EXCELLENT
F-9	18000	20.6 ± 0.08	+	+	GOOD

For all n = 3 ± S.D.

Response Surface Methodology:
Table 4:- Simulation of data corresponding to surface response curve.

Formulation Code	Level of factor 1 (propyline glycol) (g)	Level of factor 2 (carbopol) (g)	Response 1 (Viscosity) (cps)	Response 2 (Spreadability) (mm^2/g)
F-1	0	0	19000	16
F-2	0	1	20000	18.5
F-3	0	-1	16000	16.7
F-4	-1	0	18000	17.9
F-5	-1	1	17000	18.6
F-6	-1	-1	18000	19.6
F-7	1	0	21000	17.3
F-8	1	1	22000	21.4
F-9	1	-1	18000	20.6

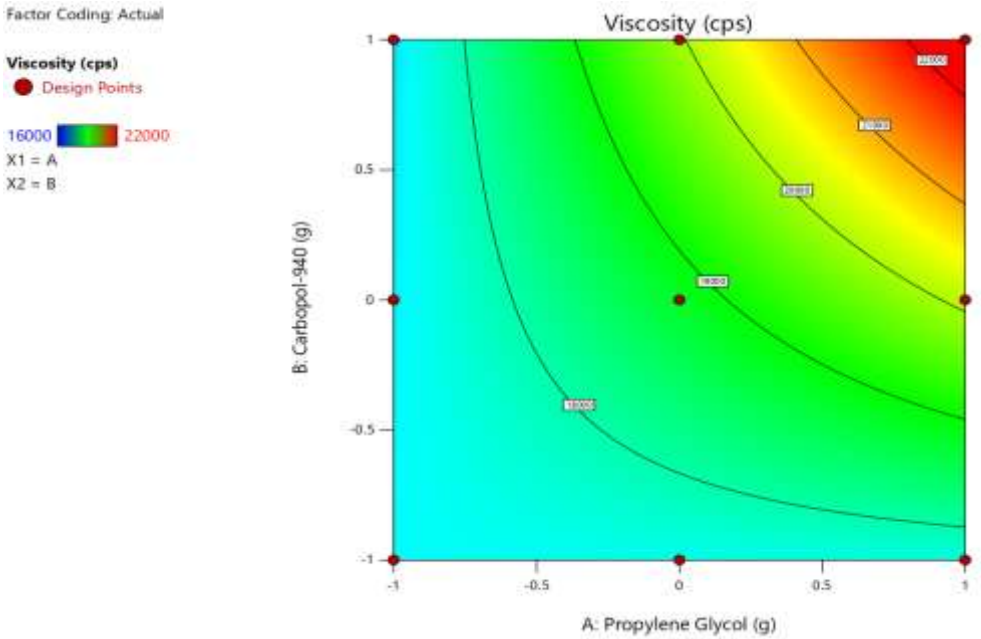

Figure 5:- Contour Plot for Viscosity with respect to amount of Carbopol 940 and Propylene Glycol.

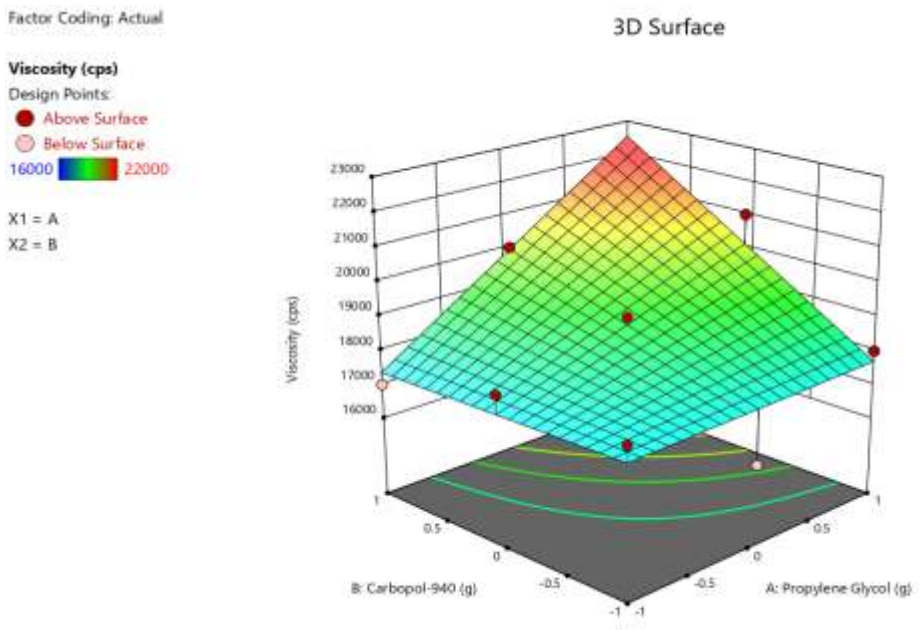

Figure 6:- 3D Curve for Viscosity with respect to amount of Carbopol 940 and Propylene Glycol.

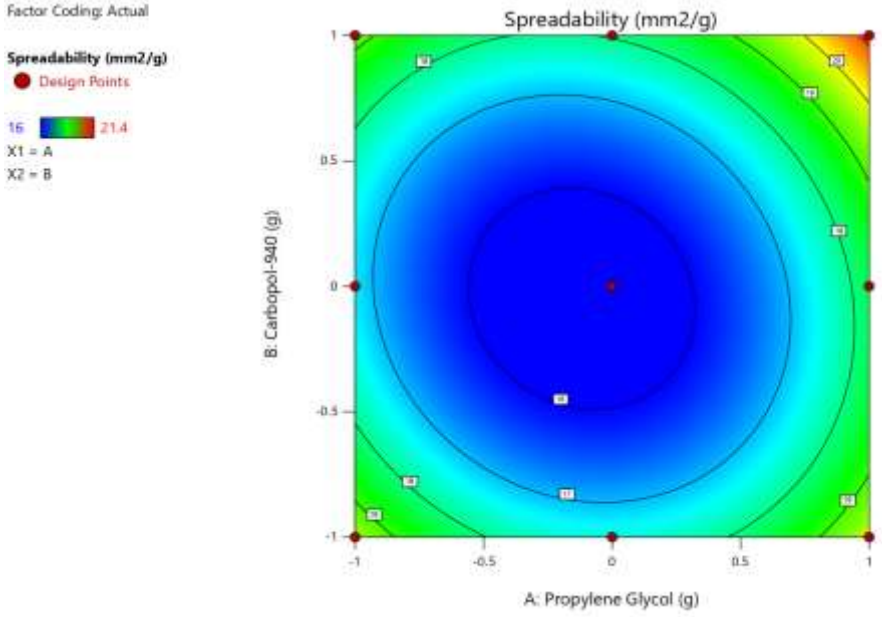

Figure 7:- Contour Plot for Spreadibility with respect to amount of Carbopol 940 and Propylene Glycol.

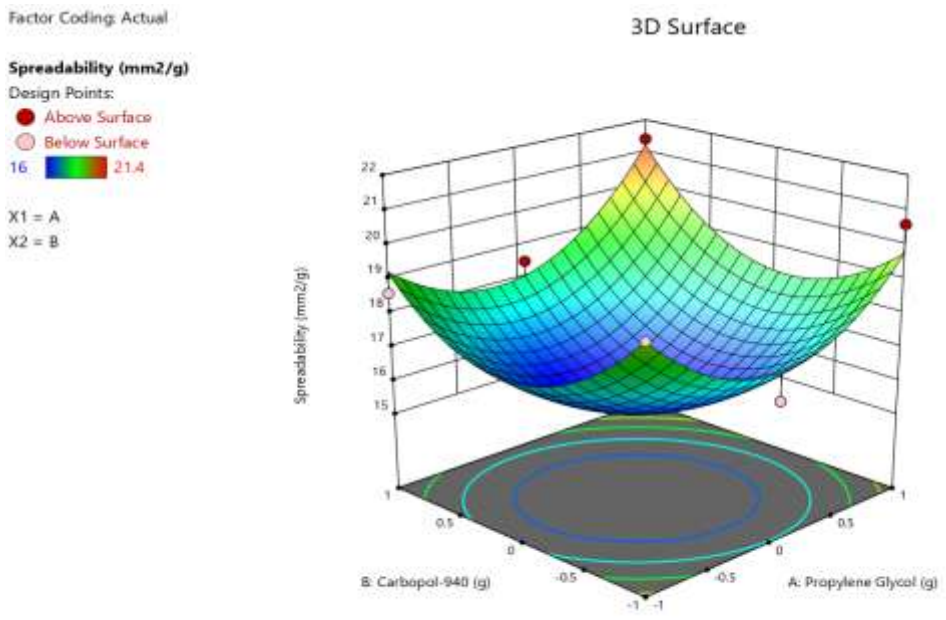

Figure 8:- 3D Curve for Spreadibility with respect to amount of Carbopol 940 and Propylene Glycol.

Conclusion:-

As the herb *Cissus quadrangularis* is reported to contain Alkaloids, Poly Phenols, Terpenoids, Sterol and other pharmacogenetic phytochemical, singly; and also, as closely related structural Phyto-moiety in numbers; hence it seems mandatory to evaluate as total active herbal ingredients in terms of Herbal-Reference-Standards; such as, Quercetin, Gallic Acid, Ellagic Acid, Chebulinic Acid, Cholesterol and other isolated purified compounds.

At 271 nanometre, the UV Detector-Response as i) Retention Time, ii) Height and iii) Area of Peak (with Tailing Factor less than 2) were considered for the area under curve value, as recorded by the software during a determinant Run-Time of sixty minutes.

During twelve days of Observation of the macro physico parameters and micro chemo parameters, it can be concluded that the F-1 formulation has a Viscosity of 19000 cps, Spreadibility of 16mm^2, excellent homogeneity and good clarity and extrudability. Out of all the nine formulations this formulation is superior in case of its physical properties. This indicates a promising herbal – gel for bone regeneration. It can be an asset as an alternative for a topical or transmembrane formulation.

Future prospects:

A systematic animal studies and human clinical trial must be required to study. More advanced Method-Development is a need for the RP-HPLC Analysis. Gradient RP HPLC could be an alternatively similar or better choice. Other alternatively available modern methods of extraction should be studied. More research is needed to fully understand the mechanisms of action of herbal gels and their potential benefits and risks. Additionally, larger clinical trials are needed to validate their effectiveness and safety for the treatment of various health conditions. Despite the need for further investigation, herbal gels represent a promising avenue for the development of natural remedies for a range of ailments. As *Cissus quadrangularis* is being used since more than 5,000 years, it may be tried with Plant Tissue Culture for a better and quicker Bone-Healing property.

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