PROJECT REPORT

DEVELOPMENT AND EVALUATION OF MULTIPURPOSE HERBAL GEL OF NELUMBO NUCIFERA AND ANANAS COMOSUS

Submitted to

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Certificate

This is to certify that ***Mr./~~Ms.~~ KAMANI PRIVAL HIRJIBHAI*** *with Enrollment no.* ***200501025*** *of Program* ***B.Pharm****, Semester* ***VIII*** *has satisfactorily completed his/her termwork in the Course* ***PROJECT WORK****, Course Code* ***18BPHCC803*** *for the term ending in the month of* ***APRIL*** *Academic year20**23**-20**24**.*

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PROJECT WORK (18BPHCC803)

Title	Development and Evaluation of Multipurpose Herbal Gel of Nelumbo Nucifera and Pineapple Comosus
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DECLARATION

We hereby declare that this Project Report on, "DEVELOPMENT AND EVALUATION OF MULTIPURPOSE HERBAL GEL OF NELUMBO NUCIFERA AND ANANAS COMOSUS", which is being submitted as a part of the partial fulfillment for the degree of Bachelor of Pharmacy, is the result of the work carried out by us, under the supervision of Ms. Hani M. Jani Assistant Professor, School of Pharmaceutical Sciences, Faculty of Health Sciences, Kalawad Road, Atmiya University, Rajkot.

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Achievement of goal is not a one person's job. It is obtained by guidance and cooperation of others. Gratitude brought to be a way of life, something which we cannot give enough of. It can mean a smile or a thank you gesture or appreciation.

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With whole hearted sincere gratitude, would like to dedicate the entire work of mine to my facility and without whose care, support, guidance and encouragement, it would be impossible.

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ABSTRACT

The objective of the study is to formulate and evaluate a topical herbal gel containing Nelumbo nucifera and Ananas comosus extracts. Different concentrations herbal gel formulations were prepared using carbopol 940 as a gelling agent. They were evaluated were the physical appearance, pH, spreadability, antimicrobial test, anti-inflammatory test and primary skin irritation test. The stability study for the topical herbal gel formulation was done. Formulated gel were homogeneous and stable. No erythema or edema eas observed in the skin irritation test confirming the gel was non toxic and safe with satisfactory action.

Keywords: *Nelumbo nucifera, Ananas comosus, Anti inflammatory, Herbal gel*

INTRODUCTION

Gel is a two – phase elastic colloidal material where the solid phase is combined with a dispersed liquid phase. The solid, which uses the liquid's surface tension to keep it from collapsing creates the structure in which the liquid "Lives". Gels can be made by quickly reacting with a high concentration of chemicals in the liquid phase or by chilling a colloidal solution [1].

Currently available in a variety of formulations, gels offer the primary benefit of remaining stable on the treated area and preventing evaporation for a longer period of time to achieve their desired effect. For instance, certain gel formulations possess mucoadhesives properties that make them suitable for use in mucosae. This is in contrast to be effective as antimicrobials [2,3,4,5].

While others are good as anesthetics or anti – inflammatory. Additionally, several formulations have been suggested to aid in hemostasis or wound healing after skin lesions [6].

There is a huge need for disinfectant gels as a result of the recent SARS – CoV – 2 outbreak. A liquid, gel or foam that lowers the amount of infectious agents on hands is called a hand sanitizer. There are several formulas that can contain or not contain alcohol. The World Health Organizations (WHO) Essential Medicines List, which is a compilation of the drugs the organization believes to be the safest and most effective in a given health care system, includes the alcohol – based variant. The use of disinfectants has a number of benefits and drawbacks when compared to hand washing with soap and water because of the differing sanitation techniques and product compositions in the two scenarios, which allow for the various applications of each techniques [7,8].

Advantages of gel in pharmaceutics

1. Gels are effortless to prepare when compared to other formulations
2. Gel is elegant non – greasy formulations
3. Gels have excellent adherence property to application site
4. Gels are biocompatible and eco – friendly [9]

Disadvantages of gels in pharmaceutics

1. Effect of gels is relatively sustained and slower
2. The gelators or additives may cause irritation
3. Water content increase possibility of fungal or microbial attack in gel [9].

FIG. 1

FIG. 2

FIG. 3

MARKETED GEL IMAGES [61,62,63]

COMMON INGREDIENTS

Table 1.1 Common Ingredients Used In Gel

INGREDIENTS	USES
Carbopol	Gelling agent
Triethanolamine	pH adjustor
Propylene glycol	Emollient
Glycerin	Moisturizing agent
Water	Vehicle

CLASSIFICATION OF GEL

1. Classification based on their sources

a) Natural Polymer Gels

b) Synthetic Polymer Gel

2. Classification based on polymeric composition

a) Homopolymeric Polymer Gels

b) Copolymeric Polymer Gels

c) Multipolymer Interpenetrating Polymer Gels (IPN)

3. Based on type of cross-linking

a) Gels physically cross – linked

b) Gels chemically cross – linked

4. Based on physical appearance

a) Amorphous (non – crystalline)

b) Semi crystalline

c) Crystalline

5. According to network electrical charge

a) Non – ionic

b) Ionic

c) Amphoteric

d) Zwitterionic [10].

COMMON METHOD OF PREPARATION OF GEL

Gels are prepared by mixing suitable thickening agent and aqueous vehicles. Drug is dispersed in aqueous vehicle and thickening agent is added by triturating in a mortar. Trituration is carried out until a homogenous preparation is formed.

1. **Fusion Method**
2. **Cold Method**
3. **Dispersion Method**

1. Fusion Method

In this method various waxy materials employed as gellant in non polar media. Drug was added when waxy materials melted by fusion, stirred slowly until uniform gel formed.

2. Cold Method

Water was cooled to 4-10^{0}c and placed it in mixing container. Gelling agent was slowly added and agitating until solution is complete. Maintained temperature below 100^{o}c . Drug was added in solution form slowly with gentle mixing. Immediately transfer to container & allow to warm to room temperature where upon liquid becomes clear gel.

3. Dispersion Method

Gelling agent was dispersed in water with stirring at 1200 rpm for 30 min . Drug was dissolved in non-aqueous solvent with preservative. This solution was added in above gel with continuous stirring.[11]

NELUMBO NUCIFERA(LOTUS)

Nelumbonucifera is a member of the Nelumbonaceae family, which also goes by multiple botanical names, including Nymphaeanelumbo, Nelumbiumnelumbo, N. speciosa, and N. speciosum, as well as several tribal names, including Indian lotus, bean of India, Chinese water lily, and sacred lotus. In terms of history, China, India, and Egypt are the three nations that have bestowed honor upon this lovely aquatic flowering plant [12,13].

PHYSICAL CHARACTERISTICS AND DESCRIPTION

Large aquatic rhizomatous herb Nelumbo nucifera has long, slender creeping stems with nodal roots. Perennial Lotus plants have orbicular leaves that are both airborne and floating. While floating leaves have a flat appearance, aerial leaves are cup-shaped. Its petioles have noticeable prickles and are quite lengthy and tough. Flowers are solitary, hermaphrodite, and range in color from white to rose. They also have a very sweet aroma. Flowers are ovoid, glabrous, and have an average diameter of 10–25 cm. Black-colored, firm, ovoid fruit with seeds is organized in whorls; the seeds ripened and were discharged as the pod bent down toward the water. Eight inches long and two inches in diameter make up tuberous roots.

The lotus root's smooth exterior covering is green, but its interior has many large air pockets that run the length of the tuber and help it float in the aquatic system [14,15].

FIG. 4 [64]

Table 1.2 Scientific Classification Of Lotus [59]

Scientific classification	
Kingdom	**Plantae**
Clade	Tracheophytes
Clade	Angiosperms
Clade	Eudicots
Order	Proteales
Family	Nelumbonaceae
Genus	Nelumbo
Species	N. nucifera
Binomial name	Nelumbonucifera Gaertn.

ANANAS COMOSUS (PINEAPPLE)

Ananas comosus , also known as B.comosa , a. sativus, Ananassa sativa and Bromelia ananas is the common name for pineapple. The pineapple, which is cultivated in a number of tropical and subtropical nations such as the Phillipines, Thailand, Indonesia, Malaysia, Kenya, India and China , is the most widely consumed member of the Bromeliaceae family. In several native cultures it has been used as a medicinal plant [16].

Table 1.3 Scientific Classification Of Pineapple[61]

Scientific classification	
Kingdom	**Plantae**
Clade	Tracheophytes
Clade	Angiosperms
Clade	Angiosperms
Clade	Commelinids
Order	Poales
Family	Bromeliaceae
Genus	*Ananas*
Species	***A. comosus***

FIG.5 [65]

Bromelain, a crude extract from pineapple that includes several closely related proteinases and exhibites a variety of fibrinolytic , antiedematous, antithrombotic and anti – inflammatory properties both invitro and invivo , is thought to be responsible for the medicinal properties of pineapple. Since its scientific discovery in 1875, bromelain has been utilized as a phytomedical substance [17].

Because pineapple stems are inexpensive waste byproducts rather than the fruit , which is often consumed , the high bromelain concentration in them necessitates their extraction [18].

REVIEW OF LITERATURE

REPORTED PHARMACOLOGICAL ACTIVITY OF NELUMBO NUCIFERA :

1. Antioxidants

The antioxidant potential of Nelumbonucifera (HAAN) hydroalcoholic extract in both in-vitro and in-vivo settings. [19]

2. Antisteroids

Gupta et al. (1996) investigated the antisteroidogenic action of N.nucifera seed extract in the rat testis and ovary. [20]

3. Antipyretic Activity :

Using rats as an in-vivo model, Sinha et al. (2000) revealed the antipyretic effect of lotus stem ethanol extract on both normal body temp. and yeast induced pyrexia. [21]

4. Immunity :

In primary human peripheral blood mononuclear cells (PBMC) stimulated by phytohemagglutinin (PHA : a particular mitogen for T-lymphocytes), Liu et al. (2004) investigate the effect of ethyl alcohol extract of lotus to decrease the cell proliferation and cytokine generation. [22]

5. Anti-Inflammatory Activity :

The potent anti-inflammatory properties of triterpenoidbetulinic acid, which was extracted from the methanol extract of Nelumbonucifera rhizome, were assessed in relation to rat paw edema brought on by serotonin and carrageenan. [23]

6. Diabetes and Complications :

The impact of an ethanolic extract of the rhizome of N.nucifera was investigated in rats with streptozotocin induced diabetes and hyperglycemia fed glucose. [24] Nelumbonucifera stamens methanol extracts showed inhibitory action against rat lens aldose reductase[25].

7. Treatment for erectile dysfunction

In 2008, Chen and colleagues examined how extract neferine affected the baseline concentration of cyclic guanosine monophosphate and cyclic adenosine monophosphate [26]. Chen et al. further emphasized the in vitro relaxation mechanisms of neferine on rabbit corpus cavernosum tissue [27] and the in vitro effects of neferine on the concentration of cytosolic free calcium in rabbit corpus cavernosum smooth muscle cells in a different study[28].

8. Restenosis and atherosclerosis

For four weeks, the rat model's luminal area narrowing and stenosis rate were dramatically inhibited by administering N.nucifera leaf and root extract[29].

N.nucifera leaf extract demonstrated strong antiatherosclerotic efficacy in a rabbit model of a atherosclerosis brought on by a high – cholesterol diet by inhibiting VSMC proliferation and migration raising plasma cholesterol levels [30,31]. Neferine, the active ingredient in N.nucifera, inhibits angiotensin – II stimulated proliferation in VSMC by downregulating the expression of the fractalkine gene and hemeoxygenase – 1 [32,33].

9. Antiaging

An antiaging ingredient found in sacred lotus (Nelumbonucifera) seed extract helps to lessen signs of aging such as elasticity loss, acne, pores, wrinkles, fine lines, blemishes and so on. A good vehicle contains compounds with strong anti – aging properties. It encourages skin that seems younger [34]

10. Antiarrythmia

Dauricine and neferine, two phytochemicals derived from N.nucifera seeds, have pharmacological effects on the cardiovascular system. The cardiac transmembrane currents for $Ca^{+2}$and $Na^{+}K^{+}$ were inhibited by the phytochemicals of N.nucifera. Neferine is a notable chemical that greatly reduces platelet aggregation in rabbits and has anti – arrhythmic effects. Neferine , the principal alkaloid from N.nucifera, has been shown in multiple in vivo experimental tests to possess anti – arrhythmic activity [35,36].

11. Hepatoprotective activity

The hepatoprotective activity of Nelumbonucifera was reported by Sohn et al. in 2003, cell survival rate and aspartate transaminase (AST) leakage in primary cultured rat hepatocytes were used to assess the protective effects of Nelumbonucifera (ENN) seed ethanol extract against CCl_4– induced cytotoxicity [37].

12. Pulmonary Fibrosis

Xiao et al. assessed the impact of isoliensinine , a bisbenzylisoquinoline alkaloid extracted from the Nelumbonucifera seed embryo, on bleomycin – induced lung fibrosis in mice [38].

13.Antiobesity activity

In 2007, Ohkoshi and colleagues revealed that active ingredients extracted from Nelumbonucifera leaves effectively prevented obesity by inducing lipolysis in the adipose tissue of rats. The discovered constituents exhibited beta – adrenergic receptor – mediated antiobesity action [39].

14.Anticancer activity

Both invitro and invivo , several extracts and isolated compounds from distinct N. nucifera components exhibit anticancer action. Isolesinine is the most effective cytotoxic of the three major alkaloids ; it mainly induces apoptosis in triple – negative breast cancer cells by activating p38 MAPK/ JNK and producing reactive oxygen species (ROS) [40].

15.Traditional uses

Numerous scientific studies support traditional knowledge about the medicinal properties of the lotus plant. The entire plant has diuretic ,cardiotonic , sudorific , emollient , antifungal and antipyretic properties. Several elements of the lotus plant are frequently used to treat tissues inflammation , diarrhea and heostasis [41].

REPORTED ACTIVITY OF ANANAS COMOSUS :

1. Effects of Bromelain on Cardiovascular & Circulation

Transient ischemic attack (TIA) and angina pectoris are prevented or atleast less severed by bromelain. It is helpful in both thrombophlebitis therapy & prevention. It may potentially have strong fibrinolytic properties and dissolve cholesterole plaques. Bromelain & other nutrients work together to shield skeletal muscles from ischemia/reperfusion damage [42].

2. Bromelain Relieves Osteoarthritis

A dietary supplement called bromelain has the potential to treat patients in place of nonsteroidal anti-inflammatory drugs (NSAIDS)[43]. It is crucial to the pathophysiology of arthritis[44]. It is believed that the analgesic effects of bromelain stem from its direct impact on pain mediators such bradykinin.[45,46]

3. Effect of Bromelain on Immunogenicity

It has been suggested that using bromelain as an adjuvant therapeutic method can help treat autoimmune, cancerous & chronic inflammatory illness.[47]

4. Effect of Bromelain on Blood Coagulation & fibrinolysis

By boosting serum fibrinolytic activity & preventing the formation of fibrin, a protein implicated in blood coagulation[48]

5. Effects of Bromelain of Diarrhea

Research indicates that some of the effects of intestinal infections such as E – coli and Vibrio cholera , whose enterotoxin produces diarrhea in animals , may be mitigated by bromealin. It seems that bromealin has this effect [49].

6. Effects of bromelain on cancer cells

According to recent research, bromelain can alter important pathways that promote cancer. Bromelain's anticancer effect is most likely caused by its direct effects on cancer cells and the environment around them , as well as by changes to the immunological, inflammatory and hemostatic systems [50].

In these investigations, tumor regression was observed upon intraperitoneal administration of bromelain following a 24 – hour inoculation with tumor cells [51].

7. Role in bromelain in surgery

When bromelain is administered prior to surgery, the average number of days it takes for pain and postoperative inflammation to completely subside can be lowered [52,53].

Studies suggest that bromelain may help women undergoing episiotomy have less pain, bruising, and swelling [54]. These days, sports injuries and acute inflammation are treated with bromelain [55].

8. Role of bromelain in debridement burns

Debridement is the process of removing damaged tissue from wounds or second – or third – degree burns. Applying a lotion containing 35% bromelain in a basis can help remove necrotic tissue and hasten the healing process. This effect is caused by escharase, which is present in bromealin [56].

MATERIAL AND METHODOLOGY

LOTUS EXTRACT PROFILE

Table 1.4

PARAMETRS	DESCRIPTION
PHYSICAL STATE	LIQUID EXTRACT
SCIENTIFIC NAME	NELUMBO NUCIFERA
SOLUBILITY	WATER SOLUBLE
USED IN	LOTIONS , FACE CREAMS, GEL, etc.
DIRECTIONS	SLOWLY STIR DESIRED AMOUNT OF LIQUID INTO FORMULA AND MIX WELL
STORAGE	STORE IN AN AIR-TIGHT, COOL, DARK LOCATION
AVAILABLE FROM	VEDA OILS

FIG. 6 MARKETED LOTUS

LIQUID EXTRACT

PINEAPPLE EXTRACT PROFILE

Table 1.5

PARAMETRS	DESCRIPTION
PHYSICAL STATE	LIQUID EXTRACT
SCIENTIFIC NAME	ANANAS COMOSUS
SOLUBILITY	WATER SOLUBLE
USED IN	FOR SURGICAL WOUNDS, INFLAMMATION DUE TO TRAUMA AND SURGERY, DEBRIDEMENT OF DEEP BURNS
STORAGE	STORE IN A COOL, DRY, DARK CONTAINER AWAY FROM SUNLIGHT
AVAILABLE FROM	BRM CHEMICALS

FIG. 7 MARKETED PINEAPPLE EXTRACT

CARBOPOL 940 PROFILE

Table 1.6

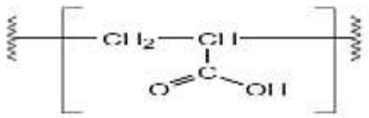
PARAMETERS	DESCRIPTION
PHYSICAL STATE	POWDER
COLOR	WHITE
ODOUR	SWEET
pH LEVEL	2.7 – 3.3
ANOTHER NAME	CARBOMER 940, POLYACRYLIC ACID
CHEMICAL STRUCTURE	
FORMULA	$(C_3H_4O_2)_n$
MOL. WT.	72.06 g/mol
SOLUBILITY	WATER SOLUBLE
USES	THICKENER IN LOTIONS, CREAMS AND GELS

FIG. 8 CARBOPOL 940

TRIETHANOLAMINE PROFILE

Table 1.7

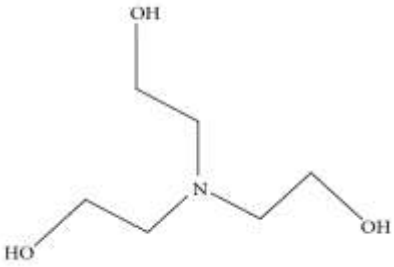
PARAMETERS	DESCRIPTION
PHYSICAL STATE	COLOURLESS VISCOUS LIQUID
GENERIC NAME	TROLAMINE
CHEMICAL STRUCTURE	
FORMULA	$N(CH_2CH_2OH)_3$
ODOUR	AMMONICAL
MOL. WT.	149.190 g/mol
SOLUBILITY	WATER SOLUBLE
USES	HELPS STABILIZE CONSISTENCY,IMPROVES TEXTURE, AND FACILITATES EASY SPREDABILITY OF THE PRODUCTS AND ALSO AS A BUFFERING AGENT AND pH ADJUSTOR

FIG. 9 TRIETHANOLAMINE

VANILLIN PROFILE

Table 1.8

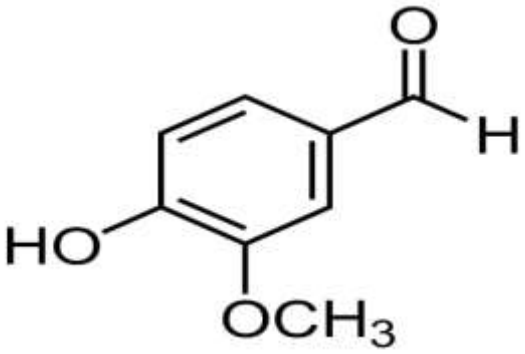
PARAMETERS	DESCRIPTION
PHYSICAL STATE	WHITE POWDER
IUPAC NAME	4-HYDROXY-3-METHOXYBENZALDEHYDE
FORMULA	$C_8H_8O_3$
CHEMICAL STRUCTURE	
MOL. WT.	152.149 g/mol
ODOUR	SWEET, SUGARY
USES	FLAVORINGS, FOODS, PERFUMES AND PHARMACEUTICALS

FIG. 10 VANILLIN POWDER

Table 1.9 Ingredients And Concentrations Used For The Final Batch

Ingredients	Concentration	Uses
Lotus extract	2 %	API
Pineapple extract	2 %	API
Carbopol 940	4.5 %	Gelling agent
Triethanolamine	0.01 %	pH adjustor
Vanillin	q.s.	Masking agent
Water	q.s.	Vehicle

METHOD OF PREPARATION

Take small quantity of water

Add 2ml of lotus liquid extract and 2ml of pineapple liquid extract

Add vanillin powder approx. 2 spatula

Add 4.5 gm of carbopol 940 into the above solution with stirring

Gel

EVALUATION

PARAMETERS OF GEL

1. Spreadability

Standard-sized glass slides were taken in two sets. Over one of the slides was the formulation for the herbal gel. The gel was sandwiched between the two slides in a region that measured 7.5 cm along the slides when the other slide was positioned on top of the gel. Gel weighing one hundred grams was applied on the upper slides, pressing it evenly to create a thin layer between the two slides. After removing the weight, the extra gel that had stuck to the slides was scraped off. The two slides in place were securely fastened to a stand so that only the upper slides could come loose from the weight that was fastened to them. Carefully, a 20 g weight was fastened to the upper slide. The amount of time it took for the upper slide to move 7.5 cm and split off from the lower slide as a result of the weight was recorded. Three repetitions of the experiment were conducted, and the mean time was recorded for computation.

Spreadability was calculated by using the following formula:

$S = m \times l/t$

where,

S= spreadability,

m-weight tied to upper slides (20 g),

l- length of the glass slide (7.5 cm),

t- time taken in sec [57].

2. pH

pH measurement of the gel was carried out using a digital pH meter by dipping the glass electrode completely into the gel system to cover the electrode. The measurement was carried out in triplicate and the average of the three readings was recorded[58]

3. Viscosity

Using a Brookfield viscometer (S-62, model LVDV-E) at 25°C and a spindle speed of 12 rpm, the viscometer's viscosity was measured for the gel [59].

4. Appearance and Homogeneity

Physical appearance and homogeneity of the prepared gels were evaluated by visual perception.

5. Antimicrobial test

The Kirby-Bauer test, also known as the Zone of Inhibition test, was utilized to assess antibiotic activity. S. aureus were the bacteria cultures that were streaked over a petri dish coated with nutrient agar. In order to promote microbial development in the culture media, a 3 cm by 3 cm piece of mustard seed extract hydrogel was cut, placed on the nutrient agar, and incubated in a petri dish for 18 to 24 hours at 36°C. Following the incubation time, the development of bacteria on culture plates was apparent as a dense yellow grass. On the test pieces, there was a noticeable clearing zone. This zone's dimensions were determined.[60]

6. Anti – inflammatory test

As per the previously documented methodology, the reaction mixture was composed of 0.2 milliliters of fresh hen's egg albumin, 2.8 milliliters of phosphate buffered saline (pH 6.4), and Two milliliters of the test extract at different concentrations resulted in concentrations (µg/ml) of 400, 800, 2000, 4000, 8000, and 16,000. This range was selected because dosages below 400 µg/ml produced very little inhibition, and concentrations above 16,000 µg/ml resulted in an excessively high value. As a control, a comparable volume of double-distilled water was used. After that, the mixes were heated for five minutes at 70°C after being incubated for fifteen minutes at 37°C ± 2°C in a biological oxygen demand incubator.

After cooling, the vehicle was used as a blank to measure their absorbance at 660 nm using a Systronix Spectrophotometer 150. Rumalaya forte and diclofenac sodium at the end Reference and traditional/herbal medication concentrations (µg/ml) of 50, 100, 250, 500, 1000, and 2000 were utilized, and they were treated in the same way for the absorbance

measurement. The test extracts were selected to stay as close as feasible to the typical therapeutic mode. Using the following formula, the percentage inhibition of protein denaturation was determined :

Inhibition percentage = 100 times ([Vt/Vc] –1)

where,

Vt is the test sample's absorbance and

Vc is the control sample's absorbance.[61]

RESULT AND DISCUSSION

1. Physical Apperance

To assess its physical characteristics , the texture , color, and aroma of the gel were examined directly. All formulas resulted in products with a transparent viscous gel with smell of vanilla.

FIG. 11 FORMULATED GEL WITH DIFFERENT CONCENTRATIONS

2. pH Value

The pH value of the formulated final batch was determined to be 6, which is close to the predicted value. It is compatible with the skin's pH, and there are no heavy metals in the formulation. The formulation is not responsible for irritation or allergic reactions.

FIG. 12 PH READING

3. Spreadability

It was anticipated that the formulation would have good spreadablity. There is a viscosity and spreadability has a linear relationship in rheological studies ; the lower the viscosity, the lower the surface tension, and the higher the spreadability.

$$S = m * l/t$$

$$S = 20g * 7.5cm/1.97sec$$

$$S = 20g * 3.30\ cm.sec^{-1}$$

$$\boxed{\mathbf{S = 76.14\ g.cm.sec^{-1}}}$$

4. Viscosity

The viscosity of the formulation was assessed using a Brookfield Viscometer, but our department Brookfield Viscometer using damage that we can't perform this study.

5. Homogeneity

We have applied gel on our hands , and the homogeneity is monitored as we can see it can be applied homogenously on our skin.

6. Stability analysis

Laboratory level we check our gel at room temperature for 3 to 4 weeks.Throughout the stability study, the product's quality, safety, and efficacy are maintained.

7. Antimicrobial study

The results of antimicrobial studies indicate that agar plate of test inoculums show similar zone of inhibition as compared to standard from 24 h grown culture. The result indicating that the formulation was acting as anti-microbial activity and safe to use.

Table 1.10 Results Of Anti – Microbial Test

		GEL					STREPTOMYCIN (0.2µG/ML)		
		1	**2**	**3**	**STD.**	**1**	**2**	**3**	**STD.**
S.AUREUS		1.5	1.65	1.5	1.5	1.4	1.45	1.3	2.2
		1.5	1.55	1.6	1.2	1.25	1.35	1.3	1.95
		1.6	1.7	1.6	1.35	0	0	0	0
	AVG.	**1.53**	**1.63**	**1.56**	**1.35**	**0.88**	**0.93**	**0.86**	**1.38**
E.COLI		1.2	1.4	1.35	0	1.2	1.25	1.6	2.35
		1.35	1.3	1.4	1.2	1.4	1.35	1.5	0
		1.25	1.25	1.35	0	1.6	1.6	1.7	0
	AVG.	**1.26**	**1.31**	**1.36**	**0.4**	**1.4**	**1.4**	**1.6**	**0.78**

FIG. 13 E – COLI

FIG. 14 S. AUREUS

8. Anti – inflammatory test

The anti – denaturation assay is the convenient method to check the anti – inflammatory activity. From the result of the present study, the extract has shown considerable anti – inflammatory activity.

Inhibition percentage = [Vc – Vt / Vc] * 100

where,

Vt is the test sample's absorbance and

Vc is the control sample's absorbance [0.210]

Table 1.11 Results Of Anti – Inflammatory Test

Solutions	Absorbance	% Inhibition
Control	0.210	-----
Standard extract	0.161	23.33
1 %	0.081	61.43
1.5 %	0.124	40.95
2 %	0.063	70

FIG. 15 ABS. OF CONTROL

FIG. 16 ABS. OF STD. EXTRACT

FIG. 17 ABS. OF 1%

FIG. 18 ABS. OF 1.5%

FIG. 19 ABS. OF 2%

RESULTS SUMMARY:

Table 1.12

SR. NO.	PARAMETERS	RESULT
1.	Color	Transparent
2.	Appearance	Viscous
3.	Homogeneity	Homogenous
4.	pH	6.4
5.	Stability	No physical change
6.	Spredebility	76.14 $g.cm.sec^{-1}$
7.	Viscosity	-----
8.	Anti – microbial activity	Satisfactory
9.	Anti inflammatory activity	Satisfactory

CONCLUSION

Nelumbo nucifera and Ananas comosus liquid extracts were used first time for the development of topical drug delivery system - Gel. Carbopol 940 used as a gelling agent. Different concentrations of the extracts were used to prepare the herbal gel. From the study, it is concluded that 2 % concentration of both the extracts is effective and safe. The prepared gel is stable and fulfill all the parameters such as pH, spreadability, anti microbial, anti inflammatory test.

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