International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 24s, 2025
https://www.theaspd.com/ijes.php

Development And In Vitro Characterization Of Hydrogel Containing A Combination Of Guduchi, Neem, And Calendula Extracts For The Treatment Of Diabetic Wound Healing

Alka Sharma[1]*, Sheetal Sharma[2], Muskan Sharma[3], Ankita[4], Ravinesh Mishra[5], Bhartendu Sharma[6]
[1*,2,3]Assistant Professor, School of Pharmacy and Emerging Sciences, Baddi University of Emerging Sciences & Technology, Baddi Makhnumajra- 173205, H.P. India.
[4]Research Scholar, School of Pharmacy and Emerging Sciences, Baddi University of Emerging Sciences & Technology, Baddi Makhnumajra- 173205, H.P. India.
[5]Professor, School of Pharmacy and Emerging Sciences, Baddi University of Emerging Sciences & Technology, Baddi Makhnumajra- 173205, H.P. India.
[6]Associate Professor, School of Pharmacy and Emerging Sciences, Baddi University of Emerging Sciences & Technology, Baddi Makhnumajra- 173205, H.P., India.

Abstract

Development And In Vitro Characterization of Hydrogel Containing a Combination of Guduchi, Neem, and Calendula Extracts for The Treatment of Diabetic Wound Healing. Managing chronic diabetic wounds is challenged by flawed angiogenesis, continuous infection, and oxidative stress. The use of herbal extracts with antimicrobial, antioxidant, and collagen-stimulating effects can be considered a possible substitution for standard wound therapies as it is safe and effective. The aim of the current study was to develop an in vitro characterization of a hydrogel containing a combination of Guduchi, Neem, and Calendula officinalis extract for the treatment of wound healing in Diabetes.To prepare the hydrogel containing the Guduchi, Neem, and Calendula officinalis extract. To characterize the various parameters of the hydrogel containing the Guduchi, Neem, and Calendula officinalis extract. To study the effect of the permeation enhancer on the drug release profile of extract extract-containing gel.A polyherbal hydrogel containing Tinospora cordifolia (Guduchi), Azadirachata indica (Neem), and calendula Officinalis, extracts was prepared and Carbopol 980 was the gel agent. UV and FTIR studies were done in preformulation to check compatibility. The pH, viscosity, spreadability, swelling index, water vapor transmission rate (WVTR), in-vitro drug release, and kinetics were determined on the hydrogel formulations.The physicochemical properties of the optimized hydrogel indicated desirable pH (6.2-6.8), good viscosity to be used as a topical, and consistency during spreading. No chemical incompatibilities were detected using FTIR. WVTR, as well as swelling index, confirmed proper moisture retention. In-vitro release data showed sustained release and was according to Higuchi kinetics. The polyherbal formulation offered synergistic wound-healing activity in terms of antimicrobial (Neem), antioxidant (Guduchi), and collagen-activating (Calendula) effects.

Keywords: *Diabetic wound, Hydrogel, Neem, Guduchi, Calendula officinalis, Polyherbal formulation.*

1. INTRODUCTION

1.1 Hydrogel: Hydrogels are polymeric networks that absorb and retain large amounts of water without dissolving. Their hydrophilic groups allow water absorption, while crosslinks provide stability. They are flexible, biocompatible, biodegradable, injectable, and responsive to stimuli like pH and temperature [1].

Advantages: Elastic, strong, transparent, modifiable, biocompatible, and can release drugs/nutrients in a controlled manner.

Properties: Sensitive to environmental changes, tunable mechanical strength, and biocompatibility (biofunctionality + biosafety) [2].

Preparation Methods: Bulk polymerization, grafting, irradiation, physical cross-linking, and complex coacervation.

Characterization: pH, SEM, FTIR, swelling index, rheology, spreadability, and skin irritancy test.

Applications: Wound healing, GI drug delivery, and transdermal drug delivery [3].

1.2 Plant Profile

• Neem (Azadirachta indica): Meliaceae family; contains compounds like azadirachtin, nimbin, and quercetin. Known for antibacterial, antioxidant, and wound-healing activities [4].

• Guduchi (Tinospora cordifolia): Menispermaceae family; rich in alkaloids, glycosides, diterpenoids, and minerals. Exhibits antioxidant, anti-inflammatory, wound healing, and antidiabetic properties [5].
• Calendula officinalis (Marigold): Asteraceae family; contains flavonoids, terpenoids, and carotenoids. Used for antimicrobial, wound/burn healing, and UV-protective effects.

1.3 Skin Anatomy

Skin has three main layers:
• Epidermis – outer layer with keratinocytes, melanocytes, and multiple strata.
• Dermis – connective tissue with sweat glands [6], hair follicles, and blood vessels.
• Hypodermis – deepest layer with fat, nerves, and vessels.

1.4 Impact of Research

Wound care is essential; improper healing causes complications and costs. WHO reports ~80% of people rely on traditional medicine, and plant-derived bioactive compounds show promise for wound healing [7].

1.5 Diabetes and Wound Healing

Diabetes leads to delayed wound healing due to hyperglycemia, oxidative stress, neuropathy, hypoxia, and prolonged inflammation[8].
• Wound Healing Phases: Hemostasis → Inflammation → Proliferation → Remodeling.
• Pathophysiology in Diabetes: Impaired oxygen delivery, nerve damage, reduced keratinocyte migration, and chronic inflammation[9].
• Current Treatments: Debridement, wound dressings, off-loading, revascularization, and infection management (antibiotics, nanomaterials).

1.6 Medicinal Plants in Wound Healing

Medicinal plants enhance wound healing through antioxidant, antimicrobial, collagen-promoting, and fibroblast-stimulating actions[10]. Active compounds include flavonoids, tannins, triterpenes, saponins, zinc, and vitamin C.

1.7 Excipient Profile

• Carbopol 980: Rheology modifier used in gels, creams, and controlled-release systems.
• Propylene Glycol: Solvent, preservative, humectant [11].
• Glycerin: Humectant, emollient, cosolvent, and sweetening agent used in topical and cosmetic formulations.

2. MATERIAL'S & METHOD'S

2.1 Materials

Tinospora cordifolia (Guduchi), Azadirachta indica (Neem) and Calendula officinalis were sourced as certified extracts. Tricarbohydrin was added as the gelling agent, glycerin and propylene glycol as humectant whilst triethanolamine served as neutralizer. All other reagents were of analytical grade.

Preparation of Calendula, Neem &Guduchi Hydrogel (Carbopol 980-based)

Carbopol 980 was dispersed in 10 mL water and allowed to swell at room temperature overnight. The next day, calendula officinalis extract, neem extract, and guduchi extract were added, stirring at 100 rpm for 30 minutes until uniform. Propylene glycol, glycerol, and methyl paraben were then incorporated during continuous stirring. The pH was adjusted to 6.0–6.5 using 1 N NaOH. Various batches with differing amounts of extracts and Carbopol 980 were prepared [12] . Their compositions are summarized below:

Table: Composition of hydrogel containing calendula officinalis extract, neem extract, and guduchi extract

S.No	Formulation code	Amount of *calendula officinalis* (% w/w)	Amount of Neem extract (% w/w)	Amount of Guduchi (% w/w)	Carbopol 980 (% w/w)	Glycerol (% w/w)	Propylene Glycol (%)	Methyl paraben (% w/w)
1	CNGH1	3	5	2	1.5	0.5	3	0.01
2	CNGH2	5	5	2	1.5	0.5	3	0.01
3	CNGH3	7	5	2	1.5	0.5	3	0.01
4	CNGH4	10	5	2	1.5	0.5	3	0.01
5	CNGH5	5	1	2	1.5	0.5	3	0.01
6	CNGH6	5	2	2	1.5	0.5	3	0.01
7	CNGH7	5	3	2	1.5	0.5	3	0.01
8	CNGH8	5	3	2	1	0.5	3	0.01

3. EVALUATION PARAMETERS

1. Physical Appearance & Homogeneity

• Visually assess color, clarity, and aggregation under light [12].

• Check homogeneity by rubbing a small amount between thumb and index finger—note any particles or lumps.

2. pH

• Dissolve 1 g hydrogel in 20 mL purified water; stir at 100 rpm for 15 min (room temperature).

• Use a calibrated pH meter; clean electrode, immerse, wait for stabilization, then record.

• Report the mean ± SD [13].

3. Viscosity

• Use a viscometer at 25 °C: place 100 g of gel under the spindle, measure at 50 rpm, let run 20 min to stabilize [14].

• Record in centipoise, in triplicate.

4. Spreadability

• Use the glass slide "drag" method: place sample between slides, pull top slide 10 cm using a 10 g weight.

• Calculate as T = 2500 / C = 1. Perform in triplicate [15].

5. Swelling Index

• Soak 1 g of hydrogel in water; remove after 1, 2, and 3 h, dry, and reweigh [16].

• Repeat in triplicate.

• Calculate:

$$\text{Swelling index (\%SW)} = \frac{(Wt - W0)}{W0} \times 100$$

6. Water Vapor Transmission Rate (WVTR)

Gel films (10 g) were mounted over glass tubes and placed in a desiccator with silica gel. The decrease in weight was used to calculate the transmission rate [17].

7. Release-Kinetics Study

Assess drug release using standard models:

- Korsmeyer–Peppas Model (diffusion-controlled release):

$$A_t/A_\infty = at^n$$

o aaa: coefficient related to matrix structure
o nnn: release exponent indicating mechanism [18].

4. RESULT & DISCUSSION

4.1 Preformulation Studies

4.1.1 Organoleptic properties

The organoleptic character of the calendula officinalis, Neem extract and Guduchi powder extract is shown in Table 1.1.

Table 1.1: Organoleptic character

S.No.	Extract	Colour	Odour
1	*Calendula officinalis* extract powder	Dark brown	Characteristics
2	Neem extract	Dark amber color powder	Characteristics
3	Guduchi powder	Light Gray color powder	Characteristics

4.1.2 UV spectroscopy

4.1.2.1 Determination of the absorption maxima

The absorption maxima of the calendula officinalis extract and neem extract were determined in methanol solvent using UV spectroscopy. On scanning of working solution 50µg/ml of calendula officinalis and 140 µg/ml of neem extract, the absorption maxima were observed to be 247nm [18] and 223nm respectively, as indicated in the UV spectrum (Figure 1.1-1.2)

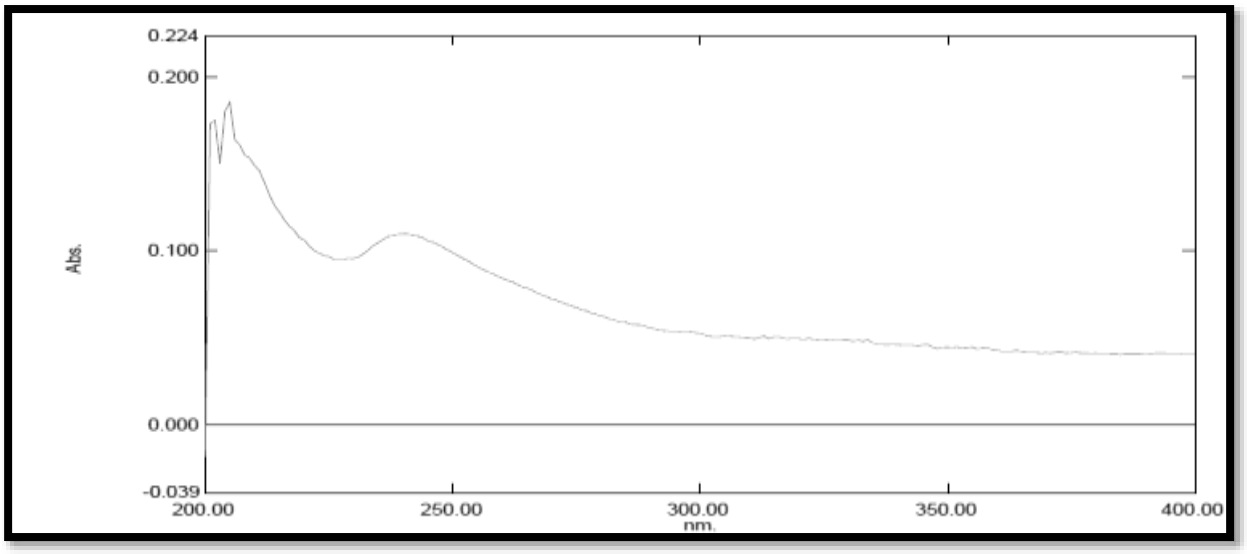

Figure.1.1: UV spectrum graph of the concentration 50µg/ml of calendula officinalis in methanol

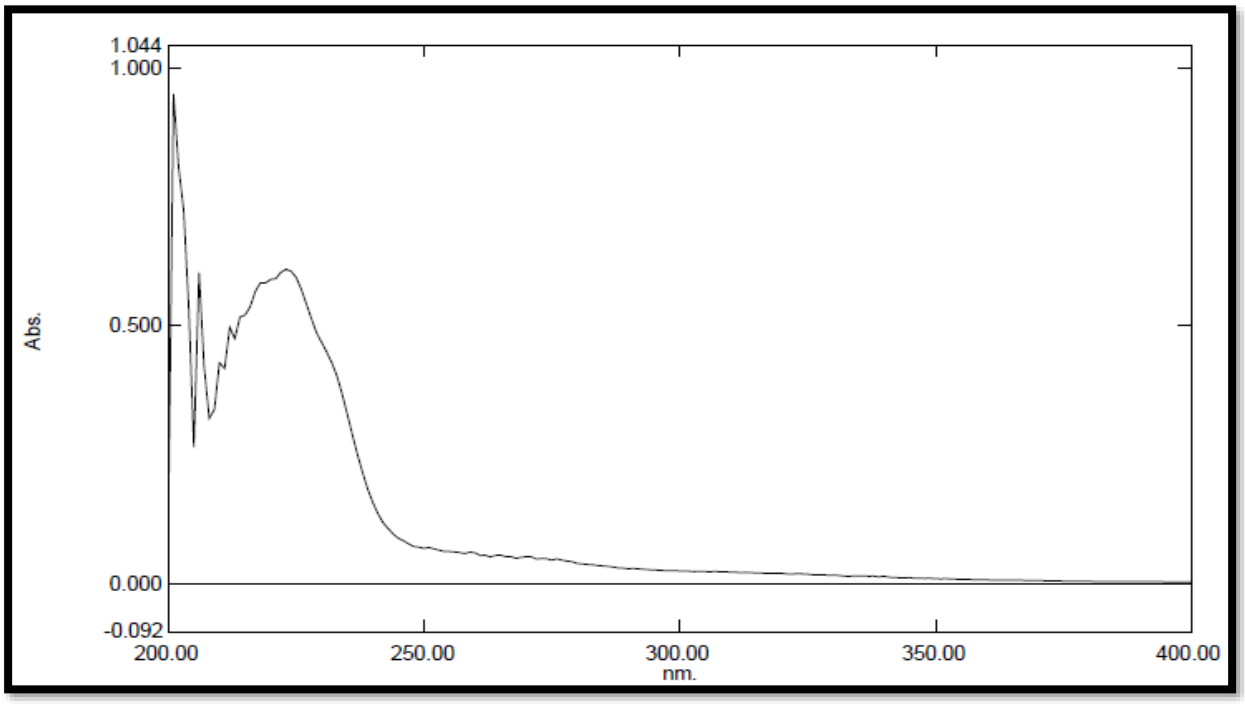

Figure 1.2: UV spectrum graph of the concentration 140µg/ml of neem extract powder in methanol

4.1.2.2 Identification of Isoabsorptive point

The isoabsorptive point of the calendula officinalis extract and neem extract was determined in methanol solvent using the overlay spectra of the both extract via UV spectroscopy. The working solution of concentrations 50µg/ml of calendula officinalis extract and 140µg/ml of neem extract was analyzed and overlay spectra was obtained. The isoabsorptive point was observed to be at 250 nm as indicated in Figure 1.3.

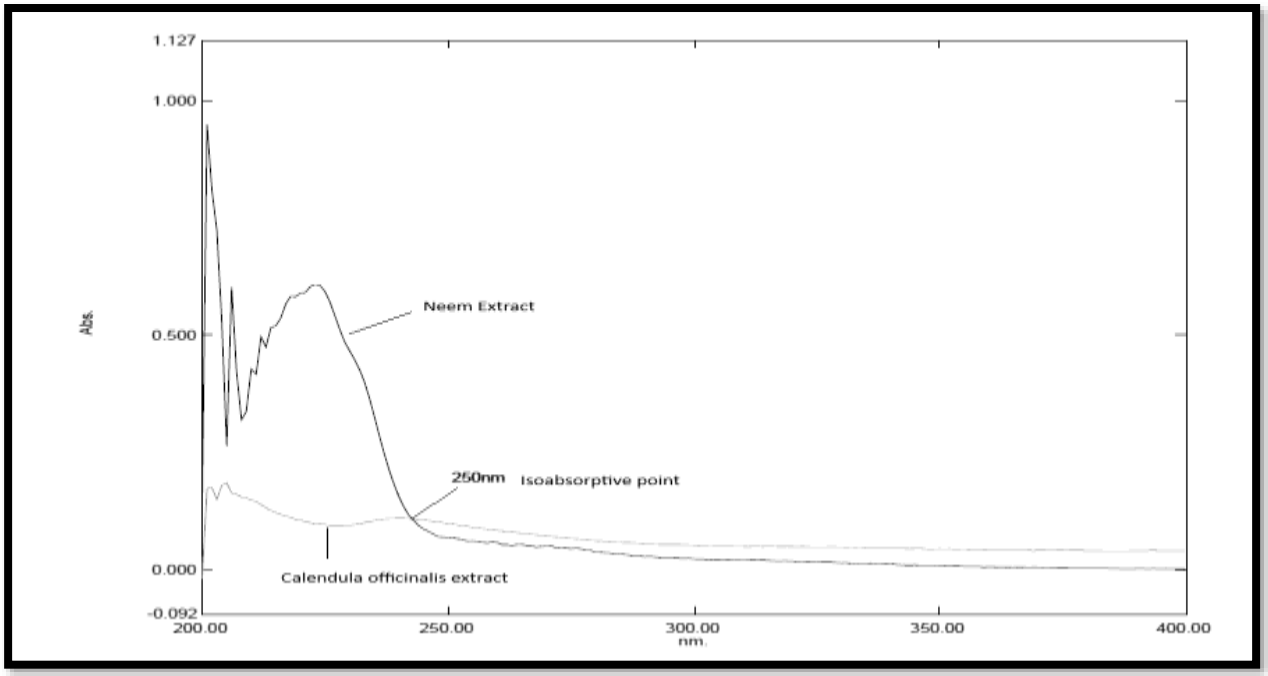

Figure 1.3: Overlay spectra of calendula officinalis extract (50µg/ml) and neem extract (140µg/ml)

4.1.2.3 Preparation of standard curve of calendula officinalis extract in methanol

For preparation of standard calibration curve a series of the working solution ranging 50-500µg/ml wereanalyzed at 247nm. The absorbance of each working solution was recorded as indicated in Table 1.2.

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 24s, 2025
https://www.theaspd.com/ijes.php

Table 1.2: Absorbance of working solution at 247nm

S.No.	Con (µg/ml)	Absorbance
1	50	0.096±0.002
2	100	0.187±0.003
3	150	0.278±0.003
4	200	0.372±0.003
5	250	0.452±0.004
6	300	0.548±0.008
7	350	0.638±0.002
8	400	0.734±0.006
9	450	0.824±0.005
10	500	0.918±0.004

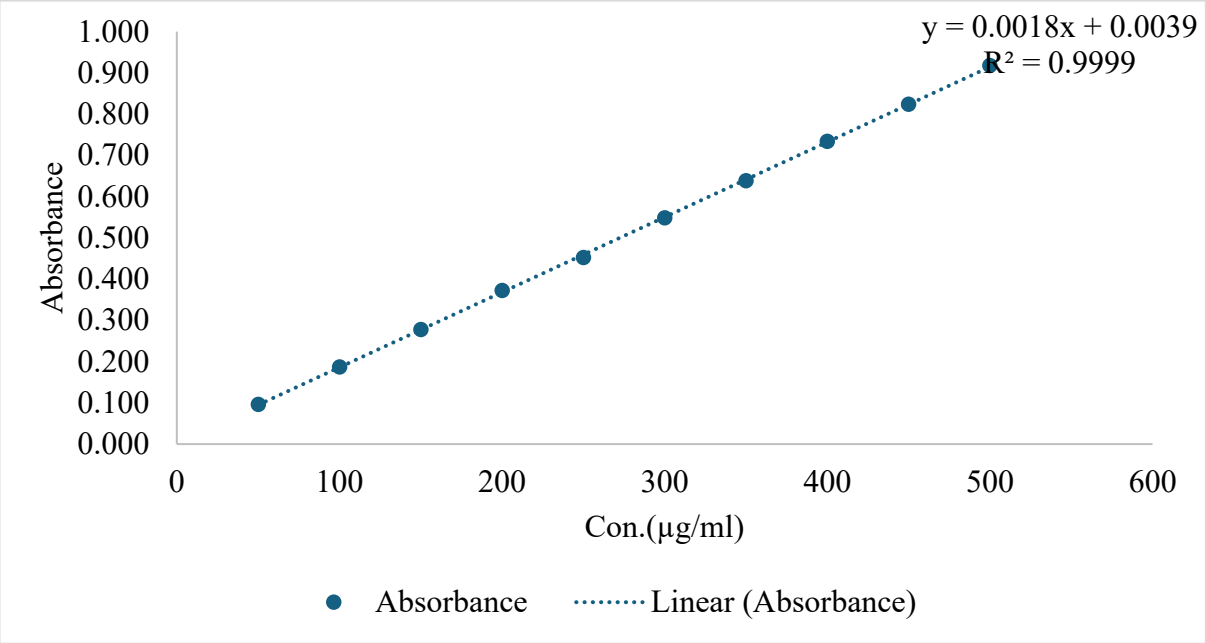

Figure 1.4: Standard calibration curve of calendula officinalis extract in methanol

The standard linearity curve for calendula officinalis extract in methanol was displayed in Figure 1.4. The standard linearity curve of calendula officinalis extract was prepared in a series of concentration ranging 50 to 500 µg/ml at 247nm using methanol solvent. The regression equation Y = 0.0018x+0.0039 and R2 value 0.999 exhibits strong linearity.

4.1.2.4 Preparation of standard curve of neem extract in methanol

For preparation of standard calibration curve a series of the working solution ranging 20-200µg/ml wereanalyzed at 223nm. The absorbance of each working solution was recorded as indicated in Table 1.3.

Table 1.3: Absorbance of working solution at 223nm

S.No.	Con (µg/ml)	Absorbance
1	20	0.093±0.004
2	40	0.170±0.006
3	60	0.249±0.005
4	80	0.332±0.007
5	100	0.409±0.004
6	120	0.495±0.005
7	140	0.590±0005
8	160	0.653±0.005

9	180	0.735±0.007
10	200	0.829±0.004

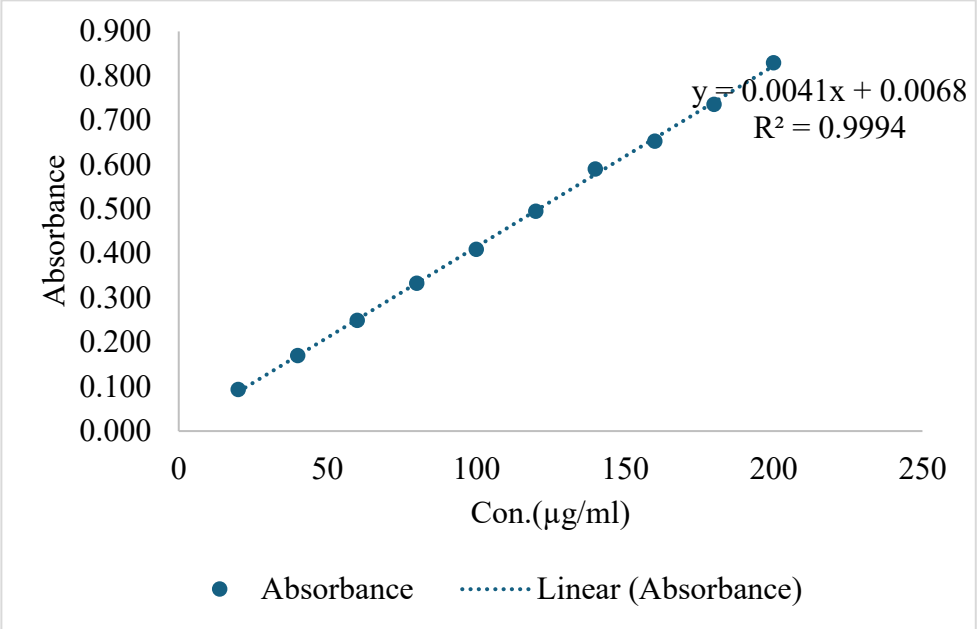

Figure 1.5: Standard calibration curve of neem extract in methanol

The standard linearity curve for neem extract in methanol was displayed in Figure 1.5. The standard linearity curve of neem extract was prepared in a series of concentration ranging 20 to 200 µg/ml at 223nm using methanol solvent. The regression equation Y = 0.0041x+0.0068and R2 value 0.999 exhibits strong linearity.

4.1.2.5 Preparation of standard curve of calendula officinalis extract at isoabsorptive point

For preparation of standard calibration curve at isoabsorptive point a series of the working solution ranging 50-500µg/ml was analyzed at 250nm. The absorbance of each working solution was recorded as indicated in Table 1.4.

Table 1.4: Absorbance of working solution at 250nm

S.No.	Con (µg/ml)	Absorbance of 250nm
1	50	0.096±0.003
2	100	0.143±0.004
3	150	0.196±0.002
4	200	0.247±0.003
5	250	0.293±0.004
6	300	0.345±0.003
7	350	0.392±0.003
8	400	0.439±0.003
9	450	0.494±0.005
10	500	0.529±0.004

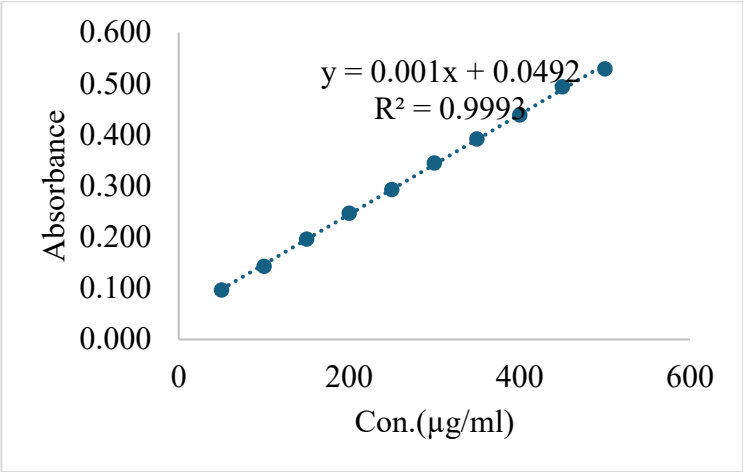

Figure 1.6: Standard calibration curve of calendula officinalis extract at isoabsorptive point.

The standard linearity curve for calendula officinalis extract in methanol is displayed in Figure 1.6. The standard linearity curve of calendula officinalis extract was prepared in a series of concentrations ranging from 50 to 500 µg/ml at 250nm using methanol solvent. The regression equation Y = 0.001x+0.0492 and R2 value 0.999 exhibits strong linearity.

4.1.2.6 Preparation of the standard curve of neem extract at the isoabsorptive point

For the preparation of a standard calibration curve at the isoabsorptive point, a series of working solutions ranging from 20-200µg/ml was analyzed at 250nm. The absorbance of each working solution was recorded as indicated in Table 1.5.

Table 1.5: Absorbance of working solution at 250nm

S.No.	Con (µg/ml)	Absorbance of 250nm
1	20	0.062±0.003
2	40	0.082±0.003
3	60	0.105±0.004
4	80	0.130±0.004
5	100	0.150±0.003
6	120	0.173±0.005
7	140	0.196±0.003
8	160	0.212±0.002
9	180	0.237±0.004
10	200	0.262±0.004

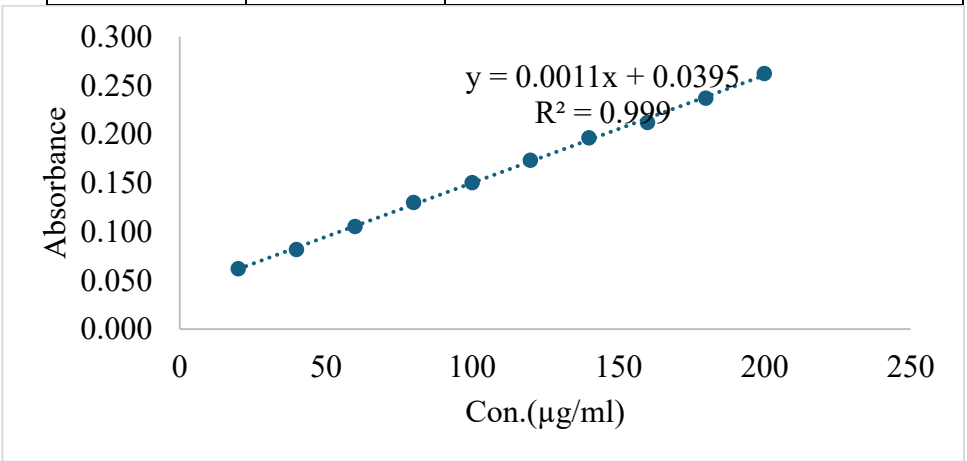

Figure 1.7: Standard calibration curve of neem extract at the isoabsorptive point.

The standard linearity curve for neem extract in methanol is displayed in Figure 1.7. The standard linearity curve of neem extract was prepared in a series of concentrations ranging 20 to 500 µg/ml at 250nm using methanol solvent. The regression equation Y = 0.0011x+0.0395 and R2 value 0.999 exhibits strong linearity.

4.1.3 Absorbance subtraction method

Absorbance subtraction measurements were made at 247 nm for calendula officinalis extract and 223 nm for neem extract, with 250 nm chosen as the iso-absorptive point at which calendula officinalis extract and neem extract could both be assessed in the presence of the others [19].Since the only requirements for this method are that both components have isoabsorptive points and that one component's spectra be prolonged, we will use their isoabsorptive point at 250 nm to identify neem extract and calendula officinalis [20]

Absorption Factor is calculated by the formula mentioned below

$$Absorption\ factor = \frac{Absorbanc\ at\ isoabsorptive\ point}{Absorbance\ at\ absorption\ maxima\ of\ drug}$$

Table 1.6: Absorbance factor of calendula officinalis

S.No.	Con.(µg/ml)	Abs at 250m	Abs at 247 nm	Absorption factor
1	50	0.096	0.096	1.000
2	100	0.143	0.187	0.765
3	150	0.196	0.278	0.707
4	200	0.247	0.372	0.663
5	250	0.293	0.452	0.648
6	300	0.345	0.548	0.629
7	350	0.392	0.638	0.614
8	400	0.439	0.734	0.598
9	450	0.494	0.824	0.703
10	500	0.529	0.918	0.666
			Average	0.699

Absorption factor for calendula officinalis extract powder was found to be 0.699.

4.1.4 Solubility

The solubility of calendula officinalis extract and neem extract was assessed in solvents as indicated in Table 1.7.-1.8.

Table 1.7: Solubility data of calendula officinalis extract

S.No.	Solvent	*Calendula officinalis* Solubility (mg/ml)	Description (Awasthi et al., 2016)
1	Water	0.836±0.039	Very slightly soluble
2	Methanol	42.136±0.404	Soluble
3	Ethanol	35.12±0.807	Soluble
4	Phosphate buffer pH 6.8	1.607±0.049	Slightly soluble

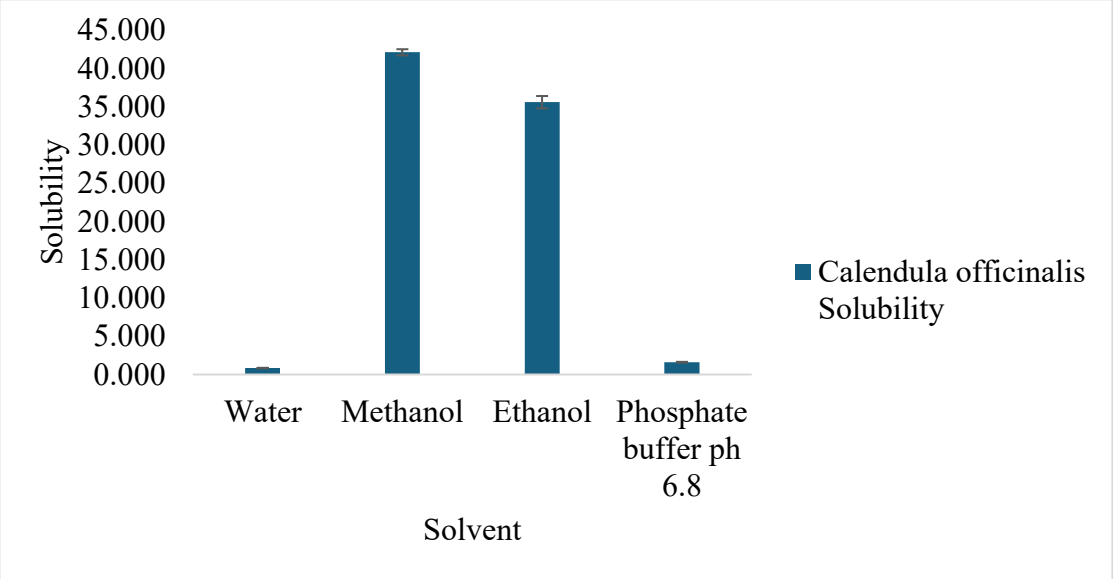

Figure 1.8: Bar graph of the solubility of the calendula officinalis extract

Table 1.8: Solubility data of neem extract powder

S.No.	Solvent	Neem extract solubility (mg/ml)	Description (Kulkarni et al., 1999)
1	Water	6.582±0.037	Slightly soluble
2	Methanol	34.392±0.310	Soluble
3	Ethanol	29.413±0.414	Sparingly soluble
4	Phosphate buffer pH 6.8	1.875±0.098	Slightly soluble

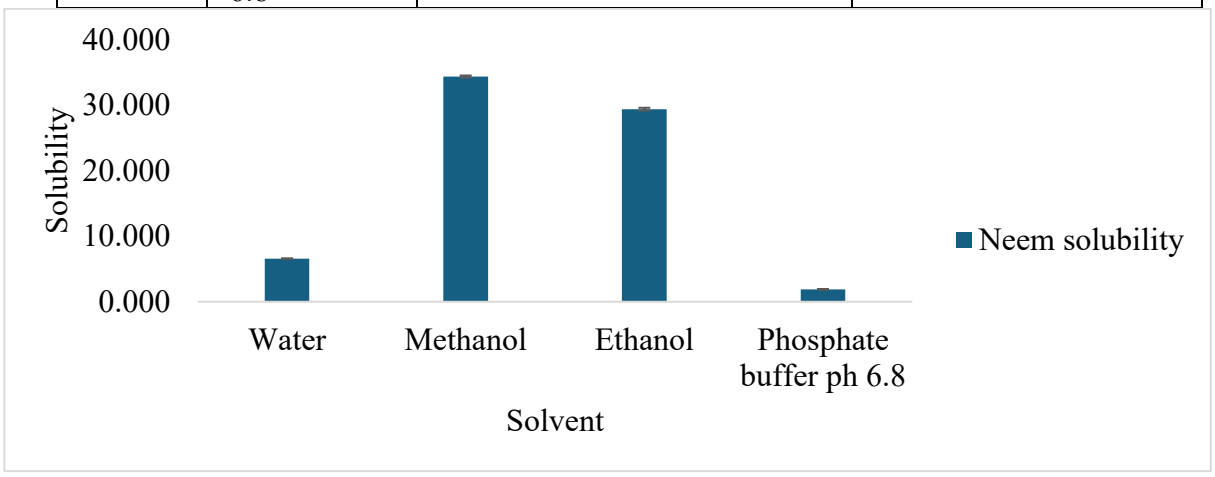

Figure 1.9: Bar graph of the solubility of the neem extract.

Figure 1.8-1.9 demonstrated that calendula officinalis extract and neem extract powder were both soluble in methanol and slightly soluble in water.

4.1.5 Partition coefficient determination

The partition coefficients of neem extract and calendula officinalis extract were determined to be 0.984±0.011 and 1.516±0.022, respectively.

4.1.6 FTIR spectroscopy

FTIR spectra of calendula officinalis extract, neem extract powder, Guduchi extract, and selected hydrogel formulations are shown in Figure 1.10-1.13

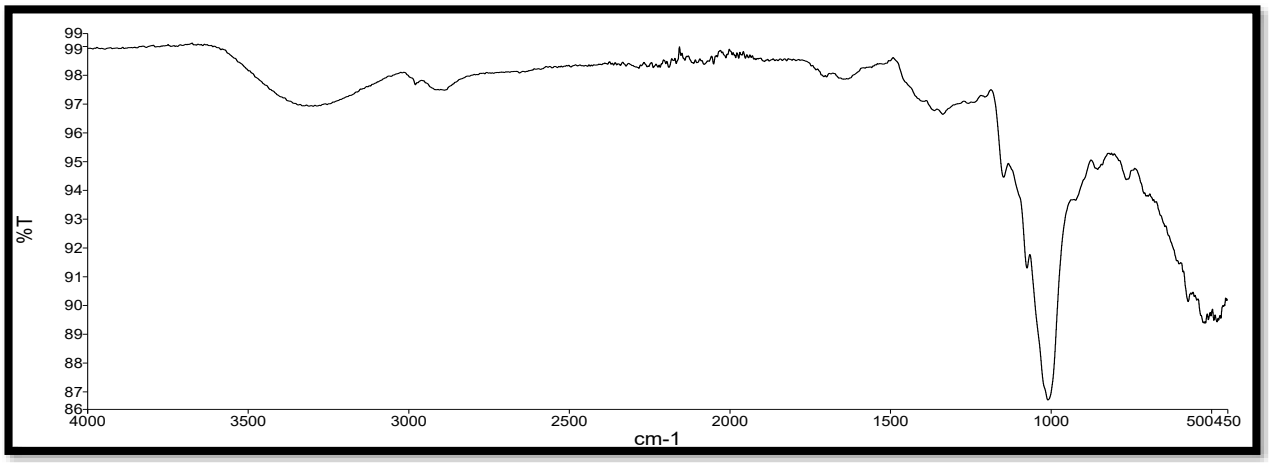

Figure 1.10: FTIR spectra of calendula officinalis

FTIR spectrum of calendula officinalis extract indicated the peaks at wavenumber of 3300cm−1, corresponds to Alkanes, H-bonded Alcohols, phenols. The peaks at wavenumber of 2880 cm−1, 1647cm−1 and 1146cm−1 were corresponding to O-H Carboxylic acid, N-H bending Amines and C–O and C C-N stretch Aliphatic amin, respectively (Al-Mussawi and Al-Hussan, 2019).

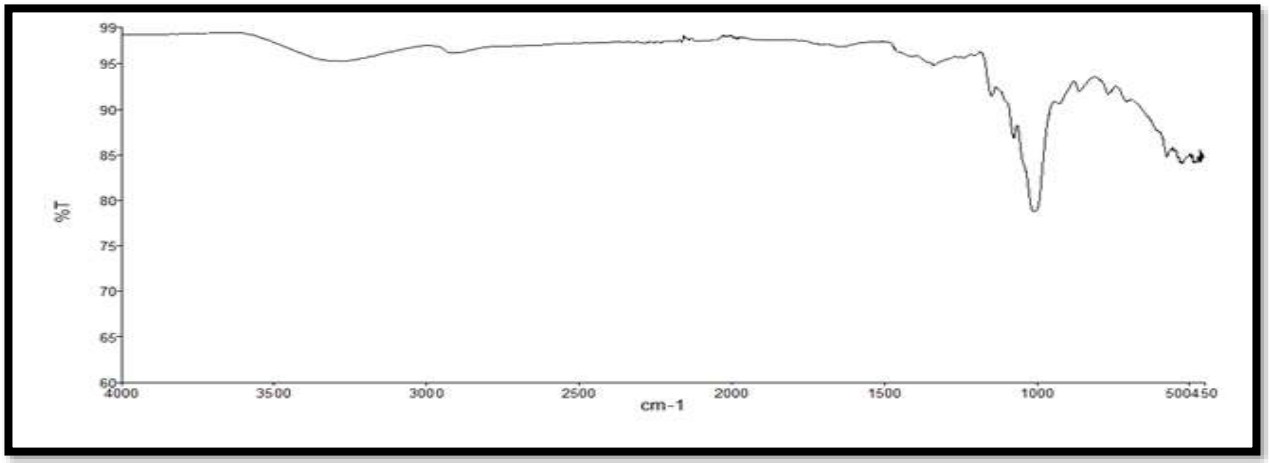

Figure 1.11: FTIR spectra of neem extract

The Figure 1.10 displays the FTIR spectra of neem extract. The peak present at 3280 cm−1 indicates (N-H stretching vibrations) primary and secondary amines; 2920cm−1 (C-H stretching vibrations) the alkanes; 1334 cm−1 (C-H bend stretching vibration) the alkanes [21].

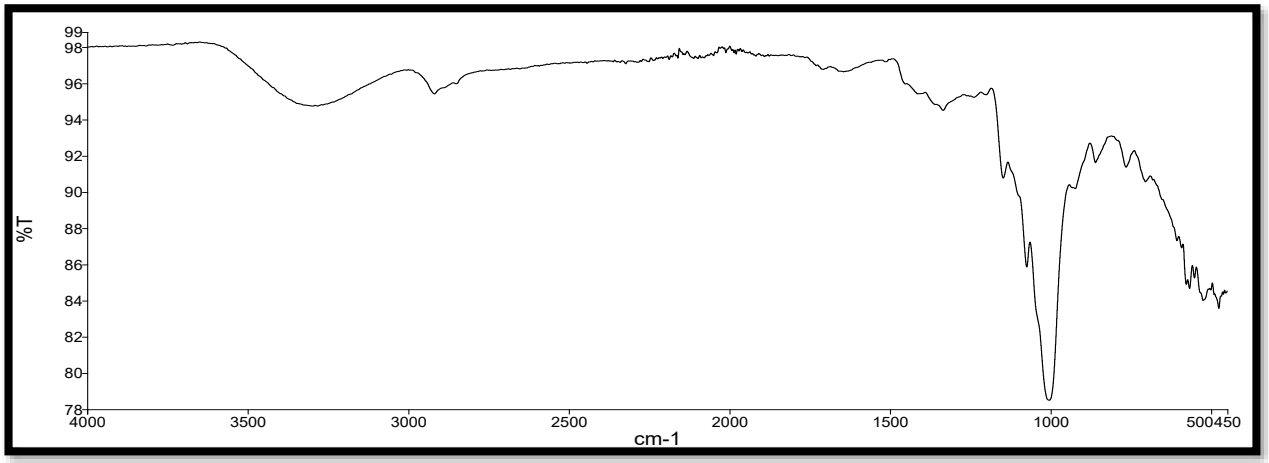

Figure 1.12: FTIR spectra of Guduchi extract

FTIR spectrum of guduchi indicated the peaks at wavenumber of 3200 cm−1, corresponding to –OH functional groups present in alkaloids, flavonoids, glycosides, steroids, and polyalcohols. The peaks at wavenumber of 2920 cm−1, 1640 cm−1, and 1004 cm−1 corresponded to C–H stretching vibrations of alkanes, C=O stretching vibration of fatty acids, and C–O–C stretching vibrations, respectively **(Gollapudi et al.**

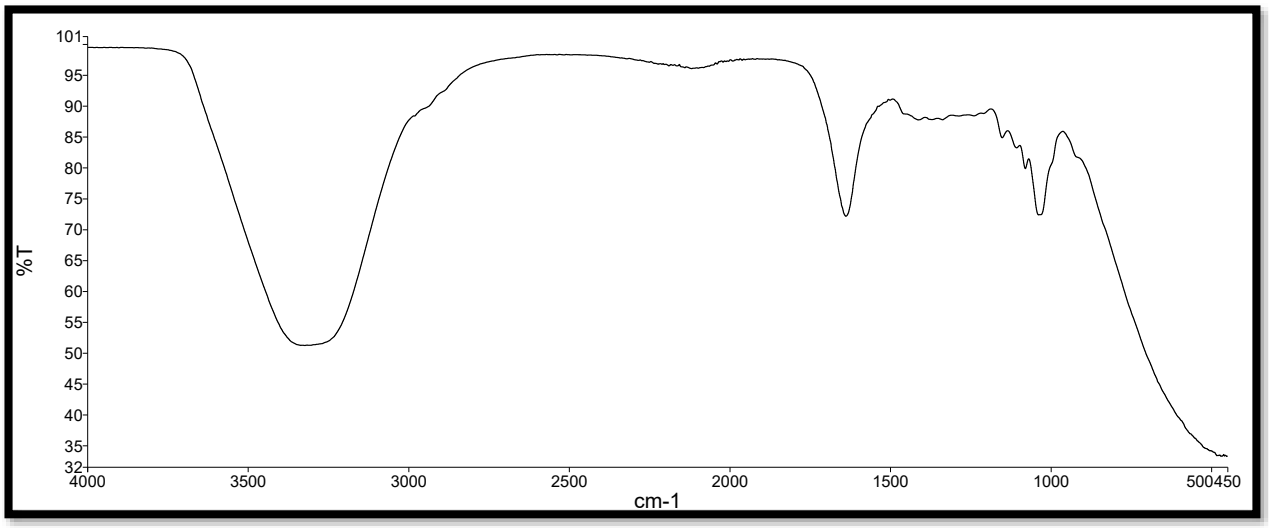

Figure 1.13: FTIR spectra of hydrogel containing calendula officinalis extract, neem extract, and guduchi extract.

FTIR spectrum of extract extract-loaded hydrogel confirmed that individual peaks of all three extracts were not identified, indicating the uniform solubilization of the extract in the gel.

4.2 Preparation of calendula officinalis extract, neem extract, and Guduchi extract incorporated hydrogel

Hydrogel's low polluting, good adherence, and spreadability make it an ideal solution for treating localized skin diseases. A hydrogel for cutaneous drug delivery has been prepared using Carbopol. The encapsulated medications nearly exhibit good skin penetration and deposition in the hydrogel. Within the research [12]. In order to transform aqueous medication solutions into hydrogels, which are ideal for topical application, a hydrogel forming agent is needed. The hydrogel-forming agent chosen for the current study is carbopol 980. The study involves the incorporation of the calendula officinalis extract, neem extract, and Guduchi extract in the carbopol hydrogel along with the propylene glycol, glycerin, and methyl paraben. The effect of various concentrations of the calendula officinalis extract, neem extract, and the amount of the carbopol was investigated on the properties of the hydrogel [12].

4.3 In-vitro characterization of calendula officinalis extract, neem extract and Guduchi extract incorporated hydrogel

4.3.1 Physical appearance

The physical appearance evaluation involves the assessment of the color, presence of lumps and clarity. Table 1.9 indicated that all prepared multiextracts containing hydrogel were dark Grseyish brown in color, clear and free from aggregation.

Table 1.9: Visually observed for the physical appearance of the prepared formulations

S.No.	Formulation code	Physical appearance
1	CNGH1	Dark Greyish brown color, clear, free from particles, viscous hydrogel
2	CNGH2	Dark Greyish brown color, clear, free from particles, viscous hydrogel
3	CNGH3	Dark Greyish brown color, clear, free from particles, viscous hydrogel
4	CNGH4	Dark Greyish brown color, clear, free from particles, viscous hydrogel

5	CNGH5	Dark Greyish brown color, clear, free from particles, viscous hydrogel
6	CNGH6	Dark Greyish brown color, clear, free from particles, viscous hydrogel
7	CNGH7	Dark Greyish brown color, clear, free from particles, viscous hydrogel
8	CNGH8	Dark Greyish brown color, clear, free from particles, viscous hydrogel
9	CNGH9	Dark Greyish brown color, clear, free from particles, viscous hydrogel
10	Placebo hydrogel	Clear, transparent and free from particles

Figure 1.14: Image of physical appearance of formulation CNGH9

4.3.2 Homogeneity and Grittiness

The observations related to the homogeneity, and grittiness of the all-prepared formulations was shown in Table 1.10.

Table 1.10: Homogeneity, and Grittiness observations for the physical appearance of the prepared formulations

S.No.	Formulation code	Homogeneity, and Grittiness
1	CNGH1	Homogenous and free from Grittiness
2	CNGH2	Homogenous and free from Grittiness
3	CNGH3	Homogenous and free from Grittiness
4	CNGH4	Homogenous and free from Grittiness
5	CNGH5	Homogenous and free from Grittiness
6	CNGH6	Homogenous and free from Grittiness
7	CNGH7	Homogenous and free from Grittiness
8	CNGH8	Homogenous and free from Grittiness

9	CNGH9	Homogenous and free from Grittiness
10	Placebo hydrogel	Homogenous and free from Grittiness

The prepared batches of the calendula officinalis extract, neem extract and Guduchi extract were evaluated for homogeneity, and grittiness. All prepared batches were Homogenous and free from Grittiness except the formulation CNGH8 have less viscosity due to the low concentration of the gelling agent. Homogeneity test of hydrogel shows the uniformity in formulation and checks for the presence of any particles or clumps. No clumps or clusters were observed in current hydrogel formulation; therefore, it is concluded that prepared gel by both methods has good homogeneity [22].

4.3.3 pH

The pH all prepared batches of multiextract containing carbomer 980 based hydrogel was shown in Table 1.11. The pH of the prepared batches of the hydrogel observed to be ranging from 6.167±0.051 to 6.330±0.108. All the prepared hydrogels that were tested obtained a pH value acceptable for its application on the skin (4.5–8) consequently, indicating a safe topical preparation [17].

Table 1.11: pH of the prepared formulations

S.No.	Formulation code	pH
1	CNGH1	6.240±0.070
2	CNGH2	6.330±0.108
3	CNGH3	6.307±0.031
4	CNGH4	6.167±0.051
5	CNGH5	6.217±0.021
6	CNGH6	6.307±0.038
7	CNGH7	6.200±0.053
8	CNGH8	6.250±0.035
9	CNGH9	6.197±0.015
10	Placebo hydrogel	6.140±0.036

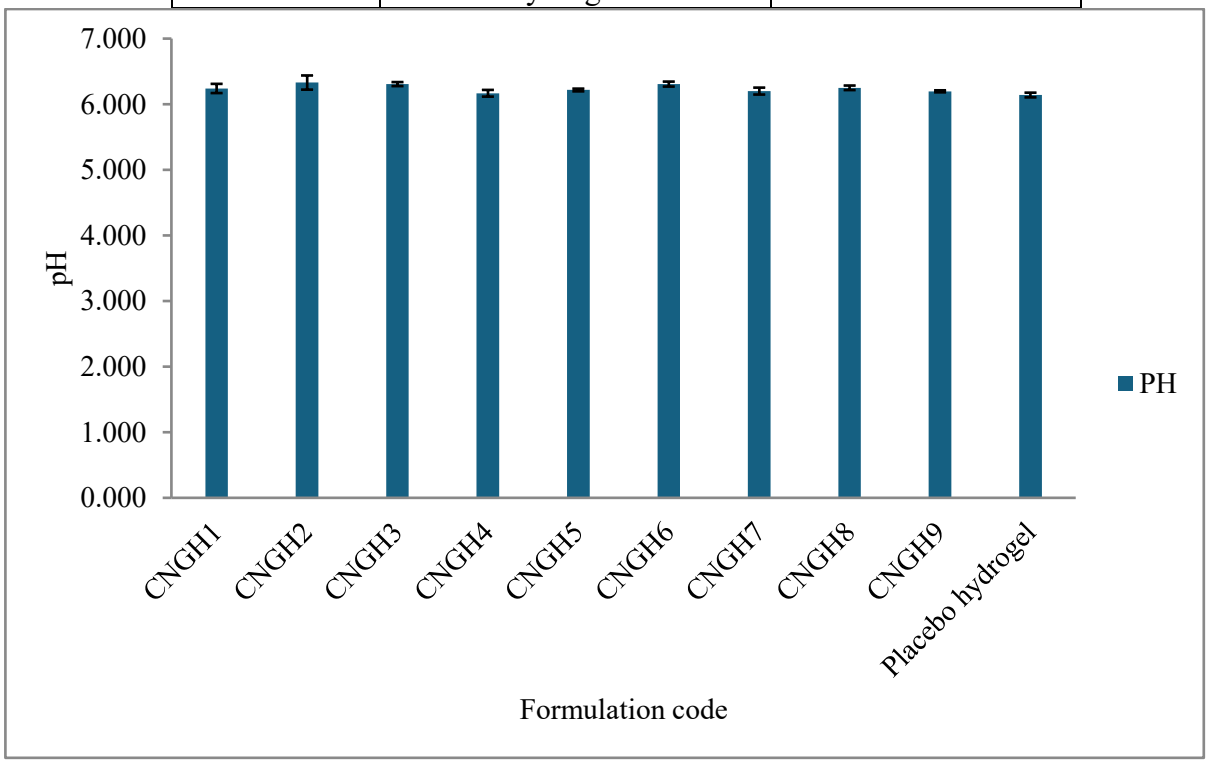

Figure 1.15: pH of the all-prepared formulation

4.3.4 Spreadability

One crucial characteristic of hydrogel topical formulations is their spreadability, which indicates how easily the gel will disperse after application with minimal shear stress. Its use will be favorable if its spreadability is good. Consistent application also contributes to consistent treatment outcomes. Table 1.12 illustrates the good spreadability of the hydrogel formulations used in this investigation, which are very easy to apply with very little shear stress [22]. The spreadability of the all-prepared batches of the multiextract containing hydrogel formulation ranged from 9.233±0.179gm.cm/sec to 30.619±0.547gm.cm/sec. In the current study amount of the extract did not significantly affect the spreadability of the hydrogel, but the concentration of hydrogel hydrogel-forming agent affected the Spreadability of the hydrogel formulation. In the present study, the formulation CNGH9 possesses the desirable spreadability close to the spreadability of the placebo hydrogel [17].

Table 1.12: Spreadability of the prepared formulations

S.No.	Formulation code	Spreadability (gm.cm/sec)
1	CNGH1	17.547±0.308
2	CNGH2	16.579±0.321
3	CNGH3	16.055±0.544
4	CNGH4	14.783±0.336
5	CNGH5	19.119±0.553
6	CNGH6	14.852±0.127
7	CNGH7	15.387±0.237
8	CNGH8	30.619±0.547
9	CNGH9	9.233±0.179
10	Placebo hydrogel	10.101±0.512

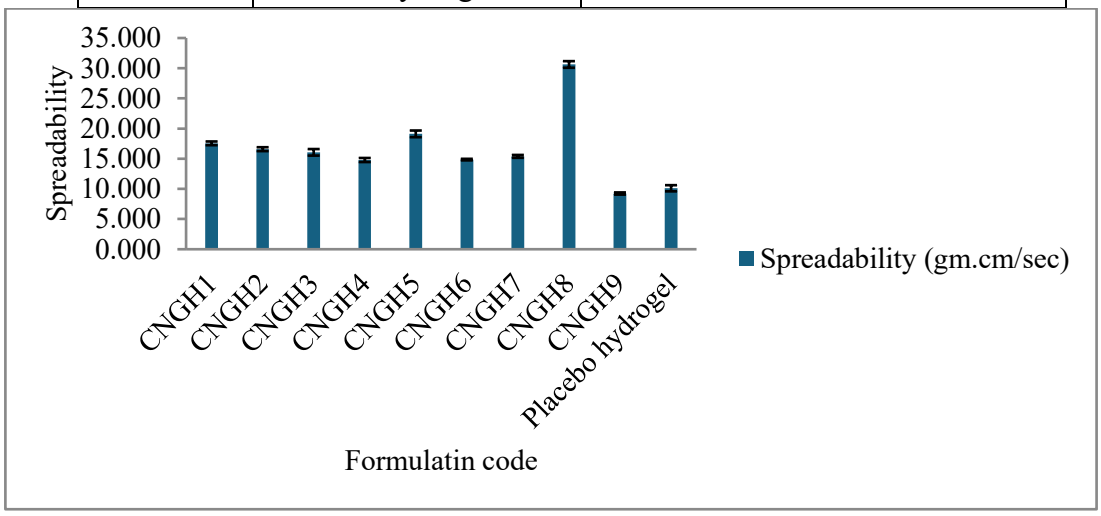

Figure 1.16: Spreadability of the all-prepared formulation

4.3.5 Swelling index

The swelling index of all prepared batches of multiextract containing carbomer 980 based hydrogel was shown in Table 1.13. The swelling index of the all prepared batches of the hydrogel observed to be ranging from 31.733±0.988% to 69.465±0.198% at 1hr, 49.233±0.729% to 84.013±0.090% at 2hr and 54.120±0.893% to 89.446±0.039% at 3hr. Table 1.13 showed that while the swelling index of the hydrogel formulation increased with increasing extract concentration, the generated hydrogel formulation's swelling was unaffected by higher extract concentrations. Likewise, the swelling of artificial hydrogels rises with increasing carbopol composition due to the high carboxylic group content in carbopol, which ionizes more at higher pH values and causes repulsion between the same group charges, which in turn causes swelling. CACG9 showed the ideal swelling index out of all the developed formulations [23].

Table 1.13: % Swelling index of the prepared formulations

S.No.	Formulation code	% Swelling index		
		1hr	2hr	3hr
1	CNGH1	53.159±0.465	66.995±0.308	74.842±0.134
2	CNGH2	59.178±0.707	69.370±0.332	76.047±0.203
3	CNGH3	61.164±0.320	71.428±0.231	77.116±0.148
4	CNGH4	60.231±0.783	70.926±0.478	77.876±0.069
5	CNGH5	63.570±0.094	54.233±0.148	68.453±0.281
6	CNGH6	65.576±0.251	77.194±0.184	80.657±0.106
7	CNGH7	66.666±0.157	78.118±0.135	82.340±0.507
8	CNGH8	31.733±0.988	49.233±0.729	54.120±0.893
9	CNGH9	69.465±0.198	84.013±0.090	89.446±0.039
10	Placebo hydrogel	65.215±0.428	80.311±0.384	88.745±0.009

4.3.6 Water vapor transmission rate

The water vapor transmission rate of all prepared formulations is shown in Table 1.14. The water vapour transfer rates (WVTRs) of all prepared batches of the hydrogel formulation were observed to be ranging from 929.989±1.589 g/m2 *day to 5096.459±0.818 g/m2*day. The water of the placebo hydrogel formulation was observed to be 3973.421±1.714 (g/m2 *day).

Table 1.14: Water vapor transmission rate of the all-prepared formulation

Sr.No.	Formulation code	WVTR (g/m^2 *day)
1	CNGH1	929.989±1.589
2	CNGH2	1372.703±1.264
3	CNGH3	2109.905±0.989
4	CNGH4	2281.854±1.816
5	CNGH5	889.361±1.040
6	CNGH6	2521.166±0.980
7	CNGH7	2793.505±1.180
8	CNGH8	401.431±1.040
9	CNGH9	3916.935±0.818
10	Placebo hydrogel	3973.421±1.714

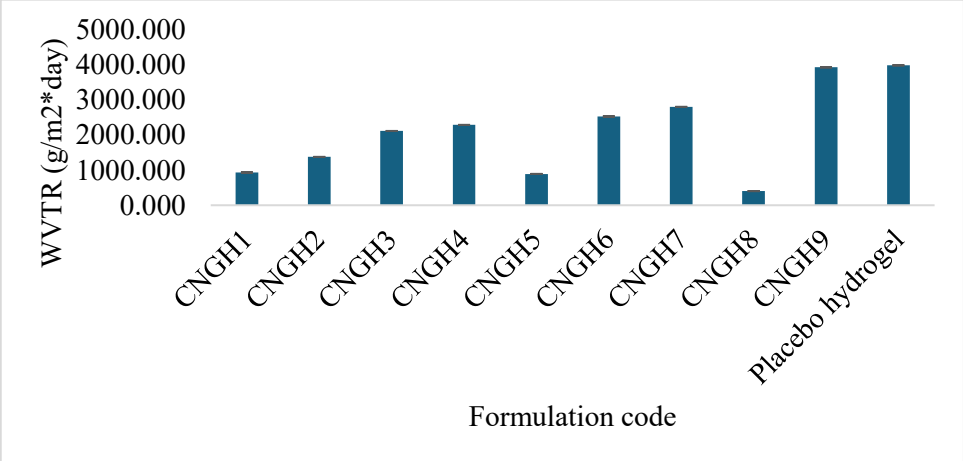

Figure 1.17: Water Vapor Transmission Rate of all prepared formulations

The WVTR of the different formulations varies with the amounts of carbopol and extract used. Hydrogel sheets with lower carbopol content exhibit a lower WVTR, whereas those with higher carbopol content demonstrate increased porosity. Therefore, the gelling agent's ability to transfer air or vapor to and from the hydrogel depends on its concentration. The WVTR is crucial for maintaining the hydrogel's moisture content. A higher WVTR results in a very moist hydrogel, while a lower WVTR leads to a drier hydrogel. Thus, a hydrogel should possess an optimum WVTR to effectively wet the wound bed and support the healing process. The WVTR of the CACG7 formulation is near the authorized range and comparable to that of the placebo hydrogel [24].

4.3.7 Percentage drug content

The observations related to the percentage drug content of all prepared formulations were shown in Table 1.15. The percentage drug content of the calendula officinalis extract and neem extract in the prepared batches of the hydrogel was observed to be 67.325±0.712% to 90.164±0.352% for calendula officinalis extract and 73.331±0.446% to 90.348±0.772% for neem extract.

Table 1.15: Percentage drug content of the prepared formulations

S.No.	Formulation code	Percentage drug content of calendula officinalis (%)	Percentage drug content of neem extract (%)
1	CNGH1	67.325±0.712	73.331±0.446
2	CNGH2	77.628±0.291	86.412±0.737
3	CNGH3	81.545±0.264	81.865±0.912
4	CNGH4	84.542±0.807	82.095±1.053
5	CNGH5	74.459±0.370	74.245±0.680
6	CNGH6	78.700±0.427	81.236±0.731
7	CNGH7	83.806±0.870	82.334±0.885
8	CNGH8	78.840±0.565	64.576±0.846
9	CNGH9	90.164±0.352	90.348±0.772

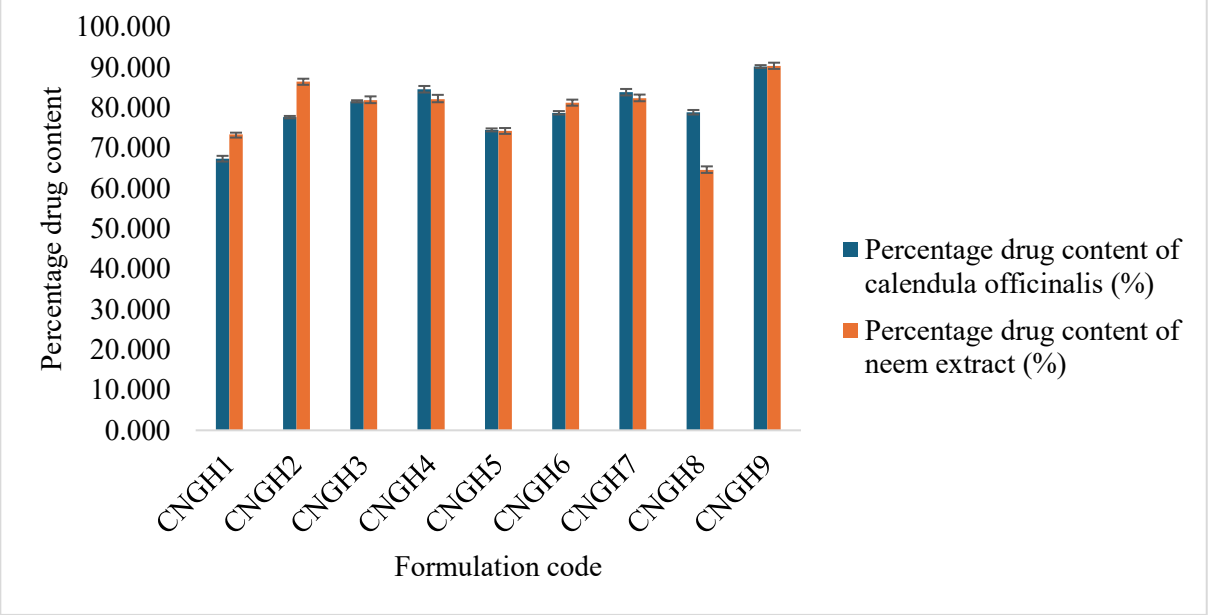

Figure 1.18: Percentage drug content of the prepared formulations

As the carbopol concentration rises, so does the extract percentage in the hydrogel (Figure 1.18). In the same way, increasing the extract's concentration results in a higher percentage of content. The percentage content of extract in hydrogel is dependent on the hydrogel's swelling index; the higher the hydrogel's swelling, the higher the extract's percentage content [23].

4.3.8 Viscosity

The observations related to the viscosity of all prepared formulations are shown in Table 1.16. The viscosity of all prepared batches of the hydrogel was observed to be 1555.333±0.577 cp to 3316.000±1.732cp. The viscosity of the placebo hydrogel was observed to be 2896.667±1.155cp.

Table 1.16 Viscosity of the prepared formulations

S.No.	Formulation code	Viscosity (Cp)
1	CNGH1	1555.333±0.577
2	CNGH2	1885.667±2.082
3	CNGH3	1913.667±1.528
4	CNGH4	2006.000±2.646
5	CNGH5	1796.667±1.520
6	CNGH6	1997.000±1.732
7	CNGH7	2052.333±2.517
8	CNGH8	1115.667±2.887
9	CNGH9	3316.000±1.732
10	Placebo hydrogel	2896.667±1.155

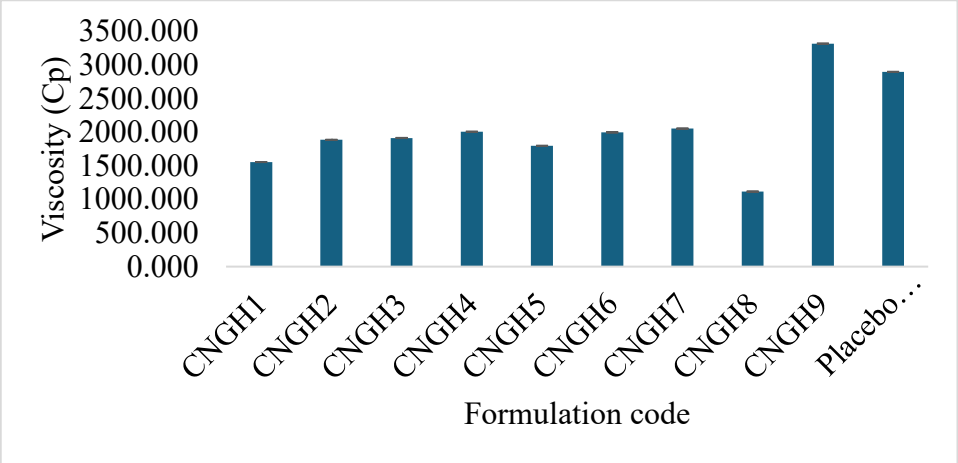

Figure 1.19: Viscosity of the all-prepared formulation

The viscosity of the prepared batches of the hydrogel was associated with the concentration of the gelling agent. As the concentration of the Carbopol increases, the viscosity of the hydrogel increases. Increasing the concentration of the gelling agent increases the interaction between the polymeric chains, resulting in a more stable three-dimensional structure. Furthermore, the formulation CNGH9 possesses the desired viscosity so that it can stay on the application site for a longer period [12,26].

Based on the findings of the above characterization parameters, the formulation CNGH9 was selected for further evaluation.

4.3.9 Percentage release of extract

Observations related to the percentage release of the calendula officinalis extract and neem extract from the hydrogel formulation CNGH9 are shown in Table 1.17.

Table 1.17: Percentage release of the extract from hydrogel formulation CNGH9

S.No.	Time (Hr.)	Percentage drug release of *calendula officinalis* from CNGH9 formulation	Percentage drug release of neem extract from CNGH9 formulation
1	0	0.000±0.00	0.000±0.00

2	0.5	7.387±0.419	9.080±0.808
3	1	18.851±0.641	18.378±1.188
4	2	36.326±0.726	32.203±0.954
5	4	57.436±0.837	75.976±0.659
6	8	78.965±1.055	81.538±0.644
7	12	98.677±0.837	99.248±0.823
8	24	97.279±0.484	98.942±0.933

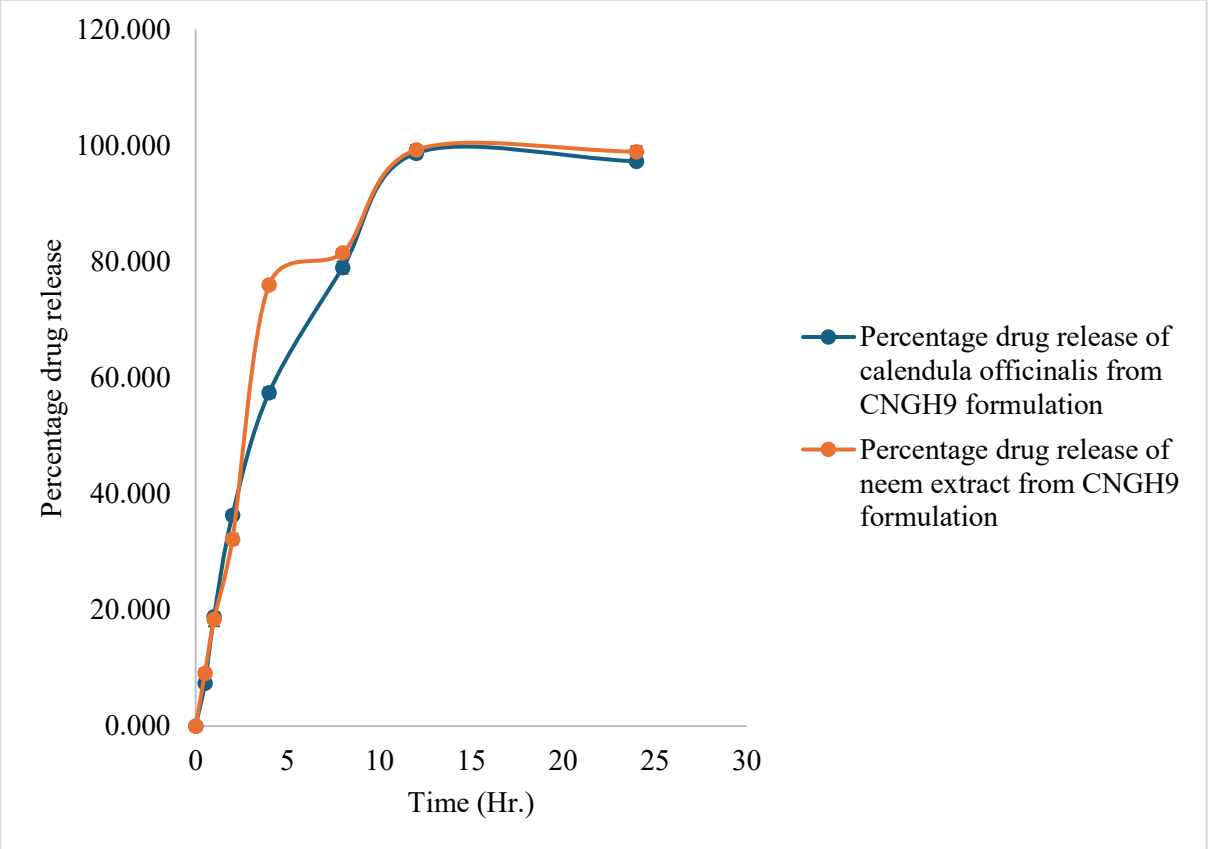

Figure 1.20: Percentage drug release of the extract incorporated hydrogel formulation CNGH9

An in vitro drug release study of the extract incorporated hydrogel CNGH9 was performed in a buffer solution of pH 6.8. At pH 6.8, a percentage release of both the neem and calendula officinalis extracts was noted. The manufactured hydrogels' pH-dependent swelling accounts for the greater release of each extract at pH 6.8. At pH 6.8, a large percentage of drug release is seen due to the deprotonation of the carbopol's COOH groups and the release of carboxylate ions at higher pH levels, which causes swelling. the percentage release of the calendula officinalis and neem extracts at 12 hours was 98.677±0.837% and 99.248±0.823%, respectively [23].

4.3.10 Drug release kinetic study

A kinetic assessment of release of each extract calendula officinalis extract and neem extract from hydrogel formulation CNGH9 was displayed in Table1.18. A variety of models, including zero order, first order, Higuchi, and Korsmeyer-Peppa's, were employed to determine the permeation mechanism using the release kinetic models.

Table 1.18: Drug release kinetic modes

S.No.	**Model**	**Regression coefficient value for *calendula officinalis* extract**	**Regression coefficient value for neem extract for formulation CNGH9**

		for formulation CNGH9	
1	Zero Order	0.7192	0.6615
2	First order	0.7557	0.8040
3	Higuchi order	0.9105	0.8660
4	Korsmeyer peppas model	0.6152	0.5959

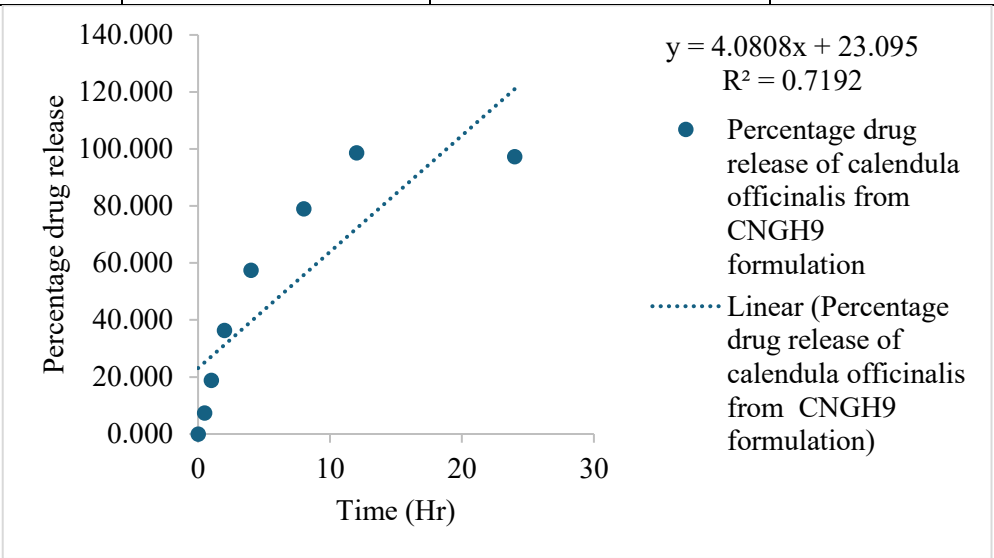

Figure 1.21: Zero order release kinetic for calendula officinalis extract from formulation CNGH9

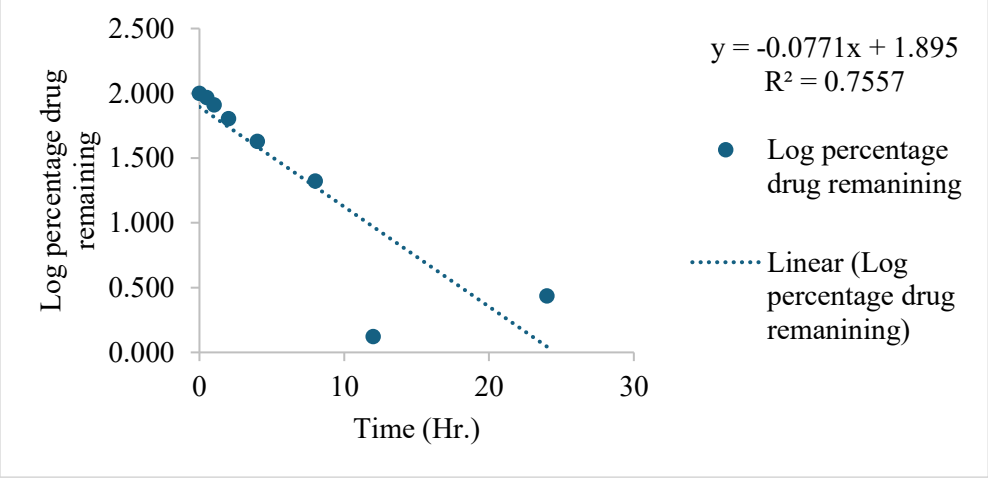

Figure 1.22: First order release kinetic for calendula officinalis extract from formulation CNGH9

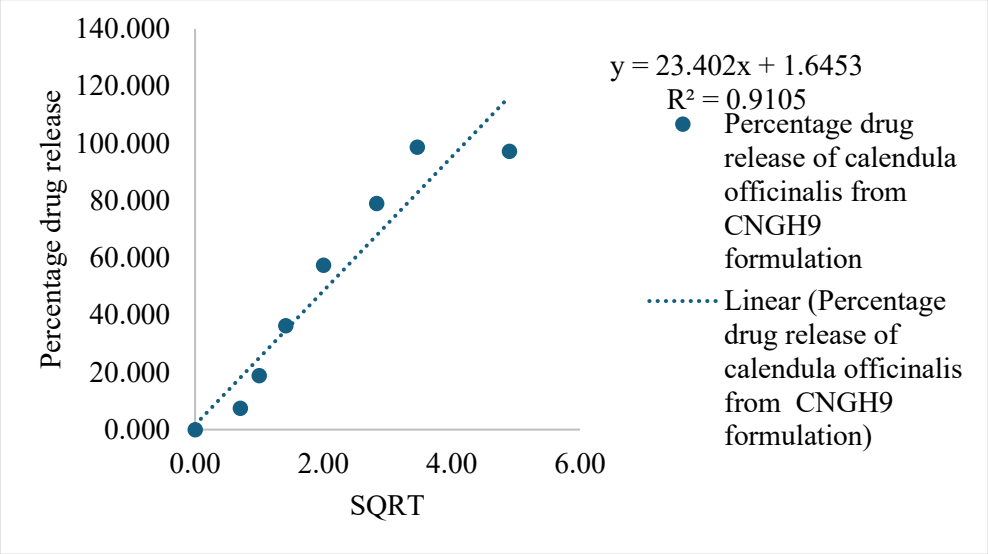

Figure 1.23: Higuchi order release kinetic for *calendula officinalis* extract from formulation CNGH9

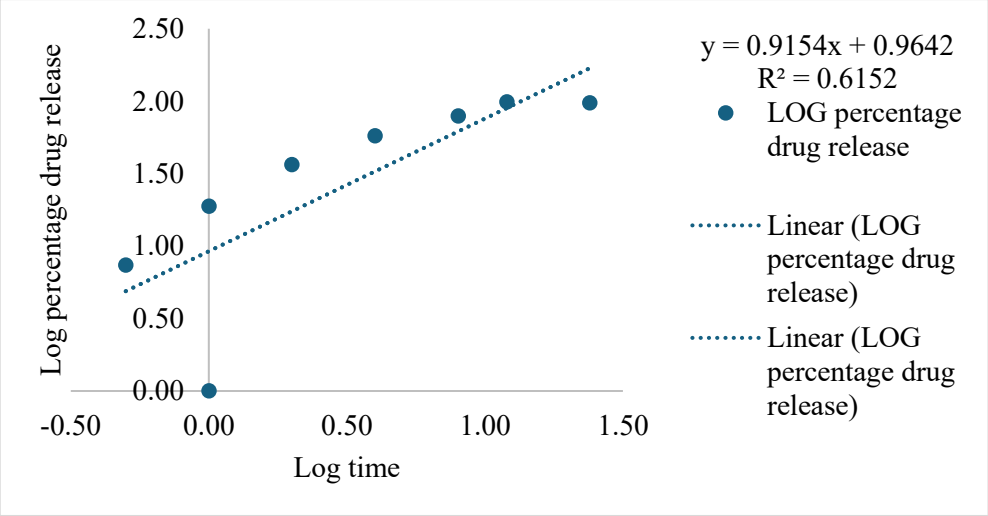

Figure 1.24: Korsmeyer Peppas order release kinetic for *calendula officinalis* extract from formulation CNGH9

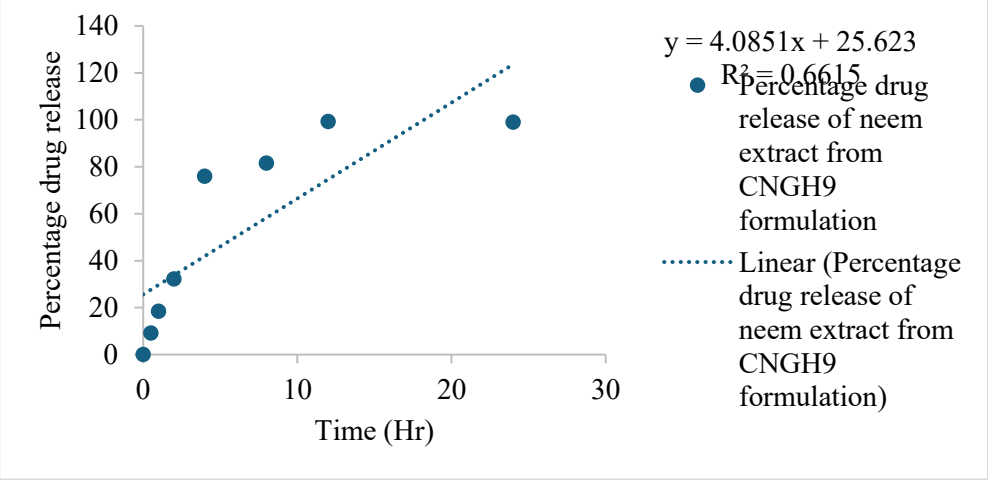

Figure 1.25: Zero order release kinetic for neem extract from formulation CNGH9

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 24s, 2025
https://www.theaspd.com/ijes.php

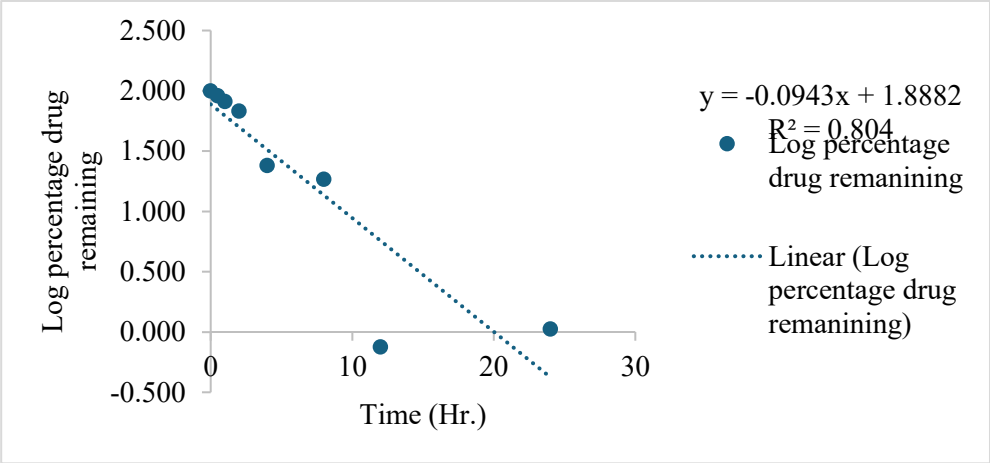

Figure 1.26: First order release kinetic for neem extract from formulation CNGH9

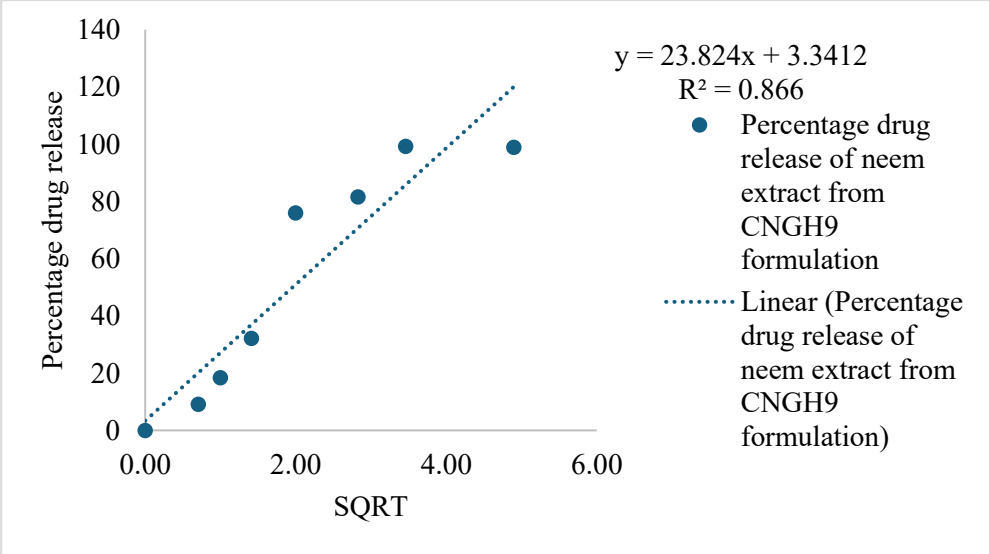

Figure 1.27: Higuchi order release kinetic for neem extract from formulation CNGH9

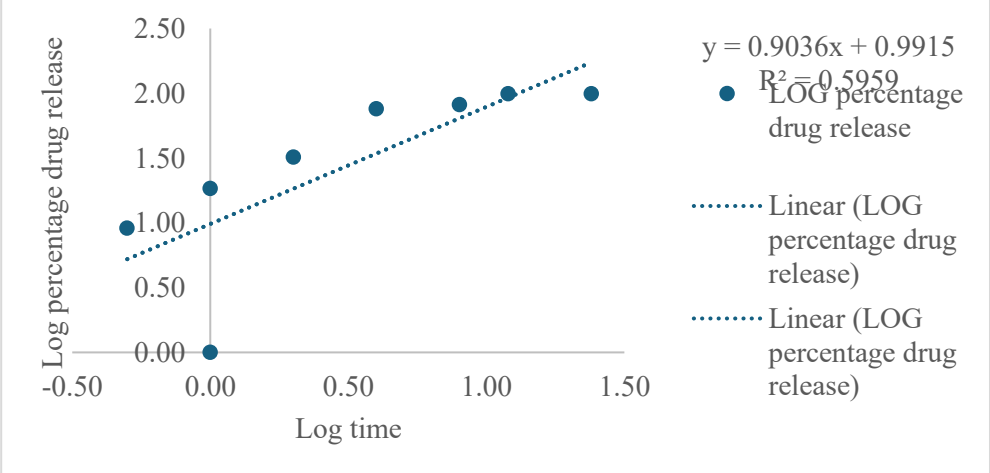

Figure 1.28: Korsmeyer peppas order release kinetic for neem extract from formulation CNGH9

Different kinetic modelling systems such as zero-order, first-order, Higuchi, and Korsmeyer Peppas, were applied on all formulations to understand the release order of both extracts from the CNGH9 hydrogels, as shown in Table 1.18. The best fit model was chosen on the basis of the closeness of the "r" value to 1. For release of calendula officinalis extract and neem extract from CNGH9 hydrogel formulations, the values of "r" were found higher for the Higuchi order kinetic with R2 values 0.9105 and 0.8660, respectively [23]

CONCLUSION

The study was able to produce and characterize carbopol-based hydrogel that contained Calendula officinalis, Neem and Guduchi extracts in healing diabetic wounds. They tested 9 formulations (CNGH1-CNGH9) using pH, spreadability, swelling index, water vapor transmission rate, drug content and viscosity. CNGH9 was one of these, with desirable spreadability, swelling index, and drug release profile. This hydrogel demonstrated an approximate 99% release of the calendula as well as neem extracts in 12 hours, in accordance with Higuchi-order kinetics, validating its abilities as a good and low-cost wound-healing preparation.

REFRENCES

1. Albulescu, M., Alexa, N., and Cojan, C. (2004). Annals of West University of Timisoara: Series Chemistry, 45-57.
2. Agyare, C., Boakye, Y.D., Bekoe, E.O., Hensel, A., Oteng, S. and Appiah, T. (2016) Review: African medicinal plants with wound healing properties. Journal of Ethnopharmacology,177(4) m 85-100. https://doi.org/10.1016/j.jep.2015.11.008.
3. Ahmed, E.M. (2015). Hydrogel: Preparation, characterization and applications: A review. Journal of advanced research, 6(2), 105–121. https://doi.org/10.1016/j.jare.2013.07.006 Ahmad, W., Jantan, I., and Bukhari, S.N.A. (2016). Tinosporacrispa (L.) Hook. f. & Thomson: a review of its ethnobotanical, phytochemical, and pharmacological aspects. Frontiers in pharmacology, 7, 59.
4. Aiyalu, R., Govindarjan, A., and Ramasamy, A., (2016). Formulation and evaluation of topical herbal gel for the treatment of arthritis in animal model, Brazilian Journal of Pharmaceutical Sciences, 52 (3), 494-507.
5. Akhtar, M.F., Hanif, M., and Ranjha, N.M (2016). Methods of synthesis of hydrogels: a review. Saudi Pharmaceutical Journal, 24, 554-559.
6. Akram, M., Hamid, A., Khalil, A., Ghaffar, A., Tayyaba, N., and Saeed, A, (2014). Review on medicinal uses, pharmacological, phytochemistry and immunomodulatory activity of plants. International journal of immunopathology and pharmacology, 27(3), 313–319.
7. Al-Mussawi, Z.K. and Al-Hussan, I.M. (2019). Phytochemical study of calendula officinalis plant by used GCMS and FTIR techniques. Plant Archives, 19, 1, 845-851
8. Ali, A., Garg, P., Goyal, R., Kaur, G., Li, X., Negi, P., Valis, M., Kuca, K., and Kulshrestha, S. (2021). A novel herbal hydrogel formulation of moringa oleifera for wound healing. Plants (Basel, Switzerland), 10(1), 25. https://doi.org/10.3390/plants10010025 (Retraction published Plants (Basel). 2024 May 30;13(11):1502.
9. Alqahtani, A., Alqahtani, T., and Ramzy, S. (2024). Utilization of absorbance subtraction and ratio difference green spectrophotometric methods for the quantification of alfuzosin hydrochloride and tadalafil in their binary mixture. BMC chemistry, 18(1), 96.
10. Alzohairy, M.A., (2016). Therapeutics role of Azadirachta indica (Neem) and their active constituents in diseases prevention and treatment. Evidence-based complementary and alternative medicine :eCAM, 2016, 7382506.
11. Alvarado-Vazquez, N., Zamudio, P., Ceron, E., Vanda, B., Zenteno, E. and Carvajal-Sandoval, G. (2003). Effect of glycine in streptozotocin induced diabetic rats. Comparative biochemistry and physiology. Toxicology &pharmacology : CBP, 134(4), 521–527.
12. Xu, H., Wen, Y., Chen, S., Zhu, L., Feng, R., and Song, Z. (2020). Paclitaxel skin delivery by micelles-embedded Carbopol 940 hydrogel for local therapy of melanoma. International journal of pharmaceutics, 587, 119626.
13. Yahyaa, E.B., and Abdulsamad, M.A. (2020). In-vitro antibacterial activity of carbopol-essential oils hydrogels. Journal of Applied Science & Process Engineering, 7, 2.
14. Yousef, H., Alhajj, M., Fakoya, A.O. (2024). Anatomy, Skin (Integument), Epidermis. In: StatPearls.
15. Yow, H., Fernandez, M. Shivashekaragowda, H., Kumar, N., Azlina, A.A., Najwa, M., and Krishnappa, P. (2023). The diabetic wound healing abilities of the aqueous ethanolic extract of tinospora cordifolia stem in streptozotocin–nicotinamide induced diabetic rats. Authorea, 1-20
16. Zahra, D., Shokat, Z., Sufyan, M., Chaudhary, Z., & Ashfaq, U. A. (2024). Exploring the potential of chitosan polyherbal hydrogel loaded with AgNPs to enhance wound healing A triangular study. International journal of biological macromolecules, 281(Pt 3), 135896.
17. Zakzak, K., Semenescu, A.-D., Moaca, E.-A., Predescu, I., Draghici, G., Vlaia, L., Vlaia, V., Borcan, F., and Dehelean, C.-A. (2024). Comprehensive biosafety profile of carbomer-based hydrogel formulations incorporating phosphorus derivatives. Gels, 10(7), 477.
18.Dubhashi, S., Pranay, V., Singaiah, M., Satwik, J., Prasad, V.V.L.N, Diwan, V.P. (2013). Studies on extraction and HPLC Analysis of Azadirachtin from Kernels of Neem Seeds. Journal of Advanced Pharmacy Education & Research, 3, 1, 27, 57-60.
19. Sebaiy, M. M., El-Adl, S. M., Nafea, A., Aljazzar, S. O., Elkaeed, E. B., Mattar, A. A., and Elbaramawi, S. S. (2023). Different methods for resolving overlapping UV spectra of combination medicinal dose forms of ciprofloxacin and metronidazole. BMC chemistry, 17(1),

20. Kumar, N., Kusuma, P.K., Nayak, B.V., and Beda, D.P. (2025). Spectrophotometric method development and validation for simultaneous quantification of Cefoperazone and Sulbactum sodium in bulk and marketed formulation: greenness profile evaluation. Green Analytical Chemistry, 13,100239. https://doi.org/10.1016/j.greeac.2025.100239.
21.Avinash, B., Venu, R., Prasad, T. N. V. K. V., Alpha Raj, M., Srinivasa Rao, K., and Srilatha, C. (2017). Synthesis and characterisation of neem leaf extract, 2, 3-dehydrosalanol and quercetin dihydrate mediated silver nano particles for therapeutic applications. IET nanobiotechnology, 11(4), 383–389. https://doi.org/10.1049/iet-nbt.2016.0095.
22.Saher, T., Manzoor, R., Abbas, K., Mudassir, J., Wazir, M. A., Ali, E., Ahmad Siddique, F., Rasul, A., Qadir, M. I., Aleem, A., Qaiser, N., Usman, A., Romman, M., and Ali, M. S. (2022). Analgesic and Anti-Inflammatory Properties of Two Hydrogel Formulations Comprising Polyherbal Extract. Journal of pain research, 15, 1203–1219.
23.Suhail, M., Wu, P. C., and Minhas, M. U. (2020). Using carbomer-based hydrogels for control the release rate of diclofenac sodium: preparation and in vitro evaluation. Pharmaceuticals (Basel, Switzerland), 13(11), 399. https://doi.org/10.3390/ph13110399.
24.Shrimali, H., Mandal, U.K., Nivsarkar, M. and Shrivastava, N. (2019). Fabrication and evaluation of a medicated hydrogel film with embelin from Embelia ribes for wound healing activity. Future Journal of Pharmaceutical Sciences, 5, 12.
25. Zarghampour, A., Jouyban, K., Jouyban-Gharamaleki, V., Jouyban, A., Rahimpour, E. (2024). A description on the shake-flask and laser monitoring-based techniques for determination of the drug's solubility. Pharmaceutical Sciences, 30(2), x-x.
26.Kostrzębska, A., Pączek, K., Weselak, A., and Musiał, W. (2023). Effect of hydrogel substrate components on the stability of tetracycline hydrochloride and swelling activity against model skin sebum. International Journal of Molecular Sciences, 24(3), 2678. https://doi.org/10.3390/ijms24032678