FORMULATION AND EVALUTION OF HYDROGEL PREPARED BY USING *SWERTIA CHIRATA* STEM EXTRACT FOR ITS ANTIMICROMIAL ACTIVITY

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Abstract:

The Indian system of medicine is rooted in Ayurveda, which uses medicinal plants for the treatment and cure of various diseases. Scientific research has broadened our understanding of the chemical effects of various phytoconstituents responsible for different therapeutic activities. *Swertia chirata*, a well-known medicinal plant native to the temperate Himalayas, belongs to the *Gentianaceae* family. It is recognized in several traditional systems of medicine and is used primarily as an antipyretic, antimalarial, antiviral, and antidiabetic agent. Additionally, *Swertia chirata* exhibits antimicrobial activity against various bacteria and fungi. This antimicrobial property is attributed to its biologically active components such as amarogentin, swerchirin, triterpenoids, xanthones, opelic acid, and gentiopicric acid. A 32-factorial design was employed for the preparation of a hydrogel. FTIR and DSC analysis of the *Swertia chirata* stem extract and excipients indicated no interaction between the extract and excipients. The hydrogel was formulated using *Swertia chirata* stem extract, carbopol 934, aloe vera gel, methyl paraben, and triethanolamine. The hydrogel was evaluated for physical characteristics, spreadability, pH, extrudability, viscosity, diffusion, in vitro antimicrobial activity, and accelerated stability. The optimized formulation (batch PF3) of the herbal hydrogel exhibited good consistency, spreadability, homogeneity, and stability. This formulation demonstrated superior antimicrobial activity due to the presence of *Swertia chirata* stem extract.

Keywords: *Swertia chirata* stem extract, Carbopol 934, *In- vitro* antimicrobial activity.

Introduction:

The proper use of appropriate medications is a key factor in the success of primary health care, and herbal medicine provides an accessible and cost-effective option for treating conditions within the primary health system.[1] Most people in developing countries have access to herbal plant medicine, which has been utilized for thousands of years. The World Health Organization reports that 80% of countries use herbal medicine.[2] Herbal medicines are more affordable than synthetic drugs and typically have no side effects.[3]

Swertia, a genus in the (F. *Gentianaceae*) tropical family of small trees and herbs. There are about 10 species in Indian subcontinent.[4] The *Swertia chirata* is an ethnomedical plant.[5] *Chirata* is a rare herb found at high altitudes in the Himalayas, typically growing at elevations of 2200 meters, from Kashmir to Bhutan. *Swertia chirata* can reach a height of up to 1 meter, with its stems covered in bark and leaves that are stalkless. This herb is commonly used to prepare medicinal remedies, with its above-ground parts being the primary source for medicinal use. The plant contains numerous therapeutic phytochemicals that can help treat various health issues.[6]

Swertia chirata is referenced in the American and British pharmacopoeias, the Indian Pharmaceutical Codex, and conventional medicine for its therapeutic uses. Traditionally, it is employed as an antipyretic, antimalarial, antiviral, and antidiabetic agent. Additionally, it demonstrates antimicrobial properties against various bacteria and fungi. The antibacterial effects of *Swertia chirata* are attributed to active compounds such as amarogentin, swerchirin, triterpenoids, xanthones, opelic acid, and gentiopicric acid. Studies on stem extracts of *Swertia chirata* have shown its antibacterial and antifungal activity, effectively targeting both gram-positive and gram-negative bacteria, as well as several types of fungi.[7]

Swertia chirata may help maintain normal blood sugar levels and could potentially offer liver-protecting benefits. It may be useful in treating asthma and might possess healing properties. Additionally, it could have beneficial effects for anemia. [8]

Due to their excellent three-dimensional structure, water solubility, and biocompatibility, hydrogels have been widely used in topical applications across various fields. Recent research has shown increased interest in hydrogels, as they exhibit remarkable chemistry that could be beneficial for treating various skin conditions. This innovation could enable the delivery of active pharmaceutical ingredients (APIs) in a way that enhances their effectiveness.[9]

Matrials and Methods:

Preformulation study:

Collection and Authentication:

The *Swertia chirata* plant stem was collected from the Belgaum area and identified and authenticated by Mr. S.S. Patil. The identification and authentication process was carried out at D.S. Kadam Science College, Gadhinglaj, affiliated with SUK.

Extraction Procedure:

The stem of *Swertia chirata* was collected and subjected to shade drying. These dried stems were then ground into a coarse powder. The coarsely ground shade-dried stems of *Swertia chirata* were used for extraction with ethanol. A 250g portion of the powder was placed in the thimble of a Soxhlet apparatus for extraction. The resulting extract was air-dried at 25-30°C and weighed. The collected sample was then evaporated. The percentage yield was calculated based on this process.

Phytochemical Screening:[10,11,12]

Phytochemical screening for alkaloids, carbohydrates, starch, glycosides, flavonoids, saponins, and proteins was carried out following the standard procedure.

Solubility of extract:

Solubility is an important physicochemical property as it affects the bioavailability of a drug. A small amount of *Swertia chirata* stem extract was placed in a test tube, followed by the addition of 5 ml of solvent (water, ethanol, chloroform, or phosphate buffer). The mixture was shaken vigorously and allowed to stand for a while. The solubility was then observed visually and recorded.

Fourier Transforms Infrared Analysis:

The potential structural modifications induced by the *Swertia chirata* stem extract sample were examined using infrared (IR) spectroscopy. The extract was analyzed within the range of 400 to 4000 cm-1. The solid form of the *Swertia chirata* stem extract was placed on the sample holder for analysis, and the spectra of the polymer were similarly recorded.

Differential Scanning Calorimetry:

Differential scanning calorimetry (DSC) analysis was performed on the *Swertia chirata* stem extract, the polymers, and the physical mixture of the drug and polymers using a differential scanning calorimeter.

Formulation of hydrogel of *Swertia chirata* stem extract:[13]

Weigh an accurate amount of Carbopol 934 and dissolve it in water overnight. Then, add the extract, aloe vera gel, and methyl paraben to the gel base while using a magnetic stirrer. Finally, add triethanolamine to adjust the pH to a range of 6.0 to 7.0, ensuring constant stirring throughout the process.

Table no.01 Formulation table

Sr. No.	Ingredients	PF1	PF2	PF3	PF4	PF5	PF6
1	*Swertia chirata* (g)	1	1	1	1	1	1
2	Carbapol 934	1	1.5	2	1	1.5	2
3	Aloevera gel	1	1	1	1.5	1.5	1.5
4	Triethanolamine	q.s	q.s	q.s	q.s	q.s	q.s
5	Methyl Paraben	0.02	0.02	0.02	0.02	0.02	0.02
6	Distilled water	100	100	100	100	100	100

Evaluation of Hydrogel:

Physical appearance: The physical appearance of the herbal hydrogel was assessed through visual inspection.[14,15]

pH: A standard digital pH meter can be used to measure the pH of a herbal hydrogel at room temperature by taking an appropriate amount of the formulation, diluting it with a suitable solvent, and placing it in an appropriate beaker. [16,17]

Viscosity: The viscosity was measured using spindle no. 4 at 2.5 rpm and 25°C. A wide-mouth container was then filled with an adequate amount of herbal hydrogel, ensuring that the

viscometer could be fully immersed in the container. The sample was allowed to settle for 30 minutes at a constant temperature of 25°C before taking the measurement.[17]

Spreadability: The herbal hydrogel was placed between two petri dishes. One gram of herbal hydrogel was placed in a petri dish, and another petri dish was placed on top. A 100 g weight was then placed on the upper petri dish for 60 seconds. After 60 seconds, the diameter of the circles formed by the spread herbal hydrogel was measured in triplicate. The average of these readings was used to calculate the value using the following formula.[18,19]

$$\textbf{Spreadability (S)} = M.L/T$$

Extrudability: The herbal hydrogel formulation was packaged into aluminum collapsible tubes. The tube was then pressed to extrude the material, and the extrudability of the herbal hydrogel formulation was assessed.

Antimicrobial activity:[20]

The antimicrobial study was conducted using the agar well diffusion method. The activity was evaluated against *E. coli*, *Staphylococcus aureus*, *Aspergillus niger*, and *Candida albicans* as test organisms. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using the broth dilution method.

Agar well diffusion method:

Antibacterial activity: The media was prepared by adding 3.8 g of agar powder to 100 ml of distilled water, and the mixture was then boiled. The solution was autoclaved at 121°C for 15 minutes and cooled to 50°C in a water bath. It was subsequently poured into sterile plates, allowed to cool and solidify under sterile conditions, and incubated for 24 hours at 37°C to ensure no bacterial contamination. Wells with a diameter of 6 mm and a depth of 5 mm were created in the solidified agar using a sterile borer. Cultures of *E. coli*, *S. aureus*, *Candida albicans*, and *Aspergillus niger* were separately inoculated onto the agar before solidification, then transferred to each Petri dish. Extracts with concentrations of 20, 40, 65, and 80 mg/ml were prepared, along with a 20 mg/ml solution of AquaClob-GM cream as a standard. The bacterial plates were incubated at 37°C for 24 hours. The sensitivity of the test organisms to all three extracts was determined by measuring the diameters of the zones of inhibition around the wells.

Antifungal activity: The media was prepared by dissolving 20 g of potato extract, 2 g of dextrose, and 1.5 g of agar-agar in 100 ml of water. The solution was autoclaved at 121°C for 15 minutes and then cooled to 50°C in a water bath. It was transferred into sterile plates, allowed to cool and solidify under sterile conditions, and incubated at 37°C for 24 hours to ensure no bacterial contamination. Wells of 6 mm in diameter and 5 mm in depth were made in the solidified agar using a sterile borer. Cultures of *E. coli*, *S. aureus*, *Candida albicans*, and *Aspergillus niger* were separately inoculated onto the agar before solidification, then transferred to each Petri dish. Extracts with concentrations of 20, 40, 65, and 80 mg/ml were prepared, and 20 mg/ml of AquaClob-GM cream was used as a standard. The bacterial plates were incubated at 37°C for 72 hours. The sensitivity of the test organisms to all three extracts was determined by measuring the diameters of the inhibition zones surrounding the wells.

Minimum inhibitory concentration: The hydrogel was serially diluted in concentrations ranging from 2 mg to 0.25 mg. The inoculum of single cultures was prepared in a broth medium, and the cultures were incubated. They were then serially diluted to achieve a cell density of 2×10^4 cells per ml, with cell counting performed using a hemocytometer. Two milliliters of nutrient broth were dispensed into tubes, and 100 µL of the cell culture was inoculated into each tube.

Next, 100 µL of hydrogel at different concentrations was added to each tube. Each experiment was conducted in triplicate, and a growth control was included for comparison in each trial.

Minimum Bactericidal Concentration: Five tubes from the MIC test were plated and incubated for 24 hours, followed by colony counting on the following day.

Stability: The prepared herbal hydrogel was packaged in 10g containers and subjected to stability studies at a temperature of 40°C ± 2°C and 75 ± 5% relative humidity for a period of 45 days. Samples were withdrawn at 15-day intervals and evaluated for physical appearance, pH, spreadability, and other parameters.

Results:

Preformulation study:

Organoleptic properties of extract:

Table no. 02 Organoleptic properties

Sr.no	Name of plant	Color	Odour	Taste.
1	*Swertia chirata* stem extract	Yellowish	Woody	Bitter

Phytochemical investigation of extract:

Table no. 03 Phytochemical screening of extract

Sr.no	Phyto-constituent	Reagents/ Test	*Swertia chirata* stem extract /Inference
1	Carbohydrate	Molish test Fehling test	-- –
2	Alkaloids	Dragendorff's Test Mayer's Test Hager's Test Wagner's Test	+ + + +
3	Saponin	Foam Test	+
4	Proteins	Biuret test	–
5	Amino acids	Ninhydrin test-	+
6	Steroids	Salkowski reaction	+
7	Anthraquinone glycosides	Borntreager's test for Anthraquinone glycosides	+
8	Flavonoids	Shinoda Test- Ferric chloric test	+ +
9	Tannins	Lead acetate test	+

Solubility determination of extract:

Table no. 04 Solubility of extract in different solvent

Solvent	Solubility (mg/ml)
water	soluble
chloroform	slightly soluble
Phosphate buffer (6.8)	soluble
ethanol	soluble

FTIR Spectroscopy:

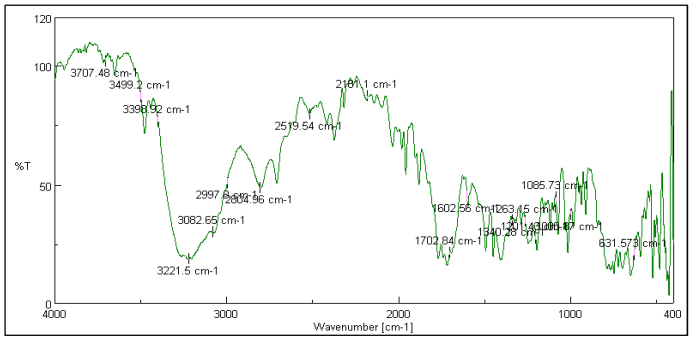

Fig. no. 01 FTIR of Pure extract

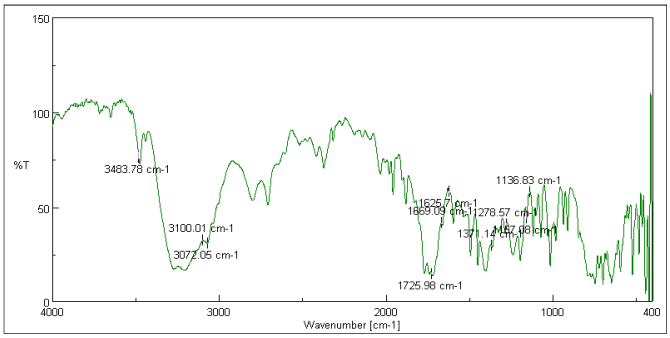

Fig no. 02 FTIR of Physical mixture

DSC:

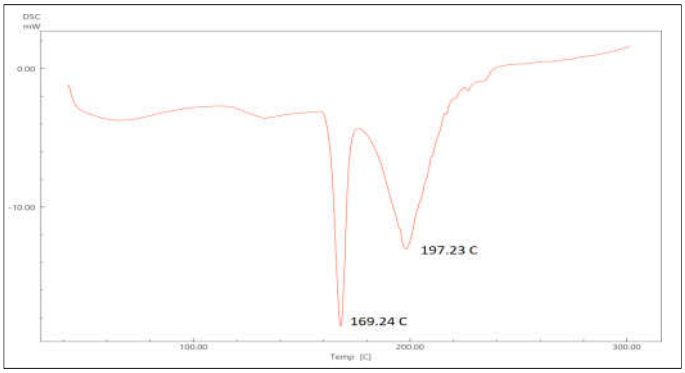

Fig no. 03 DSC of Physical mixture

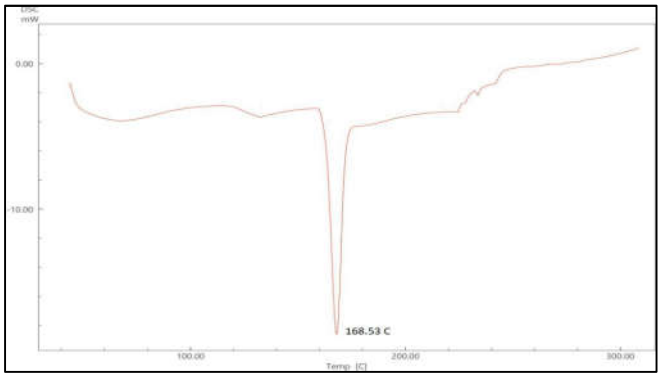

Fig. no. 04 DSC of Pure extract

Evalution of hydrogel:

The herbal hydrogel formulations were evaluated for color, odor, consistency, pH , viscosity, spreadability, extrudability. The results for all evaluation parameter of all 6 batches are presented in Table No. 7.14.

Table no. 05. Evaluation parameter of hydrogel

Batch no.	Colour	Odour	Consistency	pH	Viscosity (Cps)	Spreadability (gm.cm/sec.)	Extrudability (g/cm^2)
PF1	Brown	Pleasant	Excellent	6.16±0.05	9533±44.8813	7.5±0.05	1.34±0.09
PF2	Brown	Pleasant	Good	6.20±0.02	8763±55.7882	8.1±0.04	1.76±0.011
PF3	Brown	Pleasant	Excellent	6.63±0.04	7354±54.9454	9.1±0.03	1.98±0.13
PF4	Brown	Pleasant	Average	6.23±0.03	8643±41.6213	8.3±0.08	2.12±0.17
PF5	Brown	Pleasant	Average	6.26±0.03	9436±57.2741	7.9±0.02	1.33±0.15
PF6	Brown	Pleasant	Average	6.22±0.04	8354±43.6501	8.8±0.06	1.88±0.10

Antimicrobial activity:

In-vitro antimicrobial activity was assessed using the agar well diffusion method. *E. coli*, *S. aureus*, *Candida albicans*, and *Aspergillus niger* were used to test the antimicrobial effects. The zones of inhibition were measured to determine the inhibitory concentration of the extract and herbal hydrogel against the bacteria and fungus. The results are presented in Tables No. 06 and 08.

Antimicrobial activities of *Swertia chirata* stem extract-

Table no.06 Antimicrobial activity of *Swertia chirata* stem extract

Sr. no	Name of organism	20mg/ml	40mg/ml	60mg/ml	80mg/ml
1	Staphyloccocus aureus	S	S	S	S
2	*E- coil*	S	S	S	S
3	*Candida albicans*	S	S	S	S
4	Aspergillus niger	R	R	R	R
5.	Standard (Aqua Clob- GM)	S	S	S	S

S: Sensitive **R: Resistance**

Zone of inhibition of *Swertia chirata* stem extract -

Table no. 07 Zone of inhibition of *Swertia chirata* stem extract

Sr. no	Name of organism	20mg/ml	40mg/ml	60mg/ml	80mg/ml
1	*Staphyloccocus aureus*	18 mm	18.4mm	20mm	21 mm
2	*E- coil*	17.5mm	18.4mm	18.9mm	19mm
3	*Candida albicans*	17mm	18mm	19mm	19.8mm
4	*Aspergillus niger*	-	-	-	-

5	Standard (Aqua Clob- GM)	20mm	21mm	24mm	25mm

Fig no. 05 *S. Aureus* S.C. stem extract

Fig no.06 *E- Coil* S.C.stem etract

Fig no. 07 *Candida albicans* S.C. stem extract

Fig.no 08 *A. niger* S.C. stem extract

Antimicrobial activity of herbal hydrogel -

Table no. 08 Antimicrobial activity of herbal hydrogel

Sr. no	Name of organism	20mg/ml	40mg/ml	60mg/ml	80mg/ml
1.	*Staphyloccocus aureus*	**S**	**S**	**S**	**S**
2.	*E- coil*	**S**	**S**	**S**	**S**
3.	*Candida albicans*	**S**	**S**	**S**	**S**
4.	*Aspergillus niger*	**R**	**R**	**R**	**R**
5.	Standard (Aqua Clob- GM)	**S**	**S**	**S**	**S**

S: Sensitive **R: Resistance**

Zone of inhibition of herbal hydrogel

Table no. 09 Zone of inhibition of herbal hydrogel

Sr. no	Name of organism	20mg/ml	40mg/ml	60mg/ml	80mg/ml
1.	*Staphyloccocus aureus*	19mm	19.5mm	22mm	23mm
2.	*E- coil*	18mm	18.4mm	19mm	20mm
3.	*Candida albicans*	19mm	20mm	20.4mm	21mm
4.	*Aspergillus niger*	-	-	-	-
5.	Standard (Aqua Clob- GM	20mm	21mm	23mm	23.4mm

Fig no. 09 *S. aureus* Herbal hydrogel

Fig.no 10 *E-coil* herbal hydrogel

Fig no.11 *Candida albicans* Herbal hydrogel

Fig no.12 *A. niger* Herbal hydrogel

MBC of herbal hydrogel -

Table no. 10 MBC of herbal hydrogel

Test of compound	MBC (µg/ml)		
	S. aureus	***E-coil***	***Candida albicans***
Hydrogel	**75**	**75**	**75**
Control (water)	**-**	**-**	**-**

MIC of herbal hydrogel-

The sample was tested at concentrations ranging from 10 µg/ml to 80 µg/ml to determine the minimum inhibitory concentration. At 10 µg/ml, the sample showed a low concentration required to inhibit 50% of the growth of the bacterial strain E. coli.

Observation of *E.coli*-

Table no. 11 Observation of *E.coli*

	Concentration µg/ml	OD	Mean	Percent inhibition	MIC
Control		0.80 0.85 0.82	0.82		
Sample – Hydrogel	80	0.35 0.33 0.36	0.34	78.53	10µg/ml
	40	0.33 0.32 0.30	0.31	72.19	
	20	0.27 0.26 0.28	0.27	67.07	
	10	0.31 0.29	0.30	63.41	

		0.30			

Observation of *S. aureus*-

Table no. 12 Observation of *S. aureus*

	Concentration	OD	Mean	Percent inhibition	MIC
Control		0.50 0.55 0.52	0.52		
Sample - Hydrogel	80	0.40 0.45 0.42	0.42	79.23	10µg/ml
	40	0.35 0.33 0.38	0.35	72.69	
	20	0.33 0.29 0.28	0.30	65.30	
	10	0.18 0.20 0.21	0.19	63.46	

Observation of *Candida albicans* -

Table no. 13 Observation of *Candida albicans*

	Concentration µg/ml	OD	Mean	Percent inhibition	MIC
Control		0.65 0.68 0.60	0.64		
Sample – Hydrogel	80	0.44 0.42 0.40	0.42	7.8.37	10µg/ml
	40	0.41 0.40 0.38	0.39	74.68	
	20	0.38 0.35 0.33	0.35	65.31	
	10	0.23 0.29 0.25	0.25	60.93	

Stability study:

Stability studies on the optimized batch (PF3) were conducted over 45 days, and the herbal hydrogel maintained its drug levels throughout the accelerated stability testing period.[17]

Table no.14 Stability study

Evaluation parameter	Initial	15 day	30day	45 day
pH	6.63 ± 0.04	6.62 ±0.04	6.62±0.04	6.61±0.04
Spreadability	9.1± 0.03	9.0± 0.03	9.0± 0.03	8.9± 0.03

Conclusion:

In the present study, a hydrogel formulation containing *Swertia chirata* stem ethanol extract was developed using Carbopol 934 as a polymer along with other excipients. The formulation was evaluated for various parameters, including pH, physical characteristics, viscosity, spreadability, extrudability, in vitro antimicrobial activity and stability.Phytochemical analysis of *Swertia chirata* stem extract revealed the presence of alkaloids, saponins, amino acids, steroids, anthraquinone glycosides, flavonoids, and tannins.Solubility testing showed that the *Swertia chirata* stem extract is soluble in ethanol and phosphate buffer, while slightly soluble in chloroform and water .FTIR and DSC studies indicated no significant interaction between the *Swertia chirata* stem extract and the excipients. The hydrogel formulations (PF1 to PF6) were created using Carbopol 934 and other excipients, with batch PF3 identified as the optimized formulation. Antimicrobial testing showed that the herbal hydrogel exhibited good activity with zones of inhibition against *S. aureus*, *E. coli*, and *Candida albicans*.

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