FORMULATION, DEVELOPMENT AND IN-VITRO CYTOTOXICITY OF SARACA INDICA BARK HYDROGEL FOR ANTIMICROBIAL TREATMENT

Parul Pal[1], Seraj Alam Siddique[2], Sonam Singh Parmar[3], Dr. Prashant Kumar Katiyar[4]

[1]Research Scholar, Kanpur Institute of Technology and Pharmacy, Kanpur, UP, India
[2]Assistant Professor, Kanpur Institute of Technology and Pharmacy, Kanpur, UP, India
[3]Assistant Professor, Kanpur Institute of Technology and Pharmacy, Kanpur, UP, India
Director, Kanpur Institute of Technology and Pharmacy, Kanpur, UP, India

Corresponding Author-Parul Pal
pal96parul@gmail.com[1], seraj.siddique@kit.ac.in[2], sonam.parmar@kit.ac.in[3], prashant.katiyar@kit.ac.in[4]

Abstract

This study focused on the formulation, development, and in vitro evaluation of hydrogels containing Saraca Indica bark extract for potential antimicrobial application. Three different hydrogel formulations were prepared using different polymers: Carbopol 934 (F1), Carbopol 940 (F2), and HPMC (F3). The hydrogels' organoleptic properties, physical attributes, pH, viscosity, spreadability, drug content, and in vitro drug release were investigated. Results revealed favorable organoleptic and physical properties for all formulations, with pH values compatible for skin application. Variations in viscosity, spreadability, and drug content across the formulations were observed, likely due to differences in polymer properties. Phytochemical screening indicated the presence of several bioactive compounds, including alkaloids, flavonoids, glycosides, phenols, saponins, steroids, tannins, reducing sugars, carbohydrates, and amino acids in the extract, affirming its therapeutic potential. The in vitro drug release study demonstrated the highest release rate in the F1 formulation. The findings suggested that Saraca Indica bark extract hydrogels could serve as a promising antimicrobial treatment. However, further in vivo experiments and clinical trials are required to establish their real-world efficacy and safety.

Keywords: Saraca Indica, Hydrogel formulation, In vitro evaluation, Phytochemical screening, Carbopol 934, Carbopol 940, HPMC, Spreadability.

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INTRODUCTION

In recent years, the use of natural products and traditional medicines has gained considerable attention for treating various health issues, with a surge in the development of phytochemical-

based antimicrobial treatments. Plants harbor a diverse array of bioactive compounds with antimicrobial properties that can be harnessed for therapeutic purposes [1].

Saraca Indica, commonly known as Ashoka tree, is a plant native to India and holds immense significance in traditional Indian medicine [2]. The bark of the Saraca Indica tree is especially noteworthy due to its rich content of various phytochemicals, including alkaloids, flavonoids, glycosides, phenols, saponins, steroids, tannins, reducing sugar, carbohydrates, and amino acids, making it a potent source of medicinal ingredients [3].

In this research, we aim to formulate a hydrogel incorporating the bark extract of Saraca Indica. Hydrogels are three-dimensional, hydrophilic, polymeric networks capable of absorbing large amounts of water or biological fluids. Due to their high water content, porosity, and soft consistency, they can mimic natural living tissue more than any other type of synthetic biomaterials, making them ideal vehicles for drug delivery. We developed three different formulations of hydrogels using different polymers - Carbopol 934, Carbopol 940, and Hydroxypropyl Methylcellulose (HPMC) [4, 5].

The formulated hydrogels were then subjected to various physical and chemical tests. The study focused on evaluating the organoleptic properties, pH, viscosity, spreadability, drug content, and in-vitro drug release of these hydrogels. These parameters are critical in determining the effectiveness and patient acceptability of the formulated hydrogels [6].

Hydrogels are a class of biomaterials that have gained considerable attention in the field of drug delivery, tissue engineering, and wound healing due to their unique and highly desirable properties [7]. These polymeric materials are capable of absorbing large amounts of water or biological fluids, resulting in a gel-like structure that closely mimics the characteristics of living tissues. The hydrophilic nature of these materials makes them well-suited for biocompatible applications as they are able to interact favorably with biological systems, reducing the risk of adverse immune responses [8].

One of the main advantages of hydrogels is their versatility. They can be designed and formulated to respond to specific environmental triggers such as temperature, pH, or the presence of certain molecules [9]. This makes them suitable for targeted drug delivery applications where the drug can be released in a controlled and sustained manner, improving the drug's effectiveness and reducing potential side effects. In addition to drug delivery, hydrogels have also shown promise in tissue engineering where they can provide a scaffold for cell growth and tissue regeneration. The inherent porosity of hydrogels facilitates nutrient transport and waste removal, crucial for cell survival and function. Overall, the flexibility in design and the diverse range of applications make hydrogels a powerful tool in the field of biomedicine [10].

The ultimate goal is to develop a phytochemical-based antimicrobial treatment that can effectively deliver the bioactive compounds present in the Saraca Indica bark extract and establish its potential as a robust antimicrobial treatment [11].

Methodology

Materials [12]

The primary ingredients utilized for the formulation of the hydrogel included the extract from Saraca Indica bark, Carbopol 934, Carbopol 940, Hydroxypropyl Methylcellulose (HPMC), Propylene glycol, Methyl Paraben, Propyl Paraben, and Water. The entire chemical was purchased from the verified vendors.

Formulation of Hydrogel [13]

Three different formulations of the hydrogel were created using different polymers. In each formulation, the extract of Saraca Indica was combined with one of the polymers (Carbopol 934 for F1, Carbopol 940 for F2, and HPMC for F3) and other ingredients like propylene glycol, methyl paraben, and propyl paraben. The mixture was then filled up with water to attain the desired consistency.

Organoleptic Properties [14]

The organoleptic properties of the hydrogels, including color, odor, and taste, were examined both before and after the drying process.

Phytochemical Screening [15]

Phytochemical screening was conducted on the extracts from Saraca Indica bark dissolved in ethanol, methanol, and water. This allowed us to identify the presence of various phytochemicals, including alkaloids, flavonoids, glycosides, phenols, saponins, steroids, tannins, reducing sugars, carbohydrates, and amino acids.

Physical Examination [16]

The physical characteristics of the formulated hydrogels, such as color, odor, homogeneity, grittiness, and phase separation, were inspected.

pH Measurement [17]

The pH of the hydrogels was measured using a calibrated pH meter. Triplicate measurements were made for each formulation.

Viscosity Measurement [18]

The viscosity of the hydrogels was measured using a viscometer, and the process was replicated three times for each formulation.

Spreadability Test [19]

The spreadability of the hydrogels was evaluated by measuring how far the gel could spread under a specific load within a given time. This was done in triplicate for each formulation.

Drug Content Measurement [20]

The drug content of the hydrogels was measured using a spectrophotometer at 248nm. Measurements were taken three times for each formulation to ensure accuracy.

In Vitro Drug Release [21]

Finally, the in-vitro drug release profiles of the hydrogels were evaluated. At different time intervals, the percentage of the drug released from each hydrogel formulation was measured. This was performed over an 8 hour period.

These methods ensured a systematic evaluation of the Saraca Indica bark hydrogels' effectiveness, and all experiments were repeated three times to ensure reproducibility and accuracy. The mean and standard deviation of each set of experiments were calculated to provide a reliable representation of the data.

Results

Hydrogel Formulation

Three distinct formulations (F1, F2, F3) were prepared using different polymers. In each formulation, 1% of the Saraca Indica bark extract was used. The use of Carbopol 934 (F1), Carbopol 940 (F2), and HPMC (F3) as polymers provided different matrix characteristics to each formulation.

Table:1- Formulae of Hydrogel

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

Organoleptic Properties

All formulations displayed a transition in color from dark green before drying to light green after drying. The odor remained earthy and woody, and the taste was bitter both before and after drying. This indicates that the drying process did not significantly alter the organoleptic properties.

Phytochemical Screening

The Saraca Indica extracts showed varying concentrations of different phytochemicals in ethanol, methanol, and aqueous solutions. Alkaloids, flavonoids, phenols, reducing sugars, and amino acids were present in all extracts, while glycosides, saponins, and steroids were not found in the aqueous extract. Tannins and carbohydrates were absent in the ethanol extract but present in the methanol and aqueous extracts.

Physical Examination

The hydrogel's color varied from transparent (F1, F2) to milky white (F3), and none of the formulations had an odor. Homogeneity was excellent in F1, good in F2, and poor in F3, while grittiness and phase separation were absent in all formulations.

pH Measurement

The pH values for the formulations were slightly acidic, with F1 at 5.60±0.20, F2 at 5.80±0.10, and F3 at 6.00±0.10. These values are suitable for skin application as they are close to the skin's natural pH.

Table: 2- pH of Hydrogel (Mean & SD)

Sn.	Formulations	Triplicates	pH
1	F1	1	5.60±0.20
2		2	
3		3	
4	F2	4	5.80±0.10
5		5	
6		6	
7	F3	7	6.00±0.10
8		8	
9		9	

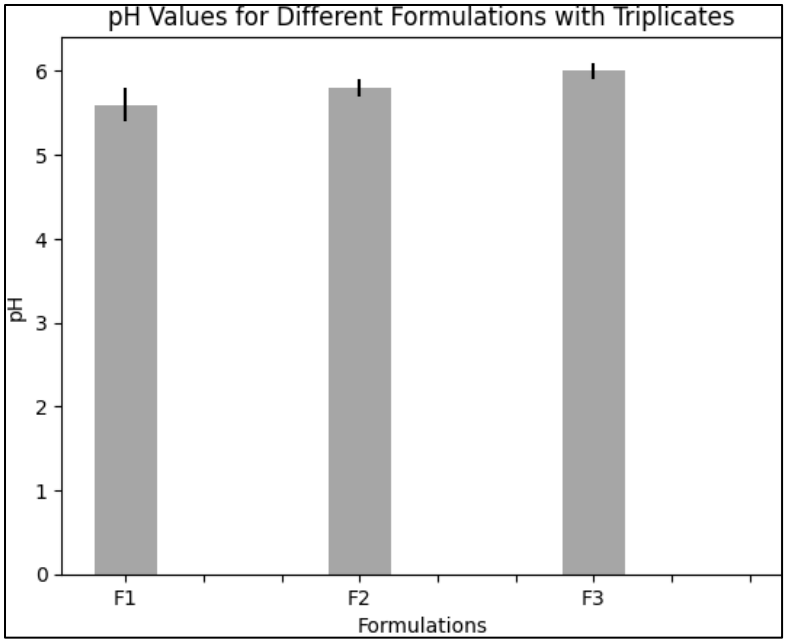

Fig: 1- pH of Hydrogel

Viscosity Measurement

The viscosity, or thickness, of the hydrogels increased from F1 to F3. This can be attributed to the different polymers used, with HPMC in F3 resulting in the highest viscosity (1172±27.6 cps) compared to Carbopol 934 in F1 (1056±37.4 cps) and Carbopol 940 in F2 (1143±9.2 cps).

Table: 3- Viscosity of Hydrogel (Mean & SD)

Sn.	Formulations	Triplicates	Viscosity (cps)
			50
1	F1	1	1056±37.4
2		2	
3		3	
4	F2	4	1143±9.2
5		5	
6		6	
7	F3	7	1172±27.6
8		8	

9		9	

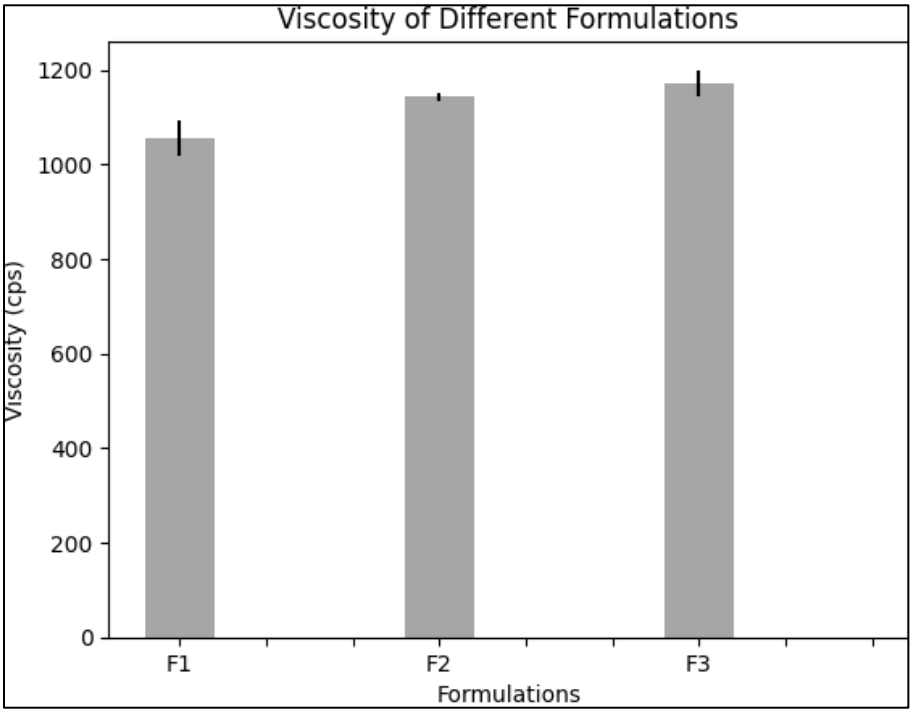

Fig: 2-Viscosity of Hydrogel at 50 RPM

Spreadability Test

Spreadability decreased from F1 to F3, suggesting that the hydrogel became less spreadable as the viscosity increased. F1 had the highest spreadability (9.24±0.08), while F3 had the lowest (8.15±0.05).

Table: 4- Spreadability of Hydrogel (Mean & SD)

Sn.	Formulations	Triplicates	Spreadability
1	F1	1	9.24±0.08
2		2	
3		3	
4	F2	4	8.36±0.04
5		5	
6		6	

7	F3	7	8.15±0.05
8		8	
9		9	

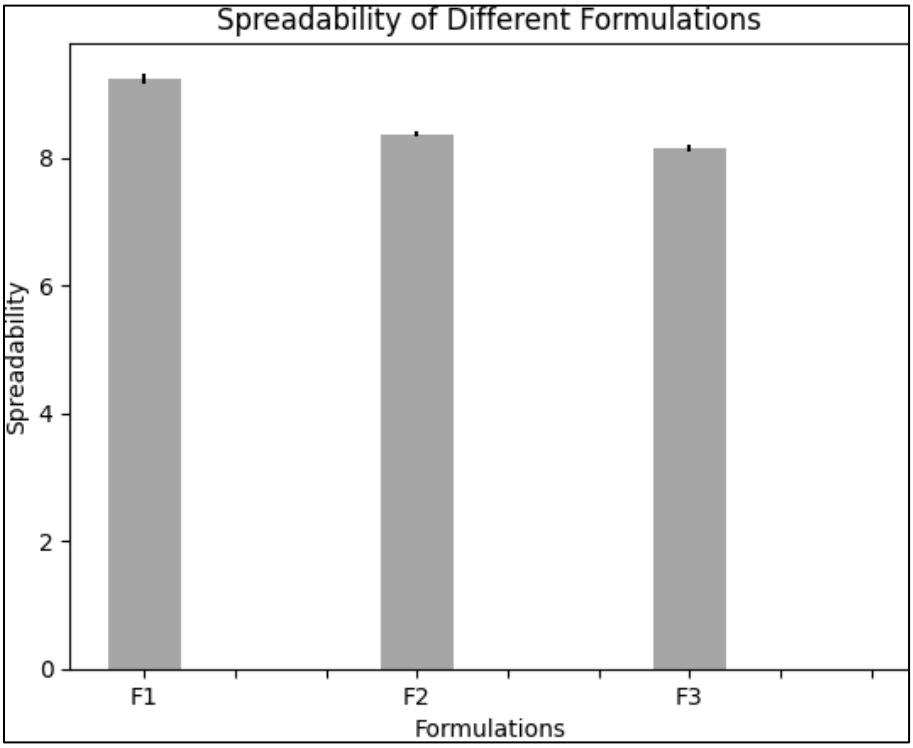

Fig: 3-Spreadability of Hydrogel

Drug Content Measurement

The drug content was highest in F1 (94.15±0.13%) and lowest in F3 (92.33±0.38%), indicating that the hydrogel formulation could have influenced the drug content.

Table: 5-Drug Content (Mean & SD)

Sn.	Formulations	Triplicates	Drug Content at 248nm
1	F1	1	94.15±0.13
2		2	
3		3	
4	F2	4	93.93±0.15

5		5	
6		6	
7	F3	7	92.33±0.38
8		8	
9		9	

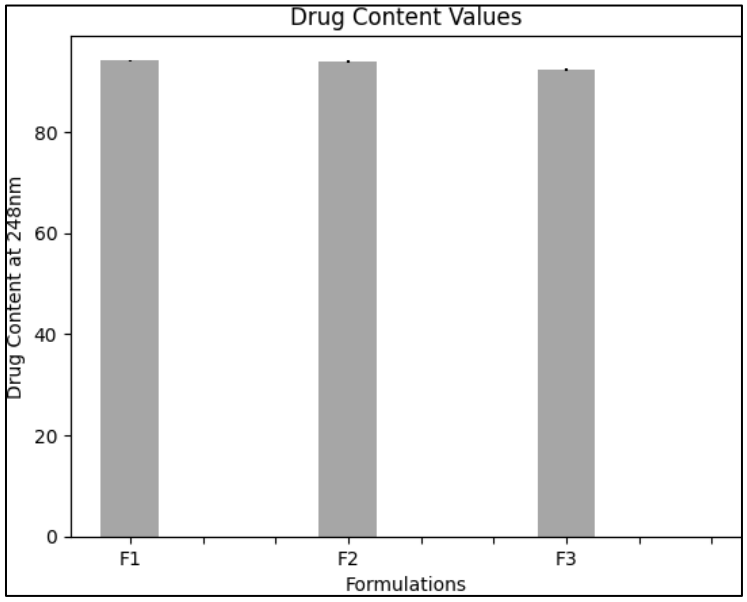

Fig: 4-Drug Content of Hydrogel

In Vitro Drug Release

The in vitro drug release data showed that all formulations displayed a sustained release over time. However, the release rate was highest in F1 and lowest in F3, aligning with the drug content results. This might suggest that the polymer used influences the drug release rate.

In summary, the results show that the formulation, specifically the type of polymer used, can significantly affect the properties of the hydrogel, such as color, viscosity, spreadability, drug content, and drug release rate. Further, the Saraca Indica bark extract retained its phytochemical properties throughout the formulation process, providing the hydrogel with potential therapeutic benefits.

Table: 6-In vitro Drug release

Sn.	Time	F1	F2	F3
1	0	0	0	0
2	1	9.85	9.50	11.89
3	2	29.71	29.36	26.90
4	3	38.85	38.50	29.93
5	4	59.42	59.07	49.78
6	5	60.94	60.59	60.54
7	6	72.58	72.23	67.97
8	7	84.85	84.50	77.97
9	8	95.26	94.91	89.34

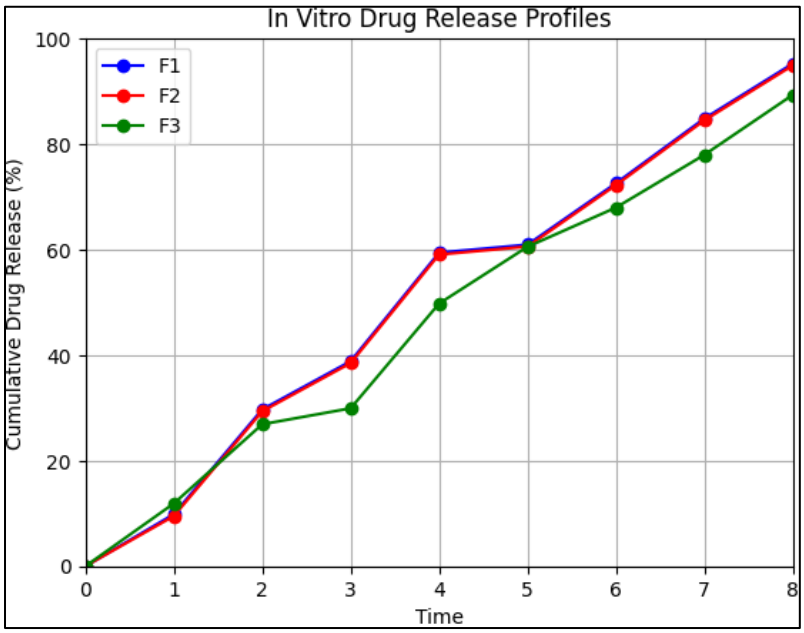

Fig: 5- In vitro drug release of Hydrogel

Conclusion

The development and in vitro characterization of Saraca Indica bark extract hydrogels were successfully carried out in this study. Three distinct formulations were prepared using different

types of polymers - Carbopol 934, Carbopol 940, and HPMC - each showing unique characteristics.

The organoleptic properties of the hydrogels - color, odor, and taste - remained largely unaltered after the drying process, which is a promising indicator for retaining the natural properties of the Saraca Indica extract. The phytochemical screening demonstrated the presence of beneficial compounds like alkaloids, flavonoids, phenols, reducing sugars, and amino acids in the Saraca Indica bark extract.

All the hydrogel formulations demonstrated favorable physical properties such as absence of grittiness, no phase separation, and suitable pH for skin applications. Although viscosity and spreadability varied between formulations, these characteristics can be controlled by altering the polymer type and concentration.

The drug content and in vitro release studies indicated that the hydrogel formulation could influence the encapsulation and release rate of the Saraca Indica bark extract. The highest drug content and release rate were observed in the F1 formulation with Carbopol 934.

In conclusion, the findings of this study suggest that Saraca Indica bark extract hydrogels, particularly the Carbopol 934-based formulation, could be a promising candidate for antimicrobial treatment applications. Further studies, including in vivo tests and clinical trials, would be necessary to validate their therapeutic efficacy and safety.

Discussion

This study focused on the development and in vitro characterization of Saraca Indica bark hydrogels, with the end goal of creating a potential new vehicle for antimicrobial treatment. The choice of Saraca Indica, a medicinal plant with well-known antimicrobial properties, as the main active ingredient laid the foundation for the research.

Formulations of hydrogels with different polymer bases were considered, including Carbopol 934 (F1), Carbopol 940 (F2), and HPMC (F3). All of these are commonly used in the pharmaceutical industry due to their favorable properties such as good viscosity and excellent stability. The results showed that the type of polymer used in the formulation significantly influenced the hydrogel's characteristics.

The organoleptic properties of the hydrogels, such as color, odor, and taste, showed minor changes after drying. This is an essential aspect, as drastic changes might indicate chemical alterations of the active ingredient, which was not the case in this study.

The phytochemical analysis highlighted the rich presence of pharmacologically active compounds in the Saraca Indica bark extract. These compounds are responsible for the extract's known therapeutic benefits, including its antimicrobial properties. The presence of these bioactive compounds in the hydrogel formulation underpins its potential efficacy as an antimicrobial treatment.

Physical examination of the hydrogels, including color, odor, homogeneity, grittiness, and phase separation, revealed favorable characteristics. The pH of all formulations was within a range suitable for skin applications, which is an essential attribute considering the topical nature of these hydrogels. The differences in the viscosity, spreadability, and drug content among the formulations can be attributed to the unique properties of the polymers used in each hydrogel formulation.

Furthermore, the in vitro drug release study showed that the F1 formulation (Carbopol 934) had the highest release rate. This implies that Carbopol 934 may be the most effective polymer for enhancing the release of the Saraca Indica bark extract from the hydrogel.

Overall, this study has provided substantial insights into the potential application of Saraca Indica bark extract hydrogels for antimicrobial treatments. While the results are promising, further studies, including in vivo experiments and clinical trials, are needed to validate these findings and determine the safety and efficacy of these hydrogel formulations in real-world conditions. In addition, further research could explore the possibility of optimizing these formulations or incorporating other bioactive compounds to enhance the antimicrobial efficacy of these hydrogels.

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