

Saraca Indica Leaf Extract Hydrogel for Wound Healing: Development and Evaluation

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ABSTRACT- Hydrophilic polymers, such as hydrogels, have long been employed as biomaterials. Because they are biocompatible, non-toxic, and extremely absorbent, they are frequently utilised in medicine and cosmetics. It has been shown that the Saraca Indica leaf extract contains both wound-healing and anti-inflammatory activities. The goal of this work was to create a hydrogel from the leaves extract of Saraca Indica that was mixed with several gelling agents, including Carbapol 934, Carbapol 940, and HPMC. The study's findings showed that both the medication and the excipients blended well with gelling agents. The outcomes were assessed using many criteria, including pH, viscosity, spreadability, and in vitro drug release. All of the settings worked well.

KEYWORDS- *Saraca Indica, Saraca Indica Hydrogel, Wound Healing*

1. INTRODUCTION

Plant source medicine is an alternative that uses plant products to treat or manage health conditions. This form of therapy dates back to ancient times, when people first began using plants for their medicinal properties [1]. Today, plant source medicine is still used by many people around the world as a complementary or alternative treatment for various conditions. Proponents of this form of therapy believe that plants contain natural substances that can help heal the body and mind. While there is scientific evidence to support some of these claims, much of the research on plant source medicine is anecdotal [2].

Wound healing is a complex process that involves the coordinated activity of many cells. The cells involved in wound healing include: fibroblasts, macrophages, neutrophils, and endothelial cells [3]. These cells work together to repair the damage caused by an injury. The first step in wound healing is inflammation. This is a response to injury that helps to protect the area from further damage and infection [4].

The next step is tissue repair, which is when the damaged tissue is replaced with new tissue. Finally, wound remodeling occurs, which is when the new tissue matures and becomes strong enough to support the body's weight [5]. Wound healing is a complex process that can take several weeks or even months to complete. However, it is an important process that helps our bodies recover from injuries and keep us healthy [6].

Hydrogels are polymeric materials that can absorb large amounts of water, and they have been shown to promote healing in several types of wounds [7]. In one study, hydrogel dressings were shown to improve the healing of diabetic foot ulcers, and in another study, they were effective in treating pressure ulcers. Hydrogel dressings are thought to work by providing a moist environment for the wound, which helps to keep the wound clean and prevents infection. They also provide a barrier against bacteria and other contaminants [8].

The Advantages of Hydrogel [9, 10, 11]

- Hydrogels are highly absorbent – can hold up to 400 times their weight in water, can be engineered to release drugs or other chemicals over time.
- Low immunogenicity – won't trigger an immune response
- Versatile – can be made into any shape or size
- Inexpensive to produce

Hydrogels have a number of advantages over other materials, which make them ideal for use in a variety of applications.

2. EXPERIMENTAL PROCEDURE

2.1. Collection of the Leaves [12]

The leaves of *Saraca Indica* were collected from the nearby nursery of the laboratory. The leaves were collected in airtight plastic bags and then rinsed with tap water to remove any dirt from the debris of the soil. The leaves were then kept for drying for a minimum of 15 days under the sunlight. The dried leaves were then crushed and grinded in the electrical grinder to make a fine powder. The powder of the leaves was then stored for further analysis, including the extractive values, extraction through the various solvents, and phytochemical analysis.

2.2. Extraction [13]

The dried leaves powder was extracted using the hot Soxhlet technique using three different extractors. Water, ethanol, and methanol were used as the extractor. Approximately 100 grams of the leaf powder was kept in the thimble attached to the container with an inlet and outlet. This glass container was fixed with the round bottom flask filled with the solvent. The round bottom flask was heated up to the temperature where the solvent started evaporating and reached the thimble. The extraction process was carried out for 4 hours continuously. The extract was then collected through steam distillation. The vaporized steam was then condensed into the liquid and collected in a separate beaker for further analysis.

2.3. Chemical and Instrument

The Chemicals and consumables were procured from different vendors. All the instruments were calibrated and validated, and the chemicals used in this study were analytical grade and extra pure.

2.4. Pharmacognostic and Physicochemical Parameters [14]

The pharmacognostic and physicochemical parameters were assessed for the authentication of the leaves of the plant which was being used in the experiment. The assessment was performed using the macroscopy and organoleptic properties. The shape, size, and color were investigated. Organoleptic parameters were assessed using the fragrance and taste of the leaves (Table-1).

2.5. Physicochemical Parameters [15]

These parameters were assessed using the Ash value, water-soluble Ash, acid insoluble ash, and loss on drying. The extractive values determine, including water-soluble extractive values, solvent soluble extractive values and yield values. The results are mentioned in table number- 2.

2.6. Phytochemical screening [16]

Phytochemical screening was carried out of *Saraca Indica* leaves extract for the presence of bioactive compounds. Aqueous and ethanolic extracts of *Saraca Indica* leaves were used in the study. Different solvents, like water, methanol, chloroform, petroleum ether, etc., were used for extraction. Phytochemical tests were performed for different constituents like alkaloids, flavonoids, tannins, saponins, etc. The results showed that both aqueous and ethanolic extracts contained alkaloids, flavonoids, tannins, and saponins, but petroleum ether extract showed no such activity. The results suggest that *Saraca Indica* leaves are a rich source of bioactive compounds which can be exploited for various medicinal purposes (Table-3).

2.7. Formulation Procedure [17]

The formulation procedure was followed as per the standards. Three different Polymers were used as a cross-linking agent, such as Carbopol 934, Carbopol 940, and HPMC. These polymers were added into the water with constant stirring and left for soaking overnight to get them completely dissolved. The polymer solution was stirred every two hours. The next day, the gel was checked for its consistency and pH. The consistency of the gel was then maintained by adding a few drops of Triethylamine. The parents of the gel were transparent and thick. The

preservatives were dissolved in propyl glycol and mixed well. The methyl paraben and propyl paraben must be mixed properly while adding to the propyl glycol. The drug was separately dissolved in purified water. At the constant temperature and RPM, all the different solutions were mixed to avoid clumping. Each formulation was prepared in triplicate (table-4).

3. EVALUATION PARAMETERS

3.1. Physical evaluation [18]

The physical evaluation of each formulation was performed individually for its physical appearance, for example, color, homogeneity, grittiness, and odor. The prepared hydrogel was colorless and odorless, with no particle observed and a smooth appearance. Phase separation was not identified in any of the formulations(table-5).

3.2. PH determination [19]

The pH plays a vital role in maintaining the acidity and basicity of the formulation. The pH was performed using the digital pH meter with the glass electrode. One gram of the gel was dissolved in 100 ml of the water and kept four to ask for settle. The electrode was dipped into the solution, and pH was recorded in triplicates for each formulation (table-6).

3.3. Viscosity [19]

Viscosity of each formulation was recorded using digital viscometer. Each test was performed at the room temperature at 50 RPM with the spindle number 3. The 100mL of the formulation was taken in the beaker under the probe of viscometer. The probe was dipped into the beaker until it reaches to the middle of the beaker. The rotation was started and reading was recorded once the reading has been stable. This process was followed for each formulation and recorded in the table-7.

3.4. Centrifuge test [18]

Centrifuge test was for each formulation; approximately 50 ml of the formulation was taken into the Falcon tubes. The Falcon tubes were fixed into the rotator of the centrifuge and rotated at the speed of 6000 RPM for 30 min to observe the phase separation. No phase separation was seen during the experiment (table-8).

3.5. Spreadability[20]

Spreadability was performed for each formulation using two glass plates pre-marked at 15 CM of diameter. Approximately 10 g of the formulation was placed over the plate and covered with the help of another plate. Approximately a hundred grams of the weight was kept on to the both plates and spread of the formulation was recorded once the process had been done. The results are recorded in the table-9.

3.6. In vitro drug release [18]

In vitro drug release study was performed to identify whether the formulation can be transported through the dialysis membrane. This test was performed using Franz diffusion cell apparatus, connected with the magnetic stirrer. All the six glass apparatus were fixed with dialysis membrane using a metal clip. The phosphate-buffered saline was filled into the container, and about one gram of the formulation was placed over the dialysis membrane and

allowed it to be transported into the phosphate-buffered saline. This test was performed for 48 hours; during each hour, the 1 ml of phosphate buffered saline and inspected using ultraviolet-visible spectrophotometry at 215 NM. The experiment was performed in triplicate for each formulation. The results are recorded in the table-10.

4. Results and Discussion

The hydrogel was prepared using the *Saraca Indica* leaves extract and three different gelling agents such as Carbopol 934, Carbopol 940, and HPMC. These crosslinking agents were used to assess the activity of in vitro drug release incorporated different excipients.

Initially, the plant leaves were collected, rinsed with water, and ground to a fine powder. The Powder was then extracted through the various solvents using the Soxhlet Apparatus. The percentage yields, as well as extractive values, were calculated. The results revealed that these values were similar to the normal plant values.

The physical appearance then includes color, grittiness, phase separation, and odor.

The formulation was inspected visually and found transparent; no phase separation was seen, particles were not observed during rubbing between the thumb and index finger, and it was odorless.

The final formulation was then inspected for the different evaluation parameters. The pH of the hydrogel was determined using the digital pH meter, and it was found to be between 6 to 6.5. This pH range is considered to be safe and effective to use in a wide range of medical settings.

The viscosity of hydrogel affects the spreading ability while applied to the skin. The viscosity was performed using the digital viscometer, which was found between 6052 and 6421.

The spreadability test was performed to see the spreading ability of the formulation once applied to the skin. All the formulations performed well. However, formulation 1 has greater spreadability with 9.24 as compared to other formulations.

In vitro drug release study revealed that formulation one showed maximum release during the 6, 7, and 8th hours compared to other formulations. The cumulative percentage release was 95.26 for formulation number 1.

Hence it could be concluded that formulation number 1, which was prepared using Carbopol 934 has more significant potential and is easily incorporated with the drug and release the drug at maximum as compared to other formulations.

5. Conclusion

Three distinct cross-linking techniques were used to create *Saraca Indica* hydrogel, which was then combined with the medication and other excipients to assess factors including pH, spreadability, viscosity, phase separation, and in vitro drug release. For each parameter, each formulation worked well. Carbopol 934 was used to create the formulation, which had a maximum drug release of over 95%. From the results, it can be inferred that Carbopol 934 is the optimal cross-linking for the creation of *Saraca Indica* leaves hydrogel.

6. Tables

Table-1: Shape, size and Texture

S. No.	Parameters	Before Drying	After Drying
1	Shape	Ovate and Oblong	Ovate and Oblong
2	Size	3-5 cm	1.5-2 cm
3	Texture	Hairy and Soft	Fibrous

Table-2: Extractive Values

Sn.	Extractive Values	Results
1	Water Soluble Extractive	20.14%
2	Alcohol Soluble Extractive	11.92%
3	Ash Value	2.14%
4	Acid Insoluble Ash	1.69%
5	Water soluble Ash	1.12%

Table-3:Preliminary Phytochemical Tests

S.No.	Chemical Tests	Extracts		
		Ethanol	Methanol	Aqueous
1	Alkaloids	++	++	++
2	Flavonoids	++	++	++
3	Glycosides	++	--	--
4	Phenols	++	++	++
5	Saponins	++	--	--
6	Steroids	++	--	++
7	Tannins	--	++	--
8	Reducing Sugar	++	++	++
9	Carbohydrates	--	++	++
10	Amino acids	++	++	++

Table-4: 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

Table-5: Physical Examination of Hydrogel

S.no	Physical Examination	Formulation Code		
		F1	F2	F3
1	Color	Transparent	Transparent	Milky white
2	Odour	None	None	None
3	Homogeneity	Excellent	Good	Poor
4	Grittiness	No grittiness	No grittiness	Tiny particles visualized
5	Phase Separation	No	No	No

Table-6: 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	

Table-7: Viscosity of Hydrogel (Mean & SD)

Sn.	Formulations	Triplicates	Viscosity (cps)
			50
1	F1	1	6128.67±81.35
2		2	
3		3	
4	F2	4	6359.00±4073
5		5	
6		6	
7	F3	7	6385.67±53.59
8		8	
9		9	

Table-8:- Centrifuge Test

Sn.	Formulations	Gel	Centrifuge	Physical Appearance
1	F1	1	6000	Not Separated
2		2	6000	Not Separated
3		3	6000	Not Separated
4	F2	4	6000	Not Separated
5		5	6000	Not Separated
6		6	6000	Not Separated
7	F3	7	6000	Not Separated
8		8	6000	Not Separated
9		9	6000	Not Separated

Table-9:- Spreadability

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	

Table-10:-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

7. REFERENCES

1. Chhabra, S., Chhabra, N., Kaur, A., & Gupta, N. (2017). Wound healing concepts in clinical practice of OMFS. *Journal of Maxillofacial and Oral Surgery*, 16(4), 403-423.
2. Zeng, R., Lin, C., Lin, Z., Chen, H., Lu, W., Lin, C., & Li, H. (2018). Approaches to cutaneous wound healing: basics and future directions. *Cell and tissue research*, 374(2), 217-232.
3. Wang, Xiaocheng, Jiang Chang, and Chengtie Wu. "Bioactive inorganic/organic nanocomposites for wound healing." *Applied Materials Today* 11 (2018): 308-319.
4. Ramos, R. M., Burland, M., Silva, J. B., Burman, L. M., Gelain, M. S., Debom, L. M., ... & Valmier, J. (2019). Photobiomodulation improved the first stages of wound healing process after abdominoplasty: an experimental, double-blinded, non-randomized clinical trial. *Aesthetic plastic surgery*, 43(1), 147-154.
5. Jin, Haiming, Zengjie Zhang, Chengui Wang, Qian Tang, Jianle Wang, Xueqin Bai, Qingqing Wang et al. "Melatonin protects endothelial progenitor cells against AGE-induced apoptosis via autophagy flux stimulation and promotes wound healing in diabetic mice." *Experimental & molecular medicine* 50, no. 11 (2018): 1-15.
6. Afkhamizadeh, Mozghan, Robab Aboutorabi, Hassan Ravari, Mohsen Fathi Najafi, Sajad Ataei Azimi, Adineh Javadian Langaroodi, Mohammad Ali Yaghoubi, and Amirhossein Sahebkar. "Topical propolis improves wound healing in patients with diabetic foot ulcer: a randomized controlled trial." *Natural product research* 32, no. 17 (2018): 2096-2099.
7. Kosaric, N., Kiwanuka, H., & Gurtner, G. C. (2019). Stem cell therapies for wound healing. *Expert opinion on biological therapy*, 19(6), 575-585.
8. Naseri, S., Lepry, W. C., & Nazhat, S. N. (2017). Bioactive glasses in wound healing: hope or hype?. *Journal of Materials Chemistry B*, 5(31), 6167-6174.
9. Desmet, Céline M., Véronique Prétat, and Bernard Gallez. "Nanomedicines and gene therapy for the delivery of growth factors to improve perfusion and oxygenation in wound healing." *Advanced Drug Delivery Reviews* 129 (2018): 262-284.
10. Goldberg, S. R., & Diegelmann, R. F. (2017). Basic science of wound healing. In *Critical Limb Ischemia* (pp. 131-136). Springer, Cham.
11. Liu, K., C. Chen, H. Zhang, Y. Chen, and S. Zhou. "Adipose stem cell-derived exosomes in combination with hyaluronic acid accelerate wound healing through enhancing re-epithelialization and vascularization." *British Journal of Dermatology* 181, no. 4 (2019): 854-856.

12. Chopin, T., & Tacon, A. G. (2021). Importance of seaweeds and extractive species in global aquaculture production. *Reviews in Fisheries Science & Aquaculture*, 29(2), 139-148.
13. Sharaf, M. H., Abdelaziz, A. M., Kalaba, M. H., Radwan, A. A., & Hashem, A. H. (2022). Antimicrobial, antioxidant, cytotoxic activities and phytochemical analysis of fungal endophytes isolated from *ocimumbasilicum*. *Applied biochemistry and biotechnology*, 194(3), 1271-1289.
14. Abbas, A., Naqvi, S. A. R., Rasool, M. H., Noureen, A., Mubarik, M. S., & Tareen, R. B. (2021). Phytochemical analysis, antioxidant and antimicrobial screening of *seriphidiumoliverianum* plant extracts. *Dose-Response*, 19(1), 15593258211004739.
15. Tanruean, K., Poolprasert, P., Suwannarach, N., Kumla, J., & Lumyong, S. (2021). Phytochemical analysis and evaluation of antioxidant and biological activities of extracts from three clauseneae plants in Northern Thailand. *Plants*, 10(1), 117.
16. Tang, Tzu-Chieh, EléonoreTham, Xinyue Liu, Kevin Yehl, Alexis J. Rovner, Hyunwoo Yuk, Cesar de la Fuente-Nunez, Farren J. Isaacs, Xuanhe Zhao, and Timothy K. Lu. "Hydrogel-based biocontainment of bacteria for continuous sensing and computation." *Nature Chemical Biology* 17, no. 6 (2021): 724-731.
17. Gao, Y., Han, X., Chen, J., Pan, Y., Yang, M., Lu, L., ... & Lu, T. (2021). Hydrogel–mesh composite for wound closure. *Proceedings of the National Academy of Sciences*, 118(28).
18. Gavan, Alexandru, LioraColobatiu, Daniela Hanganu, Catalina Bogdan, Neli Kinga Olah, Marcela Achim, and Simona Mirel. "Development and Evaluation of Hydrogel Wound Dressings Loaded with Herbal Extracts." *Processes* 10, no. 2 (2022): 242.
19. Mirzaii, Mehdi, Marzieh Yaeghoobi, MeysamAfzali, NeginsadatAmirkhalili, Majid Mahmoodi, and EsmailBabakhanzadehSajirani. "Antifungal activities of quince seed mucilage hydrogel decorated with essential oils of *Nigella sativa*, *Citrus sinensis* and *Cinnamon verum*." *Iranian Journal of Microbiology* 13, no. 3 (2021): 352.
20. Sindhu, R. K., Gupta, R., Wadhera, G., & Kumar, P. (2022). Modern Herbal Nanogels: Formulation, Delivery Methods, and Applications. *Gels*, 8(2), 97.
21. Pal N, Mandal S, Shiva K, Kumar B. Pharmacognostical, Phytochemical and Pharmacological Evaluation of *Mallotus philippensis*. *Journal of Drug Delivery and Therapeutics*. 2022 Sep 20;12(5):175-81.
22. Singh A, Mandal S. Ajwain (*Trachyspermum ammi* Linn): A review on Tremendous Herbal Plant with Various Pharmacological Activity. *International Journal of Recent Advances in Multidisciplinary Topics*. 2021 Jun 9;2(6):36-8.

23. Mandal S, Jaiswal V, Sagar MK, Kumar S. Formulation and evaluation of carica papaya nanoemulsion for treatment of dengue and thrombocytopenia. *Plant Arch.* 2021;21:1345-54.
24. Mandal S, Shiva K, Kumar KP, Goel S, Patel RK, Sharma S, Chaudhary R, Bhati A, Pal N, Dixit AK. Ocular drug delivery system (ODDS): Exploration the challenges and approaches to improve ODDS. *Journal of Pharmaceutical and Biological Sciences.* 2021 Jul 1;9(2):88-94.
25. Shiva K, Mandal S, Kumar S. Formulation and evaluation of topical antifungal gel of fluconazole using aloe vera gel. *Int J Sci Res Develop.* 2021;1:187-93.
26. Ali S, Farooqui NA, Ahmad S, Salman M, Mandal S. *Catharanthus roseus* (sadabahar): a brief study on medicinal plant having different pharmacological activities. *Plant Archives.* 2021;21(2):556-9.
27. Mandal S, Jaiswal DV, Shiva K. A review on marketed *Carica papaya* leaf extract (CPL) supplements for the treatment of dengue fever with thrombocytopenia and its drawback. *International Journal of Pharmaceutical Research.* 2020 Jul;12(3).
28. Mandal S, Vishvakarma P, Verma M, Alam MS, Agrawal A, Mishra A. *Solanum Nigrum* Linn: An Analysis Of The Medicinal Properties Of The Plant. *Journal of Pharmaceutical Negative Results.* 2023 Jan 1:1595-600.
29. Vishvakarma P, Mandal S, Pandey J, Bhatt AK, Banerjee VB, Gupta JK. An Analysis Of The Most Recent Trends In Flavoring Herbal Medicines In Today's Market. *Journal of Pharmaceutical Negative Results.* 2022 Dec 31:9189-98.
30. Mandal S, Vishvakarma P, Mandal S. Future Aspects And Applications Of Nanoemulgel Formulation For Topical Lipophilic Drug Delivery. *European Journal of Molecular & Clinical Medicine.*;10(01):2023.
31. Chawla A, Mandal S, Vishvakarma P, Nile NP, Lokhande VN, Kakad VK, Chawla A. Ultra-Performance Liquid Chromatography (Uplc).
32. Mandal S, Raju D, Namdeo P, Patel A, Bhatt AK, Gupta JK, Haneef M, Vishvakarma P, Sharma UK. Development, characterization, and evaluation of *rosa alba* l extract-loaded phytosomes.
33. Mandal S, Goel S, Saxena M, Gupta P, Kumari J, Kumar P, Kumar M, Kumar R, Shiva K. Screening of *catharanthus roseus* stem extract for anti-ulcer potential in wistar rat.
34. Shiva K, Kaushik A, Irshad M, Sharma G, Mandal S. Evaluation and preparation: herbal gel containing *thuja occidentalis* and *curcuma longa* extracts.