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Original Research Article

Anti-arthritic potentials of *Raphanus sativus* transdermal gel – An *in-vitro* study

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Abstract

Background: Rheumatoid arthritis (RA) is a chronic autoimmune disorder affecting over 18 million people globally, as reported by the World Health Organization (WHO) in June 2023. It leads to joint inflammation, pain, and progressive tissue damage, influenced by immune responses, genetic predisposition, and lifestyle factors. Current treatments range from Janus kinase (JAK) inhibitors, such as Tofacitinib and Baricitinib, to phytomedicines, which offer promising alternatives due to their bioactive compounds. Raphanus sativus (radish) has demonstrated anti-inflammatory properties, making it a potential candidate for RA management.

Objectives: This study aims to formulate and evaluate Raphanus sativus based hydrogels for in-vitro anti-arthritic activity. Objectives include assessing physicochemical properties, skin irritation potential, and efficacy using protein denaturation assays.

Methods: Hydrogels (RSG1, RSG2, and RSG3) were developed using Raphanus sativus leaf juice and carbopol, adjusted to pH 7.4. Stability, spreadability, viscosity, and washability were analyzed. Skin irritation potential was assessed, and protein denaturation assays were conducted using bovine serum albumin (BSA) and egg albumin models, with efficacy compared to Diclofenac sodium.

Results: The hydrogels exhibited stability, favorable physicochemical characteristics, and no skin irritation. Protein denaturation assays demonstrated significant anti-arthritic activity, comparable to Diclofenac sodium. The presence of flavonoids, polyphenols, and terpenoids contributed to efficacy.

Conclusion: The findings support further clinical exploration of Raphanus sativus based formulations as potential phytomedicines for RA management. However, the study is limited to in-vitro assays, and further in-vivo and long-term toxicity evaluations are necessary to confirm safety and efficacy.

Keywords: Apigenin, Arthritis, Denaturation, Luteolin, Raphanus sativus, Transdermal gel, In-vitro study.

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1. Introduction

Over the last few years, more than 18 million people worldwide have been projected to have rheumatoid arthritis (RA), with a global prevalence of 0.24–1%. Women are twice as likely to be affected as men. RA is a chronic inflammatory disease that primarily affects the joints and surrounding tissues. The exact cause of RA remains unknown, but studies suggest it may be linked to immune responses, genetic factors, and environmental influences such as age, diet, and lifestyle.[1] Immune system cells migrate to the site of inflammation and interact with adjacent synoviocytes, triggering cytokine production and stimulating mesenchymal cell release. T-helper 17 (Th17) cells secrete interleukin-17 (IL-17), prompting synoviocytes to generate IL-6, while monocytes produce IL-1 and tumor necrosis factor (TNF), leading to chronic inflammation. IL-6 contributes to hyperplasia and promotes bone degradation, resulting in RA. Synthetic treatments for RA include several medications, such asNon-steroidal anti-inflammatory drugs (NSAIDs) like Ibuprofen, Naproxen and Diclofenac and also disease-modifying antirheumatic drugs (DMARDs) like Methotrexate, Azathioprine, Leflunomide and Hydroxychloroquine. Moreover, Janus kinase (JAK) inhibitors like Tofacitinib, Baricitinib, Upadacitinib, Filgotinib and Peficitinib are also in practice in addition to biologic therapies such as TNF and IL-6 inhibitors for severe cases. Physical therapy and exercises are also being in practice to relieve joint pains.[2]

While conventional treatments are effective, they often cause notable side effects, whereas herbal medicines provide

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therapeutic benefits with minimal adverse effects. In Chinese traditional medicine, *Raphanus sativus* (radish) has been widely used for treating epigastric pain, constipation, diarrhea, coughing with phlegm, and congestion. It has also been a staple in Korean traditional medicine, serving as a laxative, expectorant, diuretic, anti-inflammatory, and anti-neoplastic agent.[3] Historically, in India, *Raphanus sativus* has been employed in treating asthma and certain cardiac conditions.[4] Commonly referred to as mooli, mullangi, red radish, and white radish. *Raphanus sativus* belongs to the Brassicaceae family and contains various bioactive compounds, including flavonoids, raphanin, isothiocyanates, vitamins, alkaloids, saponins, phenols, glycosides, proteins, steroids, carbohydrates, anthraquinones, glucosinolates, fiber, potassium, β-carotene, and calcium.[5] Several studies highlight the pharmacological properties of *Raphanus sativus* including anti-inflammatory activity,[6] antidepressant effects,[7] anxiolytic effects,[8] antinociceptive properties,[9]anti-asthmatic effects,[10] gut stimulatory activity,[11] antioxidant and anti-mutagenic activity,[12] anti-atherosclerotic effect,[13]anti-diabetic activity and intestinal motility regulation.[14-15] Furthermore, raw radish juice is believed to be effective against autoimmune conditions based on traditional claims in Andhra Pradesh, India. Although several other plant extracts have also shown potential therapeutic effects against RA, including *Vitex negundo* (leaf extract),[16] *Ricinus communis* (leaf extract),[17] *Urtica dioica* (root extract),[18]*Zingiber officinale* (rhizome extract),[19] *Albizia lebbeck* (bark extract),[20] *Ficus benghalensis* (tree sap),[21] *Rubia cordifolia* (root extract),[22] *Terminalia macroptera* (stem bark extract),[23] and *Curcuma longa* (turmeric extract).[24] With above background the studywas focused on exploring the potential of *Raphanus sativus* leaf juice as an anti-arthritic agent through the formulation of a hydrogel for transdermal application, using a suitable gelling agent (Carbopol-940).

2. Materials and Methods

Carbapol-940, HPMC, sodium benzoate, triethanolamine, propylene glycol of AR grade was procured from M/s. SD Fine Chem. Ltd., Mumbai, India. Double distilled water was utilized throughout the experiment. Experimental animals (Wistar albino rats) and feed were obtained from M/s. Sri Venkateswara Animal House, Bengaluru, India (Reg. No. 1521/PO/A/11/CPCSEA).

2.1. Preparation of leaf juice

The fresh leaves collected from local marketwere washed with distilled water and then strained to remove water completely. The fresh juice of *Raphanus sativus* leaves was prepared by cutting the leaves into smaller pieces followed by crushing with suitable mechanical force. The juice was clarified using muslin cloth.

2.2. Preparation of hydrogel

As mentioned in (**Table 1**), different quantities of ingredients were weighed on M/s. Elico DigiBal (Model: Cal357) and double distilled water (100 ml) used as vehicle to dissolve them with help of thermostatic agitation. The contents were left for hydration/swell about 6 hours. The pH of gel was adjusted to 7.4 by adding triethanolamineand sodium benzoate was added as preservative. Three clear and transparent *Raphanus sativus* leaf juice loaded hydrogels were characterized as they obtained.

2.3. Evaluation of gel

The bioactives of *Raphanus sativus* leaves loaded hydrogels were characterized and evaluated for their quality attributes.Physical appearance and homogeneity of the prepared hydrogels were visually examined and data were compiled. Concentration of the hydronium ion in gels was measured using a glass membrane electrode of digital pH meter (M/s. Elico LI 200, Hyderabad, India). The flow characteristics of the hydrogels were characterized by using Brookfield viscometer with spindle no. 63 at the rate of 10 rpm. The spreadability of the each gel formulation was calculated using the formula, $s = m \times l/t$, where 'm' is sample weight, 'l' is length of slide and 't' is time required to separate both slides against load direction. The washability of the gels was assessed by washing off the gel by hot stream of water from the applied local portion of vital area of the skin.Irritation on biological skin was evaluated by applying transparent *Raphanus sativus* leaf juice loaded hydrogels carefully on depilated portion of the experimental animal skin of six experimentalWistar albino rats. The experimentally treated animals (Wistar albino rats, n=6) were observed for the changes on their skin surface for a period of 36 h.[25-26]

2.4. In vitro anti-arthritic activity

2.4.1. Inhibition of protein denaturation by BSA method

Freshly prepared 5%w/v aqueous solution of bovine serum albumin (BSA) was utilised for in-vitro reaction mixture. About 4.5 ml of BSA and 0.5 ml of different concentrations (20, 40, 60, 80 and 100 µg/ml) of gel of *Raphanus sativus* were mixed thermostatically and resultant samples were incubated at 37°C for 180 min. The samples were diluted with phosphate buffer saline (PBS) (pH 6.3) and assayed at 220 nm using UV visible spectrophotometer (M/s. LabIndia Ltd., Chennai). A blank sample, excluding hydrogel, was used as a control to represent 100% protein denaturation. Results were compared against marketed diclofenac sodium (10 µg/ml) gel formulation. The inhibition (%) of protein denaturation was assessed using, $(A_c - A_s)/A_c \times 100$, where A_c is absorbance of control and A_s is absorbance of test sample.

2.4.2. Preparation of egg albumin solution

The outer shell of the egg was broken with the help of a glass rod and colourless liquid albumin was separated and added to a beaker containing 100 ml sodium chloride solution at constant stirring. Thermostatic stirring was continued to ensure that egg albumin in water is formed.

Undissolved/lumps of sodium chloride-albumin mixture was filtered off from the resultant solution.

2.5. Inhibition of albumin denaturation by egg albumin method

The reaction mixture was comprised of egg albumin solution (0.2 ml), PBS (2.8 ml) and about 2 ml of varying concentrations clear *Raphanus sativus* leaf juice loaded hydrogels (20, 40, 60, 80, 100 µg/ml). The reaction mixtures were incubated at 37°C upon proper dilution with double distilled water about 15 min followed by heating at 70°C for 5 min. The cooled samples were assayed at 220 nm against blank. The inhibition of albumin denaturation by egg albumin was assessed employing a protocol analogous to that utilized in the BSA denaturation assay.

3. Results

Phytomedicine has long been recognized for its diverse therapeutic applications, primarily due to the presence of secondary metabolites such as flavonoids, vitamins, alkaloids, saponins, phenols, and glycosides. In recent years, phytomedicine-based hydrogels have gained attention for their potential in rheumatoid arthritis (RA) treatment, offering localized relief with minimal side effects compared to conventional oral medications. This study formulated and evaluated hydrogels loaded with bioactives extracted from *Raphanus sativus* leaves, focusing on their physicochemical properties, stability, and therapeutic efficacy. The developed formulations viz. RSG1, RSG2, and RSG3 had exhibited optimal surface pH, spreadability, washability, viscosity, and intact physical characteristics, making them suitable for transdermal application. The hydrogels maintained a flow range of 160–180, ensuring smooth application and enhanced absorption, supporting their potential as an effective RA treatment alternative.

Table 1: The various formulation of gel preparation

Ingredients	Formulation Code		
	RSG1	RSG2	RSG3
Raphanus sativus leaf juice (ml)	5	10	10
HPMC (gm)	1	-	1.5
Propylene glycol (ml)	-	-	2
Carbapol-940(gm)	0.5	0.5	1.5
Sodium benzoate (gm)	0.2	0.2	0.2
Triethanolamine (ml)	0.5	0.5	0.5

Table 2: Data of physicochemical characterization of *Raphanus sativus* gel

Formulation	Appearance	pH	Skin irritation	Washability	Spreadability (cm)	Viscosity (cP)
RSG1	Yellow	9(±0.05)	Yes	Medium	0.55	182
RSG2	Greenish smooth	7(±0.05)	No	Good	0.7	178
RSG3	Yellowish Brown	8(±0.05)	Yes	Medium	0.6	167

Table 3: *In-vitro* anti-arthritic activity of *Raphanus sativus* gel by BSA method

Treatment	Concentration (µg/ml)	BSA method		Egg albumin method	
		Absorbance	% Inhibition	Absorbance	% Inhibition
Raphanus sativus gel	20	0.179	26.90	0.143	29.90
	40	0.197	39.19	0.175	45.14
	60	0.287	46.05	0.231	53.51
	80	0.401	49.62	0.294	55.01
	100	0.450	54.95	0.421	57.40
	IC_{50}	0.247	51.01	0.215	52.43
Diclofenac	50	0.196	38.66	0.152	36.10

The *in-vitro* anti-arthritic potential of *Raphanus sativus* leaf juice-loaded hydrogels was assessed using bovine serum albumin (BSA) and egg albumin denaturation protocols, which evaluate protein stability and inflammatory inhibition. The findings indicated that the percentage inhibition of albumin denaturation was directly proportional to the concentration of bioactives within the hydrogel, demonstrating dose-dependent anti-inflammatory activity. Additionally, RSG1, RSG2, and RSG3 showed comparable efficacy to marketed Diclofenac sodium formulations at concentrations above IC50 (24.78 µg/mL in BSA assay; 29.04 µg/mL in egg albumin assay).

4. Discussion

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease that primarily affects the joints, causing pain, swelling, and the gradual breakdown of bone and cartilage. The pathological mechanisms of RA include an increase in pro-inflammatory cytokines, immune cell infiltration into the synovial membrane, and immune system dysregulation. Previous research has shown that flavonoids such as luteolin and apigenin are commonly found in phytomedicines that possess anti-inflammatory, anti-

arthritic, and skin penetration properties. Recent scientific findings indicate that the *Raphanus sativus* tuber plant, especially its leaves, is rich in polyphenols, flavonoids, and terpenoids, making it a promising candidate for RA treatment.

The leaf juice-incorporated hydrogel formulations (RSG1, RSG2, and RSG3) are clear, homogenous, and consistent, providing a moisturizing effect on flaky skin due to the presence of highly hydrophilic polymers such as HPMC and Carbopol 940. As presented in (**Table 2**), the formulations appear clear and viscous, primarily due to the crosslinked polyacrylic acid polymer (Carbopol 940), which undergoes swelling upon contact with the aqueous leaf juice of *Raphanus sativus*. During the formulation process, HPMC, with its hydrophilic hydroxyl, methoxyl, and hydroxypropyl groups, attracted bioactive molecules from the aqueous leaf juice via hydrogen bonding. As a result, RSG1, RSG2, and RSG3 became clear, homogeneous, viscous, washable, and evenly spreadable gel medications, causing no irritation or erythema on depilated skin tissue.

Furthermore, the hydrogels demonstrated an optimal pH range of 7.0–9.0, indicating their physiological compatibility with mildly alkaline environments and their potential compatibility with excipients used in the formulations. The pH variations among the hydrogel formulations resulted from the ionization of carboxylic acid groups in Carbopol 940, forming carboxylate anions. To ensure comfortable application on biological tissue, the pH of RSG1, RSG2, and RSG3 was adjusted using triethanolamine (**Table 1**), making them suitable for transdermal use (**Table 2**).

Since RA involves chronic inflammation and protein denaturation, it leads to the generation of autoantigens, which initiate and propagate immune responses, contributing to joint inflammation and tissue destruction. Therefore, stabilizing proteins and preventing their denaturation *in-vitro* can serve as an indicator of anti-arthritic potential. Bioactive compounds capable of inhibiting heat-induced protein denaturation in bovine serum albumin (BSA) or egg albumin assays may also help prevent or reduce inflammation in RA. A higher percentage of protein denaturation inhibition suggests that RSG1, RSG2, and RSG3 may suppress autoimmune responses in arthritis by preventing the exposure of antigenic determinants, reducing immune complex formation, and minimizing inflammatory cascades within joints.

In-vitro albumin denaturation inhibition assays serve as predictive tools for identifying substances with potential anti-arthritic activity. Data in (**Table 3**) indicated that RSG1, RSG2, and RSG3 effectively prevented albumin (protein) denaturation, limiting the entry of inflammatory cells such as neutrophils, lymphocytes, and macrophages into the synovial membrane. In both BSA and egg albumin tests, protein denaturation was significantly reduced, confirming the anti-arthritic properties of RSG1, RSG2, and RSG3. These findings suggested that bioactives, such as flavonoids (luteolin and apigenin), polyphenols, and terpenoids from *Raphanus sativus* leaf-loaded hydrogels, play a key role in protein stabilization and reducing inflammation and oxidative stress, making them promising therapeutic agents for RA management, warranting further clinical exploration and optimization for patient-centric formulations.

5. Conclusion

The Indian traditional system of medicine is enriched with herbal, mineral, and phytomedicinal resources, offering a vast array of therapeutic applications. The phytoconstituents of *Raphanus sativus* have exhibited diverse antioxidant potentials, contributing to their medicinal value. In this study, the bioactive compounds extracted from the leaves of *Raphanus sativus* were successfully formulated into hydrogels using hydrophilic, gel-forming, and matrix-forming polymers, such as HPMC and Carbopol 940. The hydrogel formulations RSG1, RSG2, and RSG3 demonstrated substantial texture, clarity, optimal pH balance, and absence of skin irritation, ensuring suitability for transdermal applications. These gels effectively inhibited protein denaturation, thereby exhibiting in-vitro anti-arthritic activity against BSA and egg albumin denaturation assays. However, further *in-vivo* studies are required to comprehensively evaluate the safety and efficacy of RSG1, RSG2, and RSG3 for clinical RA management.

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7. Ethical No

1521/PO/A/11/CPCSEA.

8. Source of Funding

None.

9. Conflict of Interest

None.

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