

Development and Evaluation of Polyherbal formulation for Inflammatory conditions

Abstract

Inflammation is a localized physical condition in which the inflamed part of the body develops swelling, redness, pain, etc. in response to an infection or injury. Infections, wounds and tissue damages cannot heal without an inflammatory response.

A diverse array of natural products, spanning from crude extracts to isolated compounds, exhibit anti-inflammatory properties, making them valuable in managing inflammatory conditions. Botanicals serve as safe, natural, and economically viable alternatives to synthetic drugs. The following three herbs, *Calotropis procera*, *Rosa indica* and *Adhatoda vasica* were chosen for further studies on the basis of the literature review, ease of availability and absence of evidence regarding the presence of their extracts in any marketed herbal formulation.

This study focuses on the systematic preparation, standardization, and analysis of plant extracts of flowers of *Rosa indica*, leaves of *Adhatoda vasica*, and leaves of *Calotropis procera*. The aqueous and methanolic extracts of the plants were prepared using the Soxhlet apparatus, which provided efficient and consistent extraction of phytochemicals. Qualitative phytochemical screening was conducted to confirm the presence of key bioactive constituents such as alkaloids, flavonoids, tannins, saponins, proteins, phenolics etc. Quantitative analysis of the active compounds in respective extracts, was further performed using High-Performance Thin-Layer Chromatography (HPTLC). This method allowed for precise quantification of specific marker compounds within the extracts, ensuring the consistency and therapeutic potential of the final product.

Molecular docking studies for compounds of all three plants was performed against various receptors like COX-1, COX-2, IL-6 and TNF- α . The list of chemical constituents of plants was obtained through literature survey. Majority of phytoconstituents were obtained from IMPPAT (Indian Medicinal Plants, Phytochemistry and Therapeutics). Molecular docking was done using PyRx and AutoDock Vina. Results are obtained in the form of binding affinity of ligands towards the receptors. Ligands having highest binding affinity are sought to be most interacting ligand. Chemical constituents of all three plants like α -amyrin, β -amyrin, Syriogenin, phytosterol, Calaptropagenin, and Isosteviol exhibited relatively strong binding affinities towards all the receptors. The results of molecular docking studies showed a possible potential of anti-inflammatory activity of all three plants.

For *In-vitro* studies of extracts, THP-1 human monocytic cells were used to assess anti-inflammatory activity of herbal extracts. The cells were stimulated with the indicated concentration of LPS or in combination with aqueous and methanolic extracts of *Adhatoda vasica*, *Calotropis procera* and *Rosa indica*. Cell viability assay was performed by MTT assay for aqueous and methanolic extracts of each individual plant and mixtures of aqueous and methanolic extracts. Evaluation of anti-inflammatory activity of extracts was performed by ELISA Test of cytokines like IL-6, IL-10, IL-4 and TNF- α . The results of MTT assay indicated that aqueous extracts were comparatively safer than the methanolic extracts owing to their least cytotoxicity effect. The anti-inflammatory effect of the extracts was confirmed owing to reduced levels of inflammatory cytokines like TNF- α and IL-4 and increased levels of anti-inflammatory cytokine like IL-10.

Following the confirmation of anti-inflammatory activity in extracts of selected medicinal plants, a polyherbal gel formulation was developed using the Box-Behnken design, a statistical approach for optimizing formulations. The developed gel was then subjected to a comprehensive evaluation to ensure its quality and efficacy. Key parameters assessed included appearance, viscosity, spreadability, pH, extrudability, and assay of active components. In addition to the polyherbal gel, a polyherbal spray formulation was developed and rigorously evaluated for pH, clarity of the solution, delivery rate, spray pattern, and area covered by each spray, leakage from the container and assay of constituents by HPTLC method. Both the formulations were found to be consistent and stable.

In-vivo animal study was conducted to evaluate the safety and efficacy of two developed polyherbal formulations: Gel and Spray. Initially, a skin irritation study was performed to assess the dermal safety of both formulations. Following this, the anti-inflammatory activity of the formulations was evaluated using the carrageenan-induced paw edema model in rats. Both the gel and spray demonstrated significant anti-inflammatory effects, as evidenced by the reduction in paw edema compared to control groups. These findings suggest that the polyherbal formulations are not only safe for skin application but also effective in mitigating inflammation, supporting their potential therapeutic use. The overall results of the presented work concluded that the prepared Polyherbal Gel and Polyherbal Spray are promising formulations for different Inflammatory conditions.