9. Summary and Conclusion

The ongoing quest for new anti-inflammatory agents increasingly focuses on natural products, which show significant promise. Recent trends in drug discovery highlight a growing interest in screening phytoconstituents for anti-inflammatory properties. Many plants exhibit pharmacological effects primarily due to secondary metabolites such as flavonoids and triterpenoids. These phytoconstituents have shown activity against multiple inflammation targets at low concentrations with minimal toxic effects. Given the current drug discovery landscape and advanced molecular techniques, it is more advantageous to extend anti-inflammatory research on already known natural products rather than searching for new ones. Previous studies mainly utilized crude extracts (at high doses) or isolated phytoconstituents but have not thoroughly explored their molecular mechanisms. Therefore, a systematic screening of natural products using appropriate animal models, extensive biochemical and molecular assessments, and the development of reliable safety data is essential to uncover better anti-inflammatory agents from natural sources.

The plant materials were extracted in methanol and distilled water by Soxhlet apparatus. These extracts were then subjected to preliminary phytochemical analysis using chemical tests which revealed the presence of alkaloids, saponins, carbohydrates, glycosides, proteins and amino acids, phytosterols, flavonoid and phenolics in aqueous and methanolic extracts of *Calotropis procera*, *Adhatoda vasaka* and *Rosa indica*.

The chemical markers Gallic acid, Rutin and Vasicine were used for the standardization of leaves of *Calotropis procera*, flowers of *Rosa indica* and leaves of *Adhatoda vasica* respectively. The HPTLC methods (Method I, II and III) provide accurate and reproducible quantitative analysis for determination of Gallic acid, Rutin and Vasicine. The R_f value of standard Vasicine, Gallic acid and Rutin in the developed HPTLC chromatogram was found to be 0.31, 0.25 and 0.78 respectively. The %w/w of Vasicine, Gallic acid and Rutin were determined to be 0.716%, 0.077% and 0.319% respectively.

Molecular docking for compounds of all three plants was performed against various receptors like COX-1, COX-2, IL-6 and TNF-α. From the results it can be concluded that TNF-α had best results in terms of maximum number of ligands showing higher binding affinity followed by COX-2 and COX-1 receptors. Molecular docking results of IL-6 were least satisfactory since most ligands showed poor binding affinity towards IL-6. The comparison of the binding

capacities of various ligands to different receptors revealed that α- and β-Amyrin demonstrated the strongest binding affinity across all receptor types, making them the most effective ligands in this study. Other active compounds, such as Syriogenin, phytosterol, Calaptropagenin, and Isosteviol, also exhibited relatively strong binding affinities towards several receptors. These findings suggest that the components present in all three plants contribute to their potential anti-inflammatory activity.

The aqueous extracts of Rosa indica, Adhatoda vasica, and Calotropis procera have shown notable anti-inflammatory effects by decreasing the production of pro-inflammatory cytokines IL-4 and TNF-α in activated THP-1 cells. It is suggested that these extracts work by inhibiting the excessive production of inflammatory mediators such as TNF-α and IL-4. Moreover, these extracts have been found to increase levels of IL-10, known for its anti-inflammatory properties. This alteration in cytokine levels indicates the potential of these plant extracts as therapeutic agents for treating inflammatory conditions.

The quadratic model in the Box-Behnken Design was used to produce the optimum formulation of *Adhatoda vasica, Rosa indica* and *Calotropis procera* extracts in polyherbal gel. The optimized polyherbal gel formulation was composed of Carbopol 934 (1%), TEA (0.4%), and Extracts (3%). The optimized gel was found to have stable and desirable properties like pH of 5-6.5, a spreadability of 5-7 g.cm/s, Good Extrudability and viscosity of 4900-6300 cps. Results have shown that gel formulation was good in appearance, homogeneity, extrudability, and spread ability. The optimized Polyherbal Spray formulation was composed Water, Ethanol and Extracts (3%). The optimized polyherbal spray formulation was found to be clear and had desirable properties like pH of 6.5-7, a delivery rate 0.7-0.91 g/s, Spray pattern of 2-3 cm, area covered by each spray 5-7 cm^2 and controlled leakage from container (0.1-0.2 %). Both the formulations were found to be stable and deliverable.

The pharmacological screening was carried out by using the carrageenan-induced edema model to evaluate the possible anti-inflammatory activity of the two developed formulations. The Polyherbal gel was found to be more effective than the standard Diclofenac gel. The Polyherbal gel exhibited 67.3% of inhibition of edema which was higher than the 61.66 % of inhibition of standard Diclofenac gel. The anti-inflammatory effect of Polyherbal spray and standard Diclofenac spray was found to be comparable with 52 % and 55.5% inhibition of edema respectively.

Both the optimized polyherbal gel and spray formulations have shown significant potential as effective and safe treatments for inflammatory conditions. The systematic development process ensured that both formulations met the desired quality and efficacy standards. The use of natural herbal extracts not only enhances the therapeutic potential but also reduces the likelihood of adverse effects commonly associated with synthetic drugs. These formulations offer a viable, natural, and cost-effective alternative for patients, promoting better health outcomes and greater acceptance in the realm of herbal medicine.

Future studies may focus on further clinical evaluations and exploring the broader applications of these formulations in various inflammatory and dermatological conditions. The success of these formulations underscores the value of integrating traditional herbal knowledge with modern scientific techniques to develop innovative healthcare solutions.

Outcome of the Project

Ayurveda, a natural system of medicine, originated in India more than 3,000 years ago. The term Ayurveda is derived from the Sanskrit words ayur (life) and veda (science or knowledge). The developed formulations, owing to their derivation from natural sources, can be included under the category of Ayurvedic Proprietary medicine. The developed formulations can be used for treating different inflammatory conditions mentioned below.

Ankylosing Spondylitis, Antiphospholipid Antibody Syndrome, Autoimmune Encephalitis, Chronic Recurrent Multifocal Osteomyelitis, Gout, Juvenile Dermatomyositis, Juvenile Idiopathic Arthritis, Juvenile Lupus (SLE), Juvenile Scleroderma, Juvenile Vasculitis, Lupus (Systemic Lupus Erythematous), Mixed Connective Tissue Disease, Myositis, Poststreptococcal Inflammatory Syndromes, Psoriatic Arthritis, Reactive Arthritis, Rheumatoid Arthritis, Scleroderma, Sjogren's Syndrome, Spondylarthritis, Systemic Juvenile Idiopathic Arthritis, Undifferentiated Connective Tissue Disease, Uveitis, Vasculitis.