

Development and Evaluation of Topical herbal Formulation for anti Inflammatory Activity

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ABSTRACT

The present study was undertaken to develop and evaluate novel and conventional topical herbal formulations for anti-inflammatory activity using selected medicinal plants, namely *Curcuma longa*, *Epilobium angustifolium*, and *Agathosma betulina*. These plants were selected based on ethnomedicinal relevance and were authenticated prior to use. The extracts were subjected to pharmacognostic, phytochemical, and physicochemical standardization, confirming the presence of important bioactive constituents such as phenolics, tannins, terpenoids, and flavonoids, and ensuring compliance with WHO guidelines for safety and quality. Topical formulations including conventional creams, transdermal patches, and topical film-forming solutions were developed using a 3² full factorial design to optimize formulation variables. The prepared formulations were evaluated for physicochemical parameters such as pH, viscosity, homogeneity, spreadability, mechanical strength, moisture content, and film integrity. In vitro drug release studies were performed using suitable diffusion techniques, and release kinetics were analyzed using various mathematical models. The optimized formulations exhibited desirable physicochemical properties and demonstrated sustained and controlled drug release profiles. Kinetic modeling indicated that drug release followed diffusion-controlled mechanisms, predominantly Higuchi and Korsmeyer–Peppas models. The findings of the study suggest that the developed formulations are stable, effective, and suitable for topical delivery of herbal actives. In conclusion, the study highlights the potential of combining traditional herbal knowledge with modern formulation strategies to develop safe, efficient, and patient-compliant topical anti-inflammatory drug delivery systems.

INTRODUCTION

The present study entitled “Development and Evaluation of Topical Herbal Formulation for Anti-Inflammatory Activity” was undertaken with the objective of developing effective, safe, and patient-compliant topical drug delivery systems using selected medicinal plants. Based on ethnomedicinal information and literature survey, three plants namely *Curcuma longa*, *Epilobium angustifolium*, and *Agathosma betulina* were selected and authenticated for the study. The procured extracts were subjected to comprehensive standardization procedures. Preliminary organoleptic evaluation revealed that *Curcuma longa* extract was a yellow amorphous solid, whereas *Epilobium angustifolium* and *Agathosma betulina* extracts were clear liquids with characteristic odor. Phytochemical screening confirmed the presence of important bioactive constituents such as carbohydrates, amino acids, proteins, volatile oils, steroids, terpenoids, tannins, and phenolic compounds in all extracts, while flavonoids were absent in *Curcuma longa* but present in the other extracts. Physicochemical evaluation demonstrated that all extracts complied with WHO guidelines, showing acceptable levels of polyphenols, flavonoids, microbial load, aflatoxins, pesticide residues, and heavy metals, confirming their safety and suitability for formulation development.

Types of Chronic Inflammation

Nonspecific proliferative:

Described by the presence of a diffuse granulation tissue delivered by the development of fibroblasts, vasculature, epithelial cells, connective tissue, and the entrance of mononuclear cells (lymphocytes, macrophages, and plasma cells). Searing polyps that look like nasal or cervical polyps as well as lung abscesses are integrated into the models.

Granulomatous inflammation:

A particular sort of determined disturbance that is portrayed by the improvement of unmistakable nodular injuries or granulomas made of dynamic macrophages or its descendants cells, epithelioid cells, and normally encompassed by lymphocytes. Aschoff, Reed-Sternberg, and development beast cells are remembered for the Langhans or massive cells, which regularly structure when the macrophages or epithelioid cells inside the granulomas join together.

These are the two: Foreign body granuloma, such as silicosis, are granulomas caused by an immunological reaction to a foreign body or T-cell.

Infectious granuloma, such as those caused by leprosy and TB, are those that develop as a result of a prolonged infection.

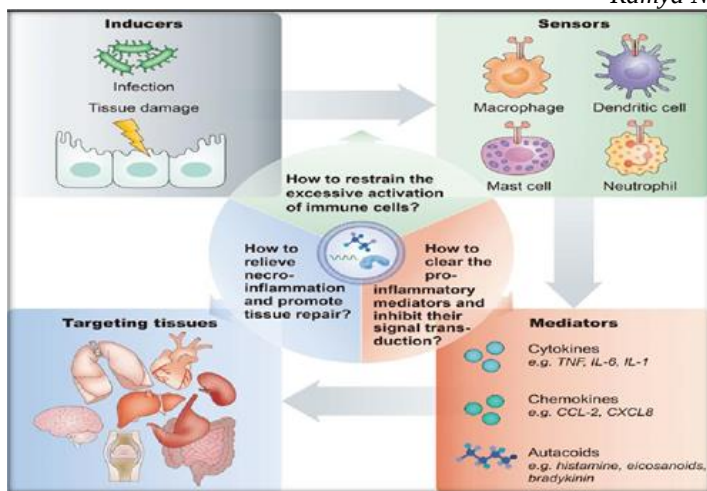


Fig.1: Components of inflammation

MATERIALS AND METHODS

Pharmacognostic investigations:

Plant material:

Based on the ethanomedical information and literature survey the plants selected for the dissertation work were *Curcuma longa* (Family : Zingiberaceae), *Epilobium angustifolium* (Family: Onagraceae), *Agathosma betulina* (Family: Rutaceae), these plant materials were identified and authenticated by Dr. K. Madhavachetty, Assistant Professor, Dept. of Botany, S V University, Tirupati.

Standardization of extracts:

Preliminary Investigations of Extracts:

The procured extracts were evaluated for organoleptic properties such as

- Nature of Extract
- Appearance
- Colour
- Odour

Phytochemical Investigations of Extracts:

Materials:

Instruments:

Heating mantle (Lab Hosp)
Hot Plate (Lab Hosp)
Hot air Oven (Lab Hosp)
Water bath (Bio Techniques)
Digital Microscope (Motic)

Chemicals:

Sulphuric acid (60% and 66%), Phloroglucinol solution, Conc. Hydrochloric acid, Sudan Red III, Ruthenium red, Dil. Acetic acid, Alc. Picric acid, Potassium chloride solution (20%), Sodium hydroxide solution (2%), Ferric chloride (5% and 10%), Solvent ether, Ammonia, Molisch's Reagent, Glacial acetic acid, Wagner's reagent, Mayer's reagent, Hager's reagent, Dragendorff's reagent, etc.

Method:

Qualitative Chemical Tests were performed for detection of the primary metabolites, secondary metabolites, organic acids like phytoconstituents by using test procedures as per standard guidelines.

Physicochemical Evaluation:

The procured extracts were screen as per WHO guidelines and COA for the parameters like Total Polyphenolic content, Total

Flavanoids content, Pesticide residue, Aflatoxins, Microbial Load, Arsenic and Toxic metals etc.; according to official methods.

Table 1: Translation of Coded Levels in Actual Units for Cream Base

Sr. No.	Ingredients	-1	0	+1
1.	X1: (Stearic acid)	4	8	12
2.	X2: (PEG-400)	1	2	3

Method of Preparation of Topical Cream:

The detail composition of topical cream preparation has been mentioned in Table No. 6.7. The oil in water emulsion- based cream was formulated using emulsifier and other oil soluble components which were dissolved in the oil phase (Part A) and heated to 75°C. The preservative and other water soluble components were dissolved in the aqueous phase using distilled water q.s. (Part B) and heated to 75°C. The extract was mixed in triethanolamine (Part C) with continuous heating till mixed completely. The Part C was completely mixed with Part B. Then oil phase (Part A) slowly added into aqueous phase (Part B) with continuous stirring until cooling of emulsifier took place. The placebo cream was prepared by same procedure.

Preparation of Conventional Herbal Formulation

The topical cream formulation was developed using a homogenizer disperser and suitable excipients as per factorial design. A 3² factorial design was employed for optimization, and the final optimized formulation was selected based on evaluation parameters such as pH and viscosity, with the eighth batch identified as the optimized formulation.

The cream was prepared using an oil-in-water emulsion technique. The oil phase, containing oil-soluble components and emulsifiers, was heated to approximately 75°C. Simultaneously, the aqueous phase containing water-soluble components and preservatives was heated to the same temperature. The plant extract was incorporated into triethanolamine and mixed thoroughly. This mixture was then added to the aqueous phase, followed by the gradual addition of the oil phase under continuous stirring. The mixture was stirred until cooling occurred and a stable emulsion was formed. A placebo formulation was prepared using the same method without the active extract.

The prepared cream was evaluated for various physicochemical and performance parameters. These included appearance (colour, texture, and smoothness), pH measurement using a calibrated pH meter, and dye test to determine the type of emulsion. Homogeneity was assessed by visual inspection and tactile examination, while viscosity was measured using a Brookfield viscometer. Additional parameters such as after-feel, type of smear, and ease of removal were also evaluated. Extrudability was determined by measuring the amount of cream expelled from a collapsible tube under applied pressure. Drug content was analyzed using appropriate analytical methods, and in-vitro drug release studies were conducted to assess the release profile of active constituents.

Preparation of Novel Formulations

Transdermal Patch (TDDS)

Transdermal patches were prepared using a 3² full factorial design to optimize formulation variables. The optimized formulation was selected based on parameters such as

percentage moisture content and moisture uptake, with the first batch identified as optimal. The patches were prepared using the solvent evaporation method, wherein a polymeric solution containing the drug and plasticizer was poured into a petri dish and allowed to dry at room temperature. The dried films were carefully removed and cut into uniform sizes ($2 \times 2 \text{ cm}^2$). Placebo patches were prepared using the same method without active extracts. The prepared patches were evaluated for physical and mechanical properties including appearance, thickness, weight variation, and folding endurance. Moisture content and moisture uptake studies were conducted to assess stability under varying humidity conditions. Water vapour transmission rate was determined using standard procedures. Drug content analysis and in-vitro drug release studies were also carried out to evaluate the performance of the patches.

Topical Film Forming Solution (TFFS)

Topical film forming solutions were developed using a similar factorial design approach, with optimization based on drying time and water vapour permeability. The fourth batch was selected as the optimized formulation. The formulation was prepared by dissolving polymers and excipients separately, followed by the addition of the drug under continuous stirring until a clear and homogeneous solution was obtained. Upon application to the skin, the solution formed a thin film after solvent evaporation. The prepared formulations were evaluated for appearance, clarity, and film-forming ability. Drying time was recorded, and stickiness was assessed using a glass slide method. Film integrity was evaluated after a specified duration, and water vapour permeability studies were conducted using standard techniques. Drug content determination and in-vitro drug release studies were performed to assess formulation efficiency.

RESULTS AND DISCUSSION



Figure 2: Herbal Extracts



Figure 3: Prepared Topical Cream Formulations

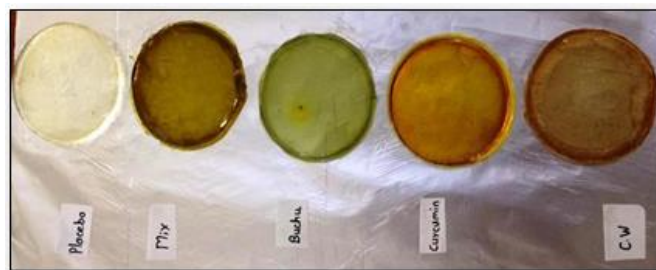


Fig.4: Prepared Topical Transdermal Patch



Fig.5: Prepared Topical Film Forming Solutions

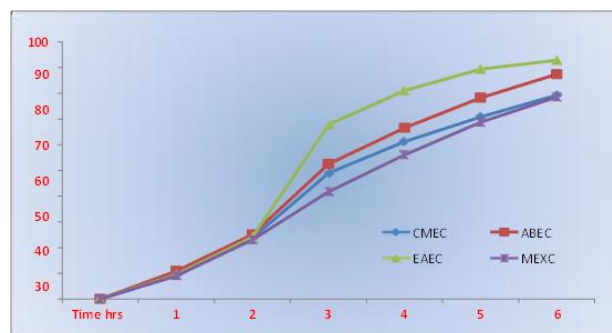


Fig.6: In-vitro Drug Release Pattern for Topical Creams

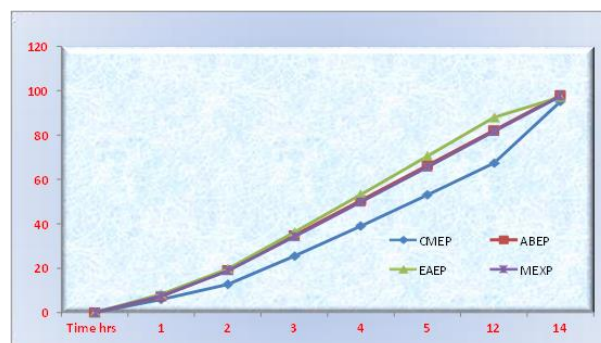


Fig.7: In vitro Drug Release Pattern for Topical Transdermal Patches

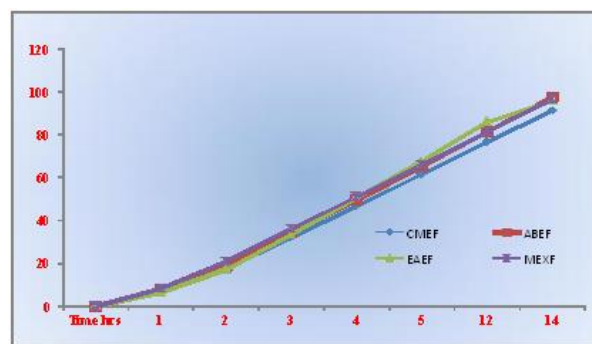


Fig.8: In vitro Drug Release Pattern for Topical Film Forming Solutions

Table 2: Preliminary Investigations of Extracts

Sr.No.	Parameters	Observations		
		<i>Curcuma longa</i> Curcuminoids Extract (CME)	<i>Epilobium angustifolium</i> Canadian Willow Extract (EAE)	<i>Agathosma betulina</i> Buchu Extract (ABE)
1.	Nature	Solid	Liquid	Liquid
2.	Appearance	Amorphous Powder	Clear Liquid	Clear Liquid
3.	Color	Yellow	Light Brown	Light Green
4.	Odor	Characteristics	Characteristics	Characteristic

Table 3: Phytochemical Screening of Extracts

Sr.No.	Phytochemicals	Observations		
		CME	EAE	ABE
1.	Carbohydrates	+	+	+
2.	Amino acids	+	+	+
3.	Proteins / Enzymes	+	+	+
4.	Volatile oil	+	+	+
5.	Steroids / Terpenoids	+	+	+
6.	Tannins and Phenols	+	+	+
7.	Flavonoids	-	+	+

Result values are expressed as + = Present and - = Absent

Table 4: Physicochemical Evaluation of Extracts

Sr.No.	Parameters	Observations		
		CME	EAE	ABE
1.	Total Polyphenolic content (mg GAE/100 gm)	0.682 $\pm$ 0.0044	26.24 $\pm$ 0.5268	16.43 $\pm$ 0.5608
2.	Total Flavonoid content (mg/gm)	-	18.33 $\pm$ 0.8819	13.46 $\pm$ 0.6767
3.	Pesticide Residues	Nil	Nil	Nil
4.	Total Aflatoxins, (B1, B2, G1, G2)	$\leq 5 \mu\text{g/kg}$	$\leq 5 \mu\text{g/kg}$	$\leq 5 \mu\text{g/kg}$
5.	Microbial Load			
	<i>E coli</i> , <i>Salmonella</i> , <i>Ps. aeruginosa</i> , <i>S. aureus</i>	Absent	Absent	Absent
	Total Aerobic Microbial Count			
	Total Bacterial Count	91 CFU/GM	85 CFU/GM	80 CFU/GM
	Total Fungal Count	< 10 CFU/GM	< 10 CFU/GM	< 10 CFU/GM
	Total Yeast and Mould count	< 10 CFU/GM	< 10 CFU/GM	< 10 CFU/GM
6.	Heavy Metals			
	Lead	0.512 ppm	0.571 ppm	0.514 ppm
	Arsenic	Nil	Nil	Nil
	Mercury	Nil	Nil	Nil
	Cadmium	Nil	Nil	Nil

Table 5: Evaluation of Prepared Topical Cream Formulations

Type of Particulars	Placebo	CMEC	EAEC	ABEC	MEXC
Colour	White	Yellow	Off White	Light Green	Mustard Yellow
Roughness	No	No	No	No	No
pH	7.2 $\pm$ 0.057	7.1 $\pm$ 0.066	7.4 $\pm$ 0.057	7.3 $\pm$ 0.033	7.4 $\pm$ 0.033
Dye Test	o/w	o/w	o/w	o/w	o/w
Removal	Good	Good	Good	Good	Good
Homogeneity	Very Good	Very Good	Very Good	Very Good	Very Good
Viscosity (cps)	12566 $\pm$ 0.316	12585 $\pm$ 3.66	12576 $\pm$ 0.836	12575 $\pm$ 1.393	12578 $\pm$ 1.655
After Feel	Emolliency	Emolliency	Emolliency	Emolliency	Emolliency
Type of Smear (Spreadability)	Very Good	Very Good	Very Good	Very Good	Very Good
Extrudability	Excellent	Excellent	Excellent	Excellent	Good

DISCUSSION

Preliminary organoleptic evaluation revealed that *Curcuma longa* extract was a yellow amorphous solid, whereas *Epilobium angustifolium* and *Agathosma betulina* extracts were clear liquids with characteristic odor. Phytochemical screening confirmed the presence of important bioactive constituents such as carbohydrates, amino acids, proteins, volatile oils, steroids, terpenoids, tannins, and phenolic compounds in all extracts, while flavonoids were absent in *Curcuma longa* but present in the other extracts. Physicochemical evaluation demonstrated that all extracts complied with WHO guidelines, showing acceptable levels of polyphenols, flavonoids, microbial load, aflatoxins, pesticide residues, and heavy metals, confirming

their safety and suitability for formulation development. Topical formulations were developed using a systematic optimization approach based on a 3² full factorial design. Both conventional and novel formulations were prepared, including topical creams, transdermal patches (TDDS), and topical film forming solutions (TFFS). The optimized cream formulation exhibited desirable physicochemical properties such as appropriate pH (7.1–7.4), excellent homogeneity, good spreadability, and high viscosity. The dye test confirmed that all creams were oil-in-water (O/W) emulsions, and extrudability was found to be excellent to good. The transdermal patches showed uniform thickness, acceptable weight variation, and good folding endurance, indicating

mechanical stability. Moisture content and moisture uptake values were within acceptable limits, ensuring stability under different environmental conditions. Similarly, TFSS formulations exhibited good film-forming properties, acceptable drying time (3–5 minutes), uniform film integrity, and controlled stickiness, indicating suitability for topical application. In-vitro drug release studies carried out using Franz diffusion cell demonstrated sustained and controlled release of active constituents from all formulations. The cumulative drug release profiles indicated that the formulations were capable of delivering phytoconstituents effectively over an extended period. Kinetic modeling revealed that most formulations followed Higuchi and Korsmeyer–Peppas models, suggesting diffusion-controlled drug release mechanisms. The correlation coefficients (R^2 values) indicated good linearity and fitting to kinetic models, confirming the reliability of the release behavior. Overall, the study successfully demonstrated the feasibility of developing stable, effective, and optimized topical herbal formulations using selected plant extracts with desirable physicochemical and release characteristics.

CONCLUSION

The present research work successfully established the development and evaluation of topical herbal formulations incorporating extracts of *Curcuma longa*, *Epilobium angustifolium*, and *Agathosma betulina*. The pharmacognostic and phytochemical investigations confirmed the presence of significant bioactive constituents and ensured the quality, purity, and safety of the extracts as per WHO guidelines. The application of a 3^2 full factorial design proved to be an efficient and reliable tool for optimization of formulations, enabling systematic evaluation of formulation variables and selection of optimized batches with desired characteristics. The developed topical cream, transdermal patch, and film forming solution exhibited satisfactory physicochemical properties, stability, and performance. The in-vitro drug release studies demonstrated controlled and sustained release of phytoconstituents, and kinetic modeling indicated that drug release followed diffusion-based mechanisms. These findings confirm the potential of the developed formulations as effective topical drug delivery systems. In conclusion, the study highlights that herbal extracts can be successfully incorporated into various topical dosage forms to achieve enhanced delivery, improved patient compliance, and therapeutic efficacy. The developed formulations show promising potential for further pharmacological and clinical evaluation as anti-inflammatory agents.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL:

Not applicable

FUNDING:

This study received no specific funding from public, commercial, or not for profit funding agencies.

AI TOOL DECLARATION

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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