Lampyrid 2023: Volume 13, 651–666
ISSN: 2041-4900
https://lampyridjournal.com

In Vitro Evaluation of Polyherbal Cream as a Promising Therapeutic Approach for Psoriasis: Assessing Anti-inflammatory and Anti-Fungal activity

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Abstract
The aim of this study was to develop and assess the effectiveness of a polyherbal cream containing extracts of Terminalia chebula, Cassia tora, Ocimum sanctum, and Cynodon dactylon for the treatment of psoriasis, a chronic autoimmune skin condition characterized by scaly patches on the skin. Given the limitations of traditional treatments, herbal medicines have gained popularity as alternative or complementary therapies for psoriasis. The physical and chemical characteristics of the polyherbal cream were evaluated after formulation using a standardized procedure. The study findings revealed significant anti-inflammatory and antioxidant properties of the polyherbal cream, both of which are crucial for the successful management of psoriasis. Furthermore, in vitro studies using the protein denaturation method and in vitro cell viability assay and anti-microbial study demonstrated the cream's anti-inflammatory activity. These results suggest that the polyherbal cream containing Terminalia chebula, Cassia tora, Ocimum sanctum, and Cynodon dactylon extracts may serve as an effective alternative or complementary therapy for psoriasis. However, further clinical trials are necessary to validate the long-term safety and efficacy of the cream.

Keywords-Polyherbal, Cream, Antifungal, Anti-inflammatory, Psoriasis**,**Terminalia chebula, Cassia Tora, Occimum Sanctum, Cynodon Dactylon.

Introduction:

Psoriasis is a chronic autoimmune skin disorder that affects millions of people worldwide. It is characterized by the rapid buildup of skin cells, resulting in the formation of scaly patches on the skin's surface. This condition can cause significant physical discomfort, itching, and pain, as well as psychological distress due to its visible nature and potential impact on self-esteem and quality of life (1).

For many decades treatment of an acute disease or a chronic illness has been mostly accomplished by delivery of drugs to patients using various pharmaceutical dosage forms, including tablets, capsules, pills, suppositories, cream, gel, ointments, liquids, aerosols and injectables, as drug carriers (2). As a result, there has been a growing interest in exploring alternative or complementary therapeutic options, such as herbal medicines, for the management of psoriasis.

One promising avenue in this regard is the use of polyherbal formulations, which combine multiple herbal extracts known for their therapeutic properties. These formulations offer the potential for synergistic effects, targeting multiple pathways involved in the pathogenesis of psoriasis. Several herbal extracts have shown promise in the treatment of psoriasis, including Terminalia chebula, Cassia tora, Ocimum sanctum, and Cynodon dactylon (3).

Terminalia chebula, commonly known as "Haritaki" in Ayurveda, has been traditionally used for its anti-inflammatory, antioxidant, and immunomodulatory properties. It has shown potential in managing skin disorders, including psoriasis, by reducing inflammation and oxidative stress (4).

Cassia tora, also known as "Senna," possesses anti-inflammatory and immunomodulatory effects. It has been used in traditional medicine for various skin ailments, including psoriasis, due to its ability to suppress pro-inflammatory cytokines and modulate immune responses (5).

Ocimum sanctum, commonly known as "Tulsi" or holy basil, has been revered in traditional medicine for its therapeutic properties. It exhibits anti-inflammatory, immunomodulatory, and antioxidant effects. These properties make it a potential candidate for the treatment of inflammatory skin conditions, including psoriasis (6).

Cynodon dactylon, known as "Durva" or Bermuda grass, has been used in traditional medicine for its wound-healing and anti-inflammatory properties. It has demonstrated the ability to inhibit pro-inflammatory mediators and modulate immune responses, suggesting its potential in managing inflammatory skin disorders like psoriasis (7).

These herbal extracts have shown promising individual effects in preclinical and clinical studies related to psoriasis treatment. However, their potential synergistic effects when combined in a polyherbal cream formulation for psoriasis management remain to be explored.

Material and method:

Table no1: Material and method

Sr.no.	Material	Supplier
1.	Cassia Tora	Amsar private limited
2.	Cynodondactylom	Amsar private limited
3.	Occimum sanctum	Amsar private limited
4.	Terminalia chebula	Amsar private limited

5.	Whitebees wax.	Analabfinechemicals,Mumbai.
6.	Liquid paraffin.	Analabfinechemicals,Mumbai.
7.	Borax.	Analabfinechemicals,Mumbai.
8.	Methylparaben.	Analabfinechemicals,Mumbai.
9.	Propylparaben.	Analabfinechemicals,Mumbai.

Equipment:

Table no 2: Equipment used

Sr.no.	**Equipment**	**ProductionCompany**
1.	Electronicbalance	AX200 Shimadzu,Japan.
2.	UV-Visiblespectrometer	Shimadzu,UV1700,Japan.
3.	PHmeter	Hannainstruments.
4.	Sonicator	Lagenrich electronicsPvt. Ltd,Vasai.
6.	Franzdiffusioncell	Fabricated.
7.	PhotoStabilitychamber	Biomedia.
8.	Brookfield Viscometer	LVDV-2.

Cream formulation:

The step-by-step process for preparing this formulation is as follows:

1.Melt beeswax, liquid paraffin, and propyl paraben in the order of increasing melting point.

2.Dissolve methyl paraben and borax in water at 75°C. If necessary, filter the solution.

3.Add the aqueous phase (containing dissolved extracts, if applicable) to the oily phase while continuously stirring.

4.Allow the mixture to cool with continuous stirring until it reaches room temperature.

5.Add perfume to the preparation at room temperature.

6.Transfer the cream to the desired container while it is still hot.

Fig no 1: Cream Batches

Table no 3: Formulation Table

INGREDIENT	F1	F2	F3	F4	F5	F6	F7	F8	F9
Terminalia chebula extract	0.2gm	0.2gm	0.2gm	0.2gm	0.2gm	0.2gm	0.2gm	0.2gm	0.2gm
Cassia tora extract	0.3 gm	0.3gm	0.3gm	0.3gm	0.3gm	0.3gm	0.3gm	0.3gm	0.3gm
Occimum sanctum extract	0.3gm	0.3gm	0.3gm	0.3gm	0.3gm	0.3gm	0.3gm	0.3gm	0.3gm
Cynodont dactylon extract	0.2gm	0.2gm	0.2gm	0.2gm	0.2gm	0.2gm	0.2gm	0.2gm	0.2gm
Beeswax	1.3gm	1.3 gm	0.9 gm	1.15 gm	1 gm	1.15 gm	1 gm	1.3 gm	1.15 gm
Liquid parafin	13ml	10 ml	11.5 ml	11.15 ml	10 ml	9.3 ml	13 ml	11.5 ml	13.6 ml
Borax	0.08gm	0.08gm	0.08gm	0.08gm	0.08gm	0.08gm	0.08gm	0.08gm	0.08gm
methylparaben	0.004 gm	0.004 gm	0.004 gm	0.004 gm	0.004 gm	0.004 gm	0.004 gm	0.004 gm	0.004 gm
propylparaben	0.004 gm	0.004 gm	0.004 gm	0.004 gm	0.004 gm	0.004 gm	0.004 gm	0.004 gm	0.004 gm
Rosewater	1ml	1ml	1ml	1ml	1ml	1ml	1ml	1ml	1ml
water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

Results:

In-Vitro evaluations of polyherbal cream

1)Appearance of Cream-gel

The acceptable criteria for colour, homogeneity, phase separation, and texture were met. None of the formulations exhibited phase separation, indicating that

they were well-blended and maintained a consistent composition. The formulations also demonstrated a pleasing texture, displaying good consistency and excellent homogeneity. These findings suggest that the formulation is of high quality and possesses exceptional stability.

Batch	**Color**	**Homogeneity**	**Phase separation**
F1	Brownish	Excellent	No separation
F2	Brownish	Excellent	No separation
F3	Brownish	Excellent	No separation
F4	**Brownish**	**Excellent**	**No separation**
F5	Brownish	Excellent	No separation
F6	Brownish	Excellent	No separation
F7	Brownish	Excellent	No separation
F8	Brownish	Excellent	No separation
F9	Brownish	Excellent	No separation

Table no. 4: Color, homogeneity, & phase separation

2)pH, Spreadability, Viscosity

Batches	pH	Spreadability g.cm/sec	Viscosity mPa.s
F1	6.3	2.14cm	14149
F2	5.7	2.23cm	15269
F3	5.9	2.22cm	15843
F4	**6.5**	**3cm**	**16112**
F5	6.1	2.11cm	14050
F6	5.7	2.43cm	14039
F7	6.4	2.04cm	15612
F8	5.6	2.33cm	15610
F9	6.2	1.99cm	15515

Table no 5: pH, Spreadability, Viscosity

3)**Observedwashability, Irritancytest, Afterfeeltest, Acceleratedstabilitystudy**

Batches	**Observedwashability**	**Irritancytest**
F1	Easilywashable	Noirritation

F2	Easilywashable	Noirritation
F3	Easilywashable	Noirritation
F4	Easilywashable	Noirritation
F5	Easilywashable	Noirritation
F6	Easilywashable	Noirritation
F7	Easilywashable	Noirritation
F8	Easilywashable	Noirritation
F9	Easilywashable	Noirritation

Batches	Afterfeeltest	Acceleratedstabilitystudy
F1	Emollient	Nochangewasobserved.
F2	Emollient	Nochangewasobserved.
F3	Emollient	Nochangewasobserved.
F4	Emollient	Nochangewasobserved.
F5	Emollient	Nochangewasobserved.
F6	Emollient	Nochangewasobserved.
F7	Emollient	Nochangewasobserved.
F8	Emollient	Nochangewasobserved.
F9	Emollient	Nochangewasobserved.

Table no 6,7:**Observedwashability, Irritancytest, Afterfeeltest, Acceleratedstabilitystudy results**

Lampyrid 2023: Volume 13, 651-666
ISSN: 2041-4900
https://lampyridjournal.com

4)Drug Content

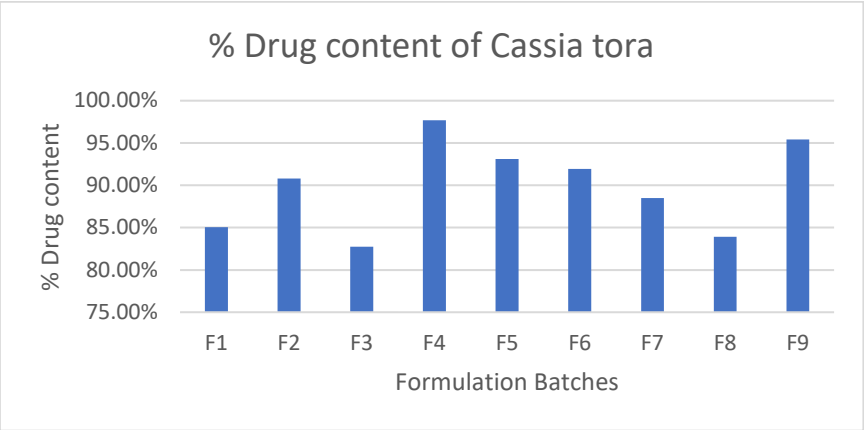

Figure no 2: % Drug content Cassia tora

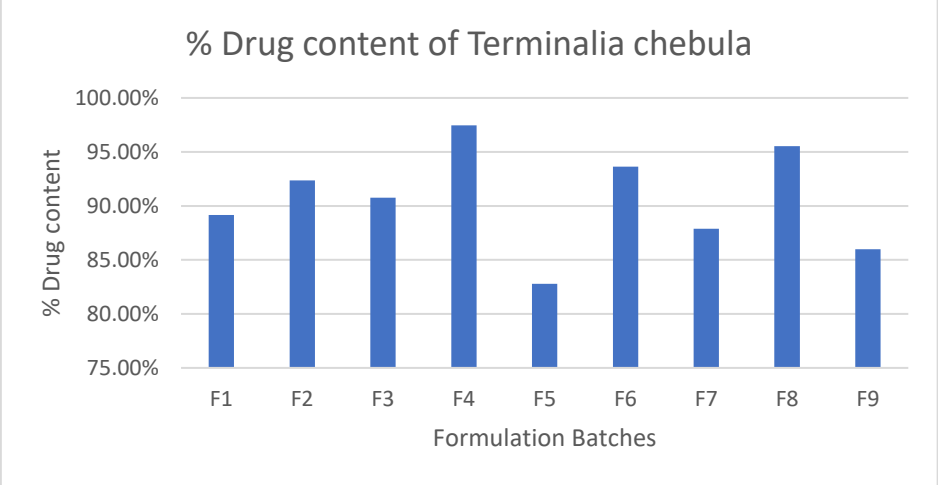

Figure no 3: % Drug content Terminalia chebula

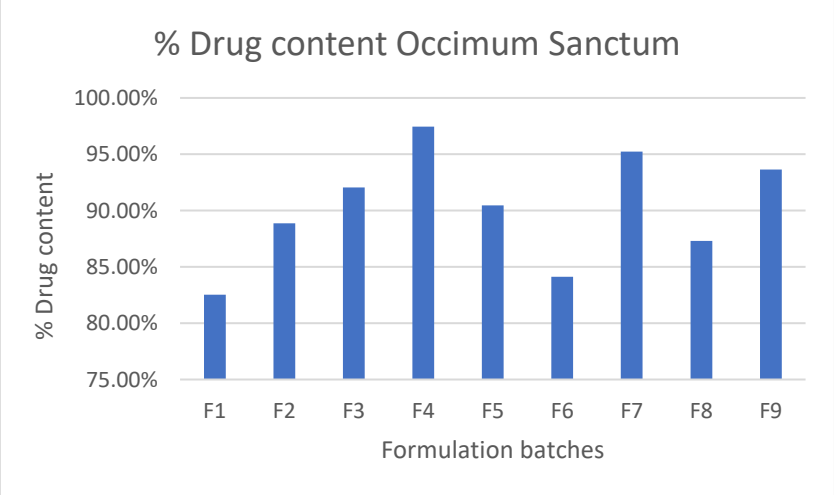

Figure no 4:% Drug content Occimum Sanctum

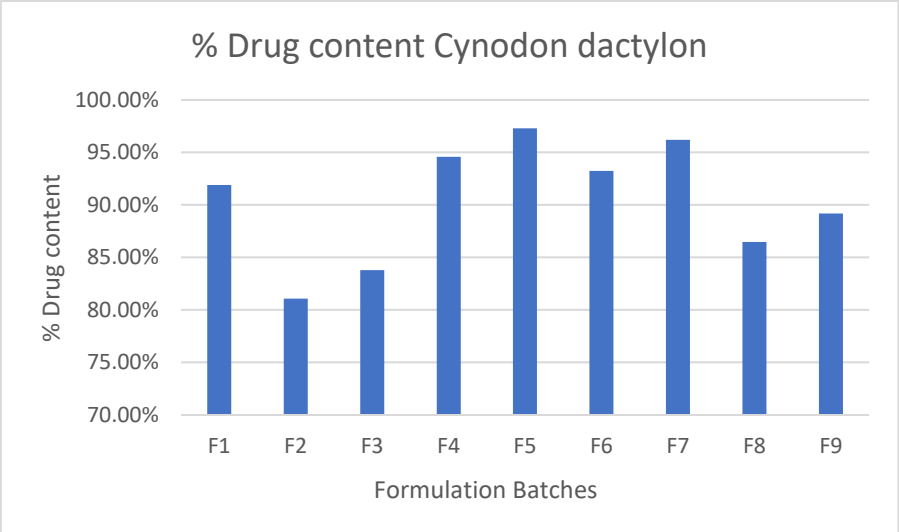

Figure no 5: % Drug content of Cynodondactylon

From the evaluation of batches, we found F4 batch has shown great results and it was used as optimised batch for further study.

5)In vitro anti-inflammatory activity by Protein denaturation method

Principle:

Protein denaturation is a process that disrupts the stability and structure of proteins. Understanding the chemistry of proteins is crucial due to their prevalence in living organisms.

Procedure:

The in vitro anti-inflammatory activity was evaluated using the protein denaturation method. The reaction mixture (1 mL) contained 0.1 mL of fresh hen's egg albumin, 0.5 mL of phosphate buffered saline (PBS, pH 6.4), and 0.4 mL of Sample A and Sample B at a concentration of 1 mg/ml. A control group with double-distilled water was also included. The mixtures were incubated at 37°C for 15 minutes, followed by heating at 70°C for 5 minutes. After cooling, the absorbance was measured at 660 nm, with the vehicle as theblank. Diclofenac sodium at a concentration of 1 mg/ml was used as a reference drug and treated similarly for absorbance determination. The percentage inhibition of protein denaturation was calculated using the provided formula.

% inhibition = absorbance of control - absorbance of test / absorbance of control x 100

Compounds	**Conc.**	**O.D.**	**Mean**	**% inhibition**
Blank		1.50 1.45 1.48	1.47	
Standard (Diclofenacsodium)	1000µg/ml	0.13 0.14	0.14	90.47

		0.15		
Samples: F4 (AB)	1000µg/ml	0.25 0.26 0.24	0.25	82.99

Table no.8: Anti-inflammatory activity of different formulation by Protein denaturationmethod.

Figure no.6:Anti-inflammatory activity of different formulation by protein denaturation method.

Conclusion:

In conclusion, All Samples F4 (AB) Were used to carry out in vitro anti-inflammatory activity by using protein denaturation inhibition assay at the concentration 1mg/ml. The all samples showed good anti-inflammatory activity as compared to standard drug (Diclofenac sodium)

6)Antimicrobial activity study:

The antifungal activity of the optimized batch (F4) of the formulation was carried out. The culture was used as Candida albicans and the antimicrobial test was performed using the agar well diffusion. The method used was the Cup plate method and the agar media used is sabouraud dextrose agar result .

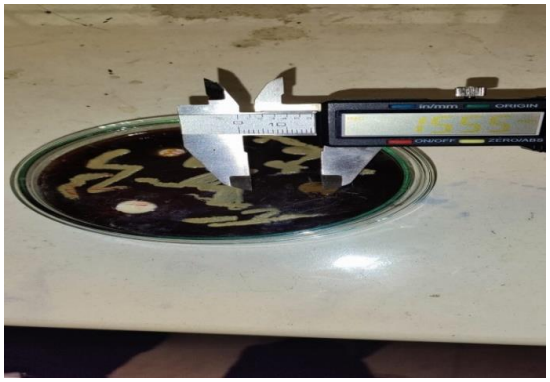

Figure no.7:Zone of inhibition of F4 batch

Formulation sample	Observed zone of inhibition
Marketed formulation	13±1 mm
Optimized batch f4	15.55±1 mm

Table no 9: Anti-fungal activity result

The zone of inhibition for marketed formulation was found to be 13 ± 1 cm and antifungal cream prepared was found to be 15.55 ± 1.50 cm

6) In vitro cell viability assay

Cell line:

L-929 (adherent type of mouse fibroblast cell line)

Media:

DMEM with high glucose (Cat No-11965-092), FBS (Gibco, Invitrogen) Cat No - 10270106 Antibiotic – Antimycotic 100X solution (Thermo fisher Scientific)-Cat No-15240062

Material required:

96 well plate	
Co2 incubatror	

Bio safety cabinet	
Elisa plate reader	

Table no 10: Material required for In vitro cell viability assay

Experimental procedure:

1.Cells were cultured at a concentration of 1 × 10^4 cells/ml in the culture medium for 24 hours at 37°C and 5% CO2.

2.The cells were seeded in 96-well tissue culture grade microplates at a concentration of 1 × 10^4 cells/well in 70 µl of culture medium. Then, 100 µl of synthesized compounds at varying concentrations (10, 40, 100 µl/ml) were added to the respective wells. Control wells were treated with 0.2% DMSO in PBS along with the cell line. All samples were prepared in triplicate.

3.The control wells containing DMSO and cells were included to determine cell survival and calculate the percentage of live cells after incubation. All samples, including controls, were incubated for 24 hours at 37°C and 5% CO2 in a CO2 incubator (Thermo Scientific BB150).

4.After the incubation period, the culture medium was completely aspirated, and 20 µl of MTT reagent (5 mg/ml in PBS) was added to each well.

5.The cells were further incubated for 4 hours at 37°C in the CO2 incubator.

6.The wells were observed under a microscope to examine the formation of dark-colored formazan crystals, which indicated viable cell presence. The yellowish MTT was reduced to formazan specifically by viable cells.
7.After the removal of the medium, 200 µl of DMSO was added to each well and incubated at 37°C for 10 minutes, covered with aluminum foil.
8.The absorbance of each sample was measured in triplicate using an ELISA microplate reader (Benesphere E21) at a wavelength of 570 nm to analyze the results

Table no.11: Effects of compound against L-929(adherent type of mouse fibroblast cell line) by MTT assay

Sr. no	Sample	Concentration(µg/ml)	OD	Mean	% inhibition	IC 50 (µg/ml))
1	Control		0.899 0.891 0.837	**0.875**		
2	Std. 5 FU	10	0.212 0.201 0.196	**0.203**	76.08	32.07
		40	0.105 0.117 0.125	**0.115**	86.85	
		100	0.078 0.093 0.100	**0.090**	89.71	
3	Sample – F4	10	0.325 0.351 0.310	**0.328**	62.51	38.19
		40	0.289 0.274 0.295	**0.286**	67.31	
		100	0.260 0.259 0.245	**0.254**	70.97	

According to Table, at the different Concentration (10 µg/ml, 100 µg/ml, 100 µg/ml) of Sample – F4 compounds carried out for anticancer activity against **L-929(adherent type of mouse fibroblast cell line).** The positive control 5

Flurouracil was used as standard drug. The Sample – B3 showed good activity as compared to standard compound.

Figure no. 8: Images of cell activity

Control

Standard 5FU

Sample – F4

Conclusion

The study successfully developed an herbal cream using Terminalia chebula, Cassia tora, Occimum sanctum, and Cynodon dactylon. The cream exhibited potent antifungal activity, alongside various other beneficial properties such as antimicrobial, antibacterial, anti-inflammatory effects. The formulated cream remained stable at room temperature, making it suitable for treating skin diseases. Furthermore, it showed potential anti-inflammatory activity, enhancing its therapeutic value. These findings emphasize the potential of herbal creams as alternative treatments and highlight the importance of natural ingredients derived from medicinal plants. Further research is necessary to validate its efficacy and safety for widespread use. Overall, this study demonstrates the promising medicinal effects of the herbal cream formulation.

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