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DEVELOPMENT AND CHARACTERIZATION OF POLYHERBAL HYDROGEL FOR DE TAN

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ABSTRACT:

Skin tanning caused by prolonged exposure to ultraviolet radiation is a common cosmetic concern and may lead to pigmentation, premature aging, and other skin-related problems. Herbal formulations are gaining importance due to their safety, efficacy, and minimal side effects. The present study aims to develop and characterize a **polyherbal hydrogel for de-tan activity** using natural extracts of **Liquorice (Glycyrrhiza glabra) root** and **Neem (Azadirachta indica) leaves**, known for their antioxidant, depigmenting, and skin-protective properties. The extracts were prepared using suitable extraction methods and incorporated into hydrogel formulations using **Carbopol 940** as a gelling agent. Four formulations (F1–F4) were prepared with varying concentrations of extracts and polymer. The prepared hydrogels were evaluated for physical appearance, pH, viscosity, spreadability, swelling index, washability, in vitro antioxidant activity (DPPH and H_2O_2 scavenging assays), in vitro SPF determination, and stability studies. All formulations showed acceptable physicochemical properties with pH suitable for topical application. Among them, **formulation F4** exhibited superior antioxidant activity, higher swelling index, good spreadability, and an SPF value of **30.6**, indicating effective photoprotective potential. Stability studies revealed no significant changes in formulation characteristics over one month. The results suggest that the developed polyherbal hydrogel is a stable, safe, and effective topical formulation for de-tan and skin protection.

KEYWORDS: Tan, Hydrogel, Liquorice, Neem.

INTRODUCTION

Tanning is a prevalent skin concern worldwide. While it is not usually a major risk, without enough protection prolonged sun exposure can lead to skin damage. When UV rays penetrate into the skin, it tends to generate a reddish-brown pigment pheomelanin. It results in tan, darkening of skin. Specialized cell in the lower layer of skin melanocytes produces the pigment melanin, leads to tanning. Skin darkening by sun exposure called as "sunbathing" or "tanning". The ultraviolet (UV) light from the exposure of sun or artificial sources were the main cause of tanning [1]. Immediate tanning is a quick tan occur within minutes of UV exposure and fades fast within some days. Delayed tanning appears hours later and last for long [2]. Several serious health risks can be caused due to sun exposure and tanning such as skin cancer, sun burn, premature aging, weak immunity and eye diseases [3]. Chemical peels, Microdermabrasion, Chemotherapy and Laser treatment are the tan removal treatments. Tan removal gels and creams with lightening actives, de tan face pack, and exfoliation products are now adays commercially available as skin care products for tan removal [4].

De-tan, refers to the skincare products or treatments that are intended to reduce undesired sun damage or tan from the skin, leaving it with a natural shine and a more even tone. The common therapies include the use of physical sunscreen alone or in combination with topical decolorizing agents. They aim to provide therapeutic effects by reducing melanin synthesis or transfer and attenuating oxidative stress response [5].

MATERIALS AND METHODS

The roots of liquorice and leaves of neem used for the study were collected from Kasaragod district, Kerala. The samples were identified and authenticated by Dr. Biju. P, Assistant professor, Department of botany, Government college, Kasaragod. Chemicals required for preparation are Carbopol 940, propylene glycol, glycerin, phenoxyethanol, triethanolamine and rose water are of AR grade and acquired from Sisco research laboratories Pvt Ltd, Nice chemical Pvt Ltd, and Isochem laboratories.

1.1 Extraction of Liquorice root (*Glycyrrhiza glabra*)

The liquorice root was washed with distilled water, dried and powdered. Using a Soxhlet apparatus, the powdered liquorice root was extracted using ethyl acetate and water in the ratio 3:1. The extract was then filtered and are evaporated to dryness [6].

Fig 1: Extraction of liquorice root.

1.2 Extraction of Neem leaves (*Azadirachta indica*)

The leaves of neem plant collected were washed with distilled water to remove dust and microbial contaminants. The leaves then shade dried at room temperature and were coarsely powdered using a mechanical grinder and it was then subjected to extraction by maceration using water and acetone in a ratio 1:1. The extracts were filtered using whatmann filter paper and are evaporated to dryness [7].

Fig 2: Extraction of neem leaves.

1.3 Preformulation studies

Pre-formulation studies assess a drug's physicochemical properties impacting its performance and dosage form development. The main goal is to develop a stable, effective dosage form by determining kinetics, compatibility, and physico-chemical parameters [8].

Fourier transform infrared (FT-IR) of drug: The stability of a formulation primarily depends on the compatibility of drug and the excipients. Hence, important to find any possible chemical/physical infractions since they can affect the bioavailability and stability of the

drug. FT-IR analysis of pure drug was carried out individually. The peaks obtained in the spectrum were compared with the reference spectrum [9].

1.4 Preparation of Hydrogel formulation

Accurately weighed quantity of Carbopol 940 is dispersed in water using a magnetic stirrer and are allowed to hydrate completely for 24 hours to ensure uniform swelling. The glycerin and propylene glycol were taken in a beaker. The extract of liquorice and neem were dissolved in the solution of glycerin and propylene glycol. The mixture is then added into the carbopol solution. The triethanolamine to adjust the pH, phenoxyethanol as preservative and rose water as perfume were added [10].

Table 1: Hydrogel formulations.

SI NO.	INGREDIENTS	QUANTITY			
		F1	F2	F3	F4
1	Liquorice extract	0.5 g	0.5 g	1 g	1 g
2	Neem extract	0.5 g	1 g	0.5 g	1 g
3	Carbopol 940	0.8 g	0.7 g	0.6 g	0.5 g
4	Propylene glycol	5 ml	5 ml	5 ml	5 ml
5	Glycerin	5 ml	5 ml	5 ml	5 ml
6	Triethanolamine	q.s	q.s	q.s	q.s
7	Phenoxyethanol	0.5 ml	0.5 ml	0.5 ml	0.5 ml
8	Rose water	0.2 ml	0.2 ml	0.2 ml	0.2 ml
9	water	q.s to100 ml	q.s to100 ml	q.s to100 ml	q.s to100 ml

1.5 Evaluation Parameters Visual Appearance

The prepared gels were visually inspected for physical appearance and homogeneity.

PH Determination

A Digital pH metre was used to measure the pH of each formulation 1 g of gel dissolved in 100 ml of distilled water and stored for 2 Hrs. The measurement of pH of each formulation is done in triplicate and average values are calculated.

Viscosity Determination

The viscosity of gel formulations evaluated by employing a Brookfield viscometer equipped with spindle no. 64 at a speed of 50 rpm and a temperature of 25 °C [11].

Spreadability

Spreadability is determined by applying the sample in between two glass slides and they were compressed to form a uniformly thick layer of gel. The upper slide was then pulled apart

horizontally with a string and pulley system.10 gm weight was tied initially to the thread and left for 5 min, and then the weight was increased at every step. The time required to separate the two slides, i.e. the time in which the upper glass slide moves over the lower plate was taken as measure of Spreadability [12].

$$\text{Spreadability} = \frac{\text{Weight} \times \text{length}}{\text{time}}$$

Washability

Washability is evaluated by applying small quantity of hydrogel on a glass slide and was observed under running normal tap water [13].

Swelling index

1 g of gel is wrapped in a porous aluminium foil, and weight recorded then put it on a beaker having 10 ml of phosphate buffer. After 24 hrs, reweighed and recorded. The study was performed in triplicate and average swelling index (%) was calculated [14].

$$\text{Swelling index} = \frac{Wt - W0}{W0} \times 100$$

Where, Wt = weight of swollen gel after time t. W0 = original weight of gel at zero time.

Invitro Antioxidant activity

A. DPPH(2,2-diphenyl-1-picrylhydrazyl) Method

1ml of alcoholic solution of DPPH was added to 2.5 ml of sample solution and standard ascorbic acid of varying concentration (50, 100, 150 and 200 µg/ml). Then kept at room temperature in dark for 30 min, and measured absorbance at 517 nm. The percentageinhibition of DPPH radical was calculated by comparing the results of the test with those of the control using the formula as indicated below.

Percentage of inhibition = [(A0 – At) / A0] × 100

Where, A0 = Absorbance of the control

At = Absorbance of the sample / standard

IC50 values were calculated for the sample and standard based on the concentration needed to scavenge 50% DPPH radicals [15].

B. Hydrogen peroxide scavenging activity

0.1 ml of sample solution / standard ascorbic acid with different concentration (50, 100, 150 and 200 µg/ml) added with 0.1 M phosphate buffer (3.4 ml) and of 40 mM H2O2 (0.6 ml). The mixture was incubated 10 mins at room temperature. then absorbance measured at 230 nm against a blank solution. The % inhibition was calculated using the equation

Percentage of inhibition = [(A0 – At) / A0] × 100

Where, A0 = Absorbance of the control

At = Absorbance of the sample / standard

IC50 values were calculated from the graph for sample and standard based on the concentration needed to scavenge 50% of hydrogen peroxide radicals [16].

Invitro SPF Determination

The developed hydrogel was weighed and dissolved in 10 ml of ethanol and shaked well for 10 min. Filter and measure the maximum absorbance using UV spectrophotometer and perform blank with ethanol. Calculate the SPF by using the Mansur equation [17].

$$SPF\,(spectrometry) = CF\,x \sum_{290}^{320} EE\,(\lambda)\,x\,I(\lambda)\,x\,abs(\lambda)$$

Stability studies

Stability is the period from production and packaging till it retains its specified potency and physical properties. The prepared hydrogel was placed in room temperature for 30 days. Batches were evaluated for parameters like organoleptic properties, PH, viscosity, and spreadability to check whether the hydrogel shows any significant changes or not [18].

2. RESULT AND DISCUSSION

Preformulation studies: Fourier transform infrared spectroscopy

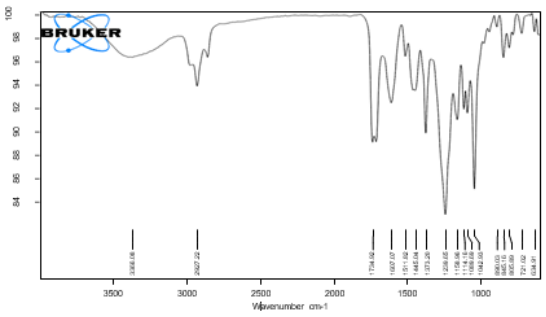


Fig 3: FTIR of Liquorice.

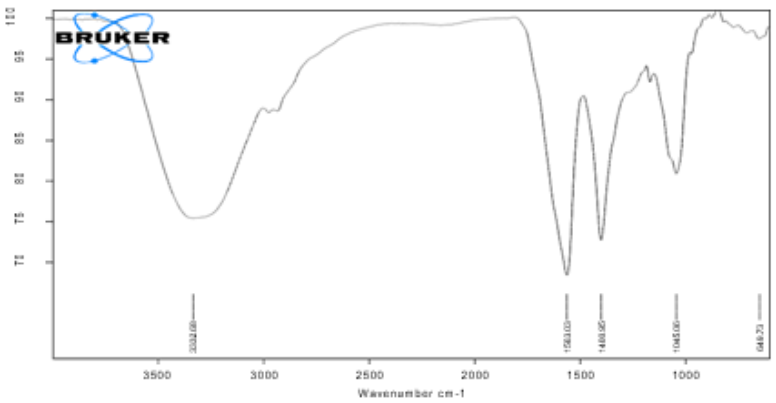


Fig 4: FTIR of Neem.

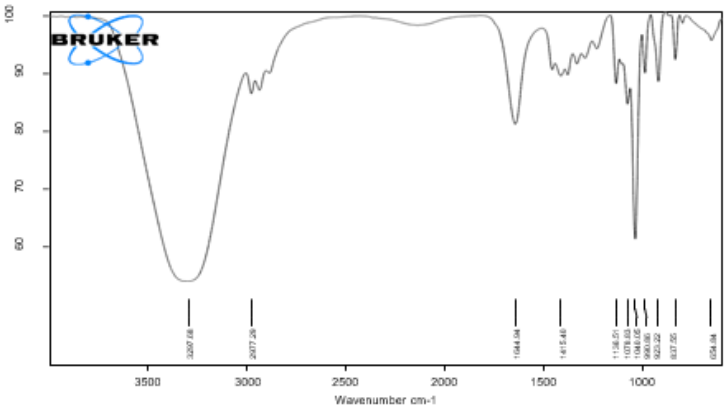


Fig 5: FTIR of Excipients.

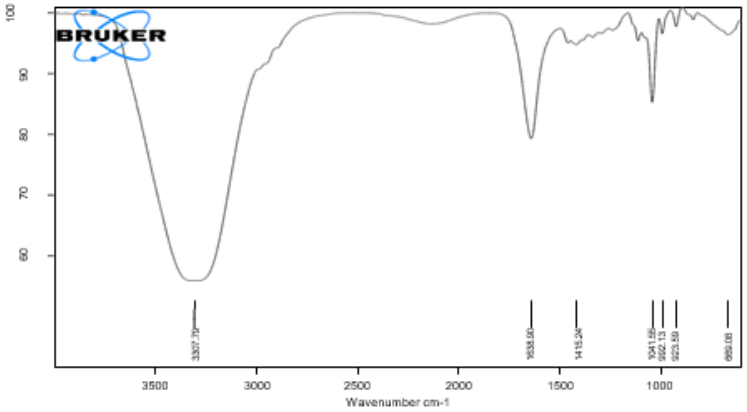


Fig 6: FTIR of drug + excipients.

Preparation of Hydrogel formulation

Fig 7: Prepared hydrogel formulation.

Totally 4 formulation F1, F2, F3 and F4 with different proportion of active pharmaceutical ingredient and Carbopol were prepared.

Evaluations

Physical appearance

Table 2: Physical appearance of all formulations.

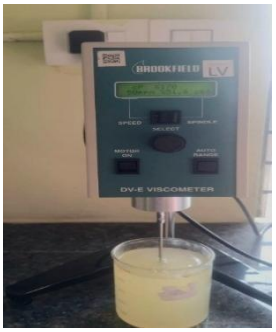
Sl no.	Characteristics	OBSERVATION			
		F1	F2	F3	F4
1	Physical state	semi solid	semi solid	semi solid	semi solid
2	Colour	pale yellow	yellow	yellow	yellowish brown
3	Odour	mild earthy	mild earthy	mild earthy	mild earthy
4	Consistency	smooth	smooth	smooth	smooth
5	Homogeneity	homogenous	homogenous	homogenous	homogenous

PH determination.

Table 3: pH observations

Sl No.	Formulation	pH Determination
1	F1	5.09
2	F2	5.11
3	F3	5.18
4	F4	5.14

All the formulation falls in between the range of 4.5 - 5.5, this pH range considered as acceptable for the topical gel formulations and indicate that the formulation is mild and non-irritant.



Viscosity determination

Table 4: Viscosity observations

Sl No.	Formulation	Viscosity (Cp)
1	F1	6342
2	F2	6170
3	F3	5920
4	F4	5835

Fig 6: Spreadability.

Table 5: Spreadabiliy observations

Sl No.	Formulation	Spreadability (g.cm/sec)
1	F1	22.05
2	F2	25.00
3	F3	26.47
4	F4	28.12

The spreadability was evaluated by using the slip and drag method to assess the ease of application and uniform spreading characteristics of the gels. The results indicate that spreadability decreased with increasing carbopol concentration. All the formulations exhibited acceptable spreadability.

Swelling index

Table 6: Swelling index observations.

Formulation	Swelling index trial			Average swelling index (%)
	1	2	3	
F1	27.62	27.77	27.96	27.45 %
F2	33.66	33.85	33.33	33.61 %
F3	35.02	35.71	35.21	35.31 %
F4	37.83	38.35	37.66	37.94 %

The result indicate that the swelling index is high in F4. All the formulation shows acceptable swelling behaviour, confirming their ability to absorb aqueous media and maintain gel consistency.

Washability

The washability of all the prepared hydrogel formulation was evaluated by applying little amount of hydrogel to the glass slide and then washing with tap water. All the formulation was found to be easily washable.

Invitro Antioxidant activity test

a. DPPH Method

Table 7: DPPH Method.

Sl No.	Sample	Concentration (µg/ml)	Absorbance (nm)	% inhibition	IC50 (µg/ml)
1	Ascorbic acid	50	0.304	40.03	87.51
		100	0.227	55.22	
		150	0.186	63.31	
		200	0.110	78.30	
2	F1	50	0.335	33.92	134.54
		100	0.311	38.65	
		150	0.225	55.62	
		200	0.184	63.70	
3	F2	50	0.322	36.48	115.27
		100	0.296	41.61	
		150	0.213	57.98	
		200	0.132	73.96	
4	F3	50	0.328	35.30	120.37
		100	0.302	40.43	
		150	0.219	56.80	
		200	0.141	72.18	
5	F4	50	0.312	38.46	105.85
		100	0.287	43.39	
		150	0.194	61.73	
		200	0.114	76.92	
	Control	-	0.507	-	-

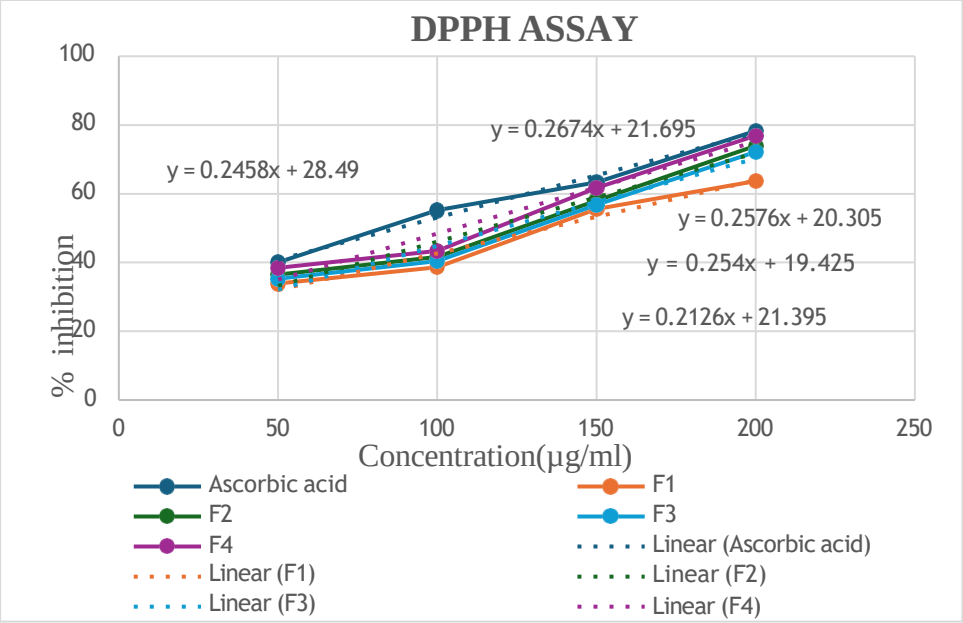


Fig 7: DPPH Assay graph.

The % inhibition of DPPH radicals increased progressively with increasing concentrations for all formulations, indicating a clear concentration dependant antioxidant activity. Among all the formulation, F4 is the most effective formulation showing antioxidant activity similar to the standard.

b. H_2O_2 Scavenging Assay

Table 8: H_2O_2 Scavenging Assay

Sl No.	Sample	Concentration (µg/ml)	Absorbance (nm)	% inhibition	IC50 (µg/ml)
1	Ascorbic acid	50	0.121	42.92	59.31
		100	0.075	64.62	
		150	0.033	84.43	
		200	0.031	85.37	
2	F1	50	0.156	26.41	128.27
		100	0.112	47.16	
		150	0.089	58.01	
		200	0.074	65.09	
3	F2	50	0.136	35.84	88.27
		100	0.095	55.18	
		150	0.056	73.58	

		200	0.047	77.83	
4	F3	50	0.144	32.07	97.29
		100	0.098	53.77	
		150	0.060	71.69	
		200	0.053	75.00	
5	F4	50	0.129	39.15	74.68
		100	0.087	58.96	
		150	0.042	80.18	
		200	0.038	82.07	
	Control	-	0.212	-	-

The antioxidant potential of the standard and formulation F1 – F4 was evaluated using the H_2O_2 radical scavenging assay at concentrations of 50, 100, 150 and 200 µg/ml. Among all the formulation, F4 is the most effective formulation showing antioxidant activity similar to the standard.

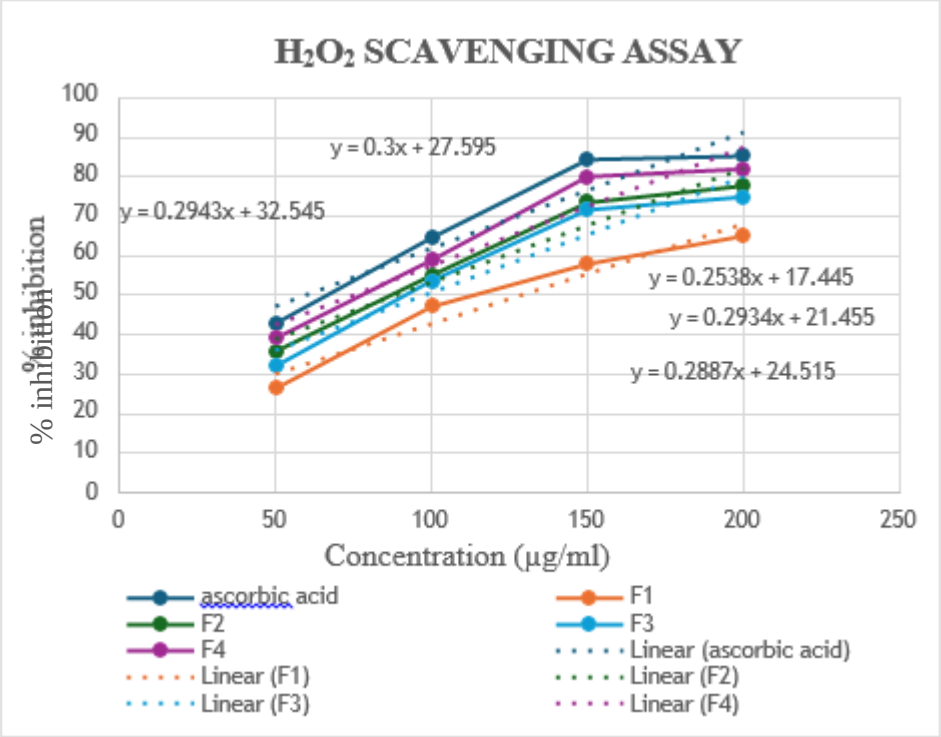


Fig 8: H2O2 Scavenging assay Invitro SPF Determination.

Table 9: Invitro SPF Determination

Wavelength	ΣEE x I	Absorbance of F4
290	0.0150	2.950
295	0.0817	3.052
300	0.2874	3.197
305	0.3278	3.107
310	0.1864	2.896
315	0.0839	2.854
320	0.0180	2.755

Spectroscopically SPF value calculated by the equation, SPF = CF x ΣEE x I x Abs. The result indicate that SPF value was found to be 30.6

Stability studies

The formulation was subjected to stability testing. Changes in the appearance, pH, viscosity, spreadability, and swelling index of the gel were investigated at a period of 1 month. No significant changes in physical appearance, pH, viscosity, spreadability and swelling index were seen. This indicates stability of prepared gel formulation.

CONCLUSION

The present study successfully developed and evaluated a polyherbal hydrogel formulation for de-tan activity incorporating Liquorice and Neem extracts. The formulated hydrogels exhibited desirable physical characteristics, suitable pH range, good viscosity, satisfactory spreadability, and excellent washability, making them appropriate for topical application. In vitro antioxidant studies confirmed concentration-dependent free radical scavenging activity, with formulation F4 showing the highest antioxidant potential, comparable to the standard ascorbic acid. Additionally, the in vitro SPF value of F4 indicates effective protection against UV radiation, supporting its de-tan and photoprotective efficacy. Stability studies demonstrated that the optimized formulation remained stable without significant changes in physicochemical properties. Overall, the results indicate that the developed polyherbal hydrogel is a promising, safe, and effective herbal formulation for the management of skin tanning, offering a natural alternative to synthetic de-tan products. Further clinical studies may help establish its therapeutic efficacy on human skin.

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