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Assessment of Flavonoid Release with Different Permeation Enhancers

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ABSTRACT
The aim of the study was to evaluate the effect of different permeation enhancers in the transdermal permeation of flavonoids. A weed plant named *Chromolaena odorata* have been employed as the flavonoid source. Six different cream formulations have been prepared with the Chromolaena extract using different permeation enhancers such as ethanol, dimethyl sulphoxide, surfactants and occlusive agents. The amount of flavonoids penetrated during a time period of four hours was noted. The highest amount of penetration was obtained with the formulation no 6 which contained a combination of different permeation enhancers. The result suggests that a synergistic combination of different permeation enhancers can be adopted for enhancement of permeation of flavonoids in topical drug delivery systems.

Keywords: *Chromolaena odorata*, flavonoids, Permeation enhancers, In-vitro skin permeation study, total flavonoid content analysis.

INTRODUCTION

Chromolaena odorata (L.) R.M. King and H. Robinson is one of the world's worst tropical weeds. It is a member of the tribe Eupatorieae in the sunflower family Asteraceae. The weed goes by many common names including Siam weed, devil weed, French weed, communist weed, hagonoy, co hoy etc. [1] Chromolaena is being used traditionally for its many medicinal properties, especially for external uses as in wounds, skin infections, inflammation etc. [2] Studies have demonstrated that the leaf extract has antioxidant, anti-inflammatory, analgesic, antimicrobial, cytoprotective and many other medicinally significant properties. [2, 4-5] The phytochemical studies have revealed the presence of a wide range of chemical entities in the plant. The leaves of this plant have be found to be a rich source of flavonoids, of which quercetin, sinensetin, sakuranetin, padmatin, kaempferol, salvagenin were isolated and identified. [3] The major pharmacological activities of the plant are attributed to the flavonoids present in the aerial parts, especially leaves. [2]
Permeation enhancers are chemicals that interact with the constituents of the biological membrane to increase the drug flux. Human skin is a remarkably efficient barrier that effectively inhibits the efforts to transfer any exogenous compound across it. This barrier property causes difficulties for transdermal delivery of therapeutic agents. [6] To-date, a vast array of chemicals has been evaluated as penetration enhancers (or absorption promoters), yet their inclusion in to topical or transdermal formulations is limited since the underlying mechanisms of action of these agents are seldom clearly defined. [7] The enhancement in the penetration is thought to be acquired by means such as increasing the diffusion coefficient, increasing the drug solubility in the skin and by increasing the degree of saturation. [8] The usual penetration enhancers used are dimethyl sulphoxide, essential oils, Fatty acids, alcohol, surfactants, pyrrolidones, azones, oxazolidinones, occlusive agents and urea. The skin permeation enhancers have been evaluated already and they were proved to be of commendable efficacy. Studies are being conducted in this field regarding their efficacy and mechanism which would significantly increase the number of drugs suitable for transdermal drug delivery, with the result that skin will become one of the major routes of drug administration in the nearby future. [9-10]
In this study, an anti-inflammatory cream is formulated using methanolic extract of *Chromolaena odorata*. Different formulations are made using various penetration enhancers such as alcohol, dimethyl sulphoxide, tween-80 and occlusive agents. The release of flavonoids form each formulation is being evaluated by an In-vitro permeation study using pig ear skin as the skin simulatory membrane. The release of flavonoids is quantified by total flavonoid content analysis using UV spectrometry. The effect of different penetration enhancers on flavonoid permeation are then analyzed by comparing the results. [8, 11]

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MATERIALS AND METHODS

The extract was prepared by percolating Chromolaena leaf powder with methanol for 6 hours using soxhlet apparatus (continuous hot percolation). Tween-80, white soft paraffin,

Stearyl alcohol, glycerin, methyl paraben, propyl paraben, butylated hydroxy toluene, alcohol and dimethyl sulphoxide were the different chemicals used for the preparation of the cream. Phosphate buffer of pH 8 and standard quercetin in different dilutions were used for the *in-vitro* release study. Sodium citrate, acetic acid and aluminium chloride were used for the total flavonoid content analysis.

Preparation of permeation membrane

Pig ear skin was used as the skin simulatory membrane. Skin from the outer surface of a freshly excised porcine ear was carefully dissected. The skin was shaved to remove the hairs before the preparation. Body fluids were removed by paper or towel. It was stored in the normal saline for preservation. It can be preserved up to 4 days in this state. To counteract microbes, antimicrobial preservatives have been added. The whole skin membrane was removed from the underlying cartilage with the help of a scalpel. The subcutaneous tissues were removed and skin was stored at -4°C for the time until use. [12-15]

Formulations

An O/W formula was created. Six different formulations were created using different penetration enhancers in the formula. Formulation 1 contained 1% Chromolaena extract without any penetration enhancers. Formulation 2 contained 5% ethanol as permeation enhancer. Formulation 3 contained excess amount (35%) of white soft paraffin in the formula as an occlusive agent. In Formulation 4, dimethyl sulphoxide was incorporated as the permeation enhancer. Formulation 5 contained an excess (5%) of tween-80 as surface acting permeation enhancer. Formulation 6 was formulated using a combination of different permeation enhancers such as ethanol, dimethyl sulphoxide and white soft paraffin for a synergistic activity. The method of preparation employed for the cream formulation is as follows:

Ingredients	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Formula 6
Chromolaena extract	1	1	1	1	1	1
Tween-80	5	5	5	5	7.5	5
White soft paraffin	25	25	35	25	25	30
Stearyl alcohol	8	8	8	8	8	8
Glycerin	12	12	12	12	12	12
Methyl paraben	0.1	0.1	0.1	0.1	0.1	0.1
Propyl paraben	0.05	0.05	0.05	0.05	0.05	0.05
Butylated hydroxy Toluene	0.02	0.02	0.02	0.02	0.02	0.02
Ethanol	-	5	-	-	-	5
Dimethyl sulphoxide	-	-	-	5	-	2.5
Water	48.83	43.83	38.83	43.83	46.33	36.33

The creams were prepared by emulsion method. The ingredients were classified in to two phases. The phase 1(aqueous phase) contained water, glycerin and methyl paraben. Phase 2 (oil phase) contained white soft paraffin, Stearyl alcohol, Propyl paraben, tween-80 and Butylated hydroxy toluene. The ingredients of the phase 1 were added to water and heated. Then glycerin was added to the phase with constant stirring. The phase was heated up to 70°C. Phase 2 was prepared by melting white soft paraffin at 70°C. Then Stearyl alcohol and tween-80 was added to it with constant stirring. The oil phase was added to the water phase at 70°C and was stirred continuously. The mixture was stirred until the temperature of 35°C was reached. Then the extract of Chromolaena was added, mixed properly and the cream was cooled by stirring to room temperature. Prepared creams were stored at room temperature in tightly closed plastic containers protected from light. [16-18]

Analytical procedure

The total flavonoid content was analyzed by UV spectrophotometric method (shimadzu 1800 model).10 ml sample solutions were withdrawn from the receptor compartment of the Franz diffusion cell during 0.5, 1, 2, 3, 4 hours. The total flavonoid content was analyzed by aluminium chloride colorimetric method. To 10 ml of the sample, 0.5 ml of 0.5% solution of sodium citrate and 2ml of $AlCl_3$ (2 g of $AlCl_3$ in 100 ml of 5% acetic acid in methanol) was added and then made up to 25 ml with 5% acetic acid in methanol. The same procedure was performed with blank sample solution but without $AlCl_3$. After 45 minutes, yellow solutions were filtered and absorbance at 425nm was measured using phosphate buffer as the blank. The concentration of the sample solutions were estimated by the standard absorbance Vs concentration graph plotted by using the different dilutions of standard quercetin. [11, 19-20]

Permeation study

The permeation studies were carried out in amber glass Franz type diffusion cell with a diffusion area of 1.86 cm^2. The porcine ear skin samples were placed between the donor and the receptor compartments of the cell, with the dermal side in contact with the receptor medium. The formulation studied (1 g) was placed in the donor compartment and the cell was covered with the aluminium foil. The receptor chamber was filled with 15 ml of phosphate buffer of pH 8 and kept at 32 ± 0.5°C by a circulating water jacket. Samples of 1 ml were withdrawn from the receptor compartment at 0.5, 1, 2, 3 and 4 hours and replaced the same volume with phosphate buffer of 32°C. Sink conditions were met in all cases. [12-14]

Table 1: Total flavonoid contents penetrated through the skin at different intervals from cream formulations

Formulation	Total flavonoid content at different time intervals (µg)				
	0.5 h	1 h	2 h	3 h	4 h
Formula 1	21	65	140	161	187
Formula 2	29	90	211	285	375
Formula 3	42	111	215	271	312
Formula 4	31	95	140	268	340
Formula 5	24	92	130	176	224
Formula 6	35	128	236	330	463

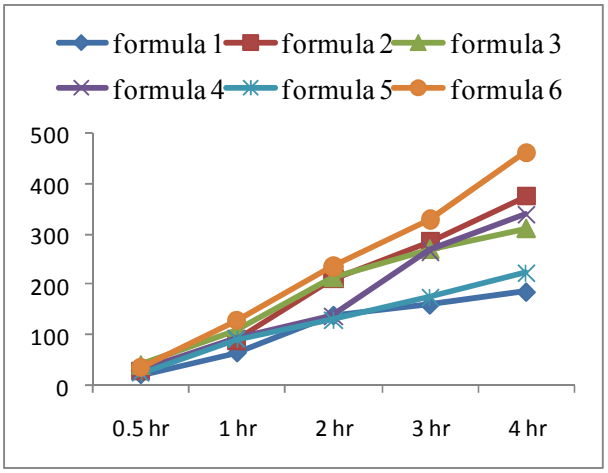

Fig. 1: Graphical representation of the flavonoid permeation

RESULTS

The flavonoids penetrated through the skin at different intervals from cream formulations are shown in the Table 1. The in vitro release profiles of flavonoids from different formulations can be compared by these representations. The amount of flavonoids permeated at 4 hr ranged between 187 µg (formula 1) and 463 µg (formula 6). In all the cases, the flavonoid permeation was enhanced by means of the permeation enhancers; when compared with the control (formula 1). The formula 6 showed the greatest penetration capability with a flavonoid content permeation of 463 µg after the time period of 4 hours. The graphical representation of the flavonoid permeation is represented in the Fig. 1.

DISCUSSION

Different release profiles of flavonoids were observed. The control formulation showed the least release rates compared to the formulations incorporated with the penetration enhancers. The highest release was obtained with the use of a combination of different penetration enhancers in the formula. This shows the synergistic action of different penetration enhancers can be of significant value in case of topical preparations containing flavonoids.

There was no evidence of phase separation, development of disagreeable odour or change in colour/ consistency in any of the preparations.

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