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Formulation and Evaluation of *Hylocereus undatus* for the Treatment of Psoriasis

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ABSTRACT: The pitaya, from the cactus family, sometimes called dragon fruit or dragon fruit, has aroused the curiosity of connoisseurs of the subject for its unique flavor, shape, and color. Also documented the functions of *H. polyrhizus* seeds, including their antioxidant capacity, and the use of various parts of *H. undatus* to promote wound healing in diabetic rats. This study aimed to determine the relationship between ethanol and *Helicobacter pylori*. Efficacy of pericarp undatus and a reformulated gel containing botanical components of *H. undatus* pericarp extract on ultraviolet-induced psoriasis in rats. Herbal remedies are widely claimed to be effective in treating various skin conditions. Unfortunately, no research supports their use in psoriasis. The acute dermal and oral toxicity of ethanolic extracts of plant bark in gels and suspensions has been studied. Acute dermal toxicity results at 1% w/w and oral toxicity data at 1000 mg/kg indicate that the gel and suspension are safe. Wistar rats developed psoriasis by exposing 10% of their body to ultraviolet light. 2.5% and 5% gels were used to test antipsoriatic activity. The severity index, histological examination, and biochemical analysis were studied. Our results indicate that the test formulation (gel) of plant bark extract has antipsoriatic activity.

Keywords: Dragon fruit, psoriasis, phytochemical screen, UV-C induced photodermatitis, Perry mouse tail model

INTRODUCTION

Psoriasis is a common inflammatory disease of human skin characterized by focal to confluent, raised patches of skin with uniform scaling and variable erythema. Typical histological features of psoriasis include epidermal hyperplasia (acanthosis) with persistent redness, epidermal granular layer discontinuity (hypo granulosa), cutaneous and epidermal parakeratosis, and leukocyte infiltration (Danilenko, 2008). Epidermal hyperplasia, cutaneous angiogenesis, activated T cell infiltration, and increased cytokine levels. (Villadsen *et al.,* 2003). Mitotic activity, keratinization, and elongation of the dermal papilla lead to the thickening of the stratum corneum, which produces silvery scales (Lakshmi *et al.,* 2020). NSAIDs that inhibit cyclooxygenase (COX)-2, corticosteroids, and immunosuppressants such as FK-506 and cyclosporin A have been used to treat psoriasis but are nephrotoxic and neurotoxic and have other side effects. NSAIDs inhibit COX-2 to suppress inflammation and suppress immunity (Shin *et al*., 2005). Natural treatments for moderate psoriasis include corticosteroids, vitamin D3 analogs, and calcineurin inhibitors, although flavonoids, tannins, glycosides, monoterpenes, and alkaloids are also effective (Nandhini and Bini 2017).

MATERIAL AND METHOD

Collection of sample. Fresh fruits of *Hylocereus undatus* were purchased from Chikhali market, Dist Buldana, Maharashtra. Media, reagents, chemicals, and solvents used in this study were obtained from Kamla Agencies Akola, Maharashtra, India.

Plant identification. *Hylocereus undatus* obtained from Chikhali market, Dist. Buldana, Maharashtra.

The sample was verified by Dr. Vanita Pochhi, head of the botany department. Chikhali, Shivaji Academy of Sciences.

Extraction. Extract the freeze-dried bark powder with various solvents such as 95% ethanol, chloroform, and n-hexane. The extraction will be carried out in an Innova 4000 incubator at 30°C for 2 hours followed by Whatman No. 4 filtration Cellulose filter paper. The filtrate was then concentrated at 40°C under a vacuum. The final concentration of the extract was standardized to 10 mg/ml. (Vandana and Shweta Pawar 2019).

Preliminary Phytochemical Screening. *Hylocereus undatus* ethanol extracts contain secondary metabolites summaries in Table 1.

Thin layer chromatography. TLC confirmed the presence of Phytochemical constituents ina preliminary test (Bhandari *et al.,* 2020).

Table 1: Preliminary Phytochemical Analysis of *Hylocereus undatus* Peel Extract.

Sr. No.	Phytochemical constituents	Methanol
1.	Carbohydrate	+
2.	Saponins	-
3.	Tannins	+
4.	Flavonoids	+
5.	Steroid and triterpenoids	+
6.	Phenols	+
7.	Alkaloids	+
8.	Glycoside	+

Table 2: Plant constituents, visualizing agents, and TLC confirmatory test results.

Component	Spray Reagent	Observation	Results
Flavonoids, Steroids	Antimony (III) chloride	Fluorescing spots in long-wave UV light	+ +
Phenols, Tannin Flavonoids	Potassium-ferricyanide-ferric chloride	Blue spots	+ +
Alkaloids	Dragendorff's Reagent	Orange spots	+ +
Cardenolides	3,5 Dinitrobenzoic acid Kedde Reagent	Red-blue violet-colored zones	- -
Coumarins, Anthraquinones, Anthrones, Phenols	Methanolic potassium hydroxide (Bornträger reagent)	Anthraquinones give orange coloration; Anthrones give yellow and Coumarins react to form blue (UV 365nm) colored zones	+ +
Anthraquinones	Magnesium acetate in methanol	Orange-colored zones	+ +
Higher alcohols Phenols, Steroids, Essential oils	Vanillin-sulfuric Acid	Colorful zones	+ +

According to TLC data, the best solvent system is n-butanol-acetic acSid-water (4:1:5). The spray reagent used to reveal stains and visible color contains flavonoids and steroids compatible with prior phytochemical analysis.

Table 3: Rf values for **various spots formed** in **solvent systems were visualized using spray reagents.**

Phytochemical	Solvent System	No. of Spots	Rf Value
Flavonoid and steroids	n-Butanol-Acetic Acid-Water	1	0.72
Phenols, Tannins, and Flavonoids	Chloroform-Acetic Acid-Water	1	0.93
Alkaloids	Chloroform-Methanol	1	0.90
Coumarins, Anthraquinones, Anthrones, and Phenols	n-Butanol-Acetic Acid-Water	1	0.60
Anthraquinones	Chloroform-Methanol	1	0.96
Higher Alcohols, Phenols, Steroids,and Essential Oils	n-Butanol-Acetic Acid-Water	1	0.98

Testing for phenols, tannins and flavonoids using different solvent systems provided only one sample with the highest Rf value observed in a mixture of chloroform-acetic acid-water (50:45:5). Tests for alkaloids and anthraquinones yielded only one spot, but tests for coumarin and phenol gave identical Rf values. Finally, test results for higher alcohols, phenols, steroids, and essential oils have been collected in one place (Panda *et al*., 2006).

Preparation of Gel. Take the dry powder dragon fruit extract and sift it to get small particles. Accurately weigh 2.5 g of dried dragon fruit powder. Dissolve in 47.5 ml of propylene glycol, stir continuously after dissolution, and let stand for 10 minutes until completely dissolved. When a dark solution is obtained, 1 g of carbazole is slowly added and stirred continuously with a mechanical stirrer so that no lumps are formed in the solution gel . Adjust the pH of the gel to 6.8 by adding triethanolamine dropwise to the gel and check the pH with a digital thermometer (Sudha *et al.,* 2017).

Gel evaluation

(a) Visual inspection. Check homogeneity the gel composition developed by dispersion test, color; After placing the gel in the container, visually inspect the gel for synapses and clots. The formulation was found to be a homogeneous reddish gel formulation (Kasar *et al.,* 2018).

(b) Spread ability Test. For dispersion pH measurement, 0.5 g of each compound was placed between two glass slides and allowed to stand for 5 minutes. The diameter of the dispersion circle was measured to compare the lubricity (Prasantha *et al.,* 2017). The spread ability of the skin extract gel showed acceptable spread ability. The spread ability of the

prepared gel was confirmed to be 21.22 g. cm/s is good compared to commercially available gel formulations.

(c) pH determination. Determination of the pH of the gel with a digital pH meter 20 Determination of drug content. Lectures were attended on average three times. The gel was found to be prepared at pH 7-8 and skin pH at pH 5.5 to avoid irritation (Sari *et al.,* 2016).

(d) Determination of drug content. The formed gel is dissolved in 100 ml of phosphate buffer. Shake mechanically for 2 hours. The solution was filtered through a Millipore filter and measured with a UV-Vis spectrophotometer at 260 nm using phosphate buffer (pH 5.5) as a control (Patel *et al*., 2008).

(e) Rheological studies. Measure the viscosity of the gel composition at 25 °C using a rotational viscometer (Panda *et al.,* 2006). The viscosity of the prepared gel was confirmed to be 3671 ± 0.58

Acute skin toxicity of extract. Acute skin toxicity according to OECD Standard No. 402. Adult Wistar rats of both sexes were used. The nine creatures were divided into three groups of three. About 24 hours prior to testing, 10% of the back hair of the test animals was removed with a depilatory c ream. Animals in group I served as controls, and animals in groups II and III received test drug at 1000 mg/kg body weight (limit test). All animals were observed for 14 days for changes in fur, eyes, behavior and toxic response. No deaths or abnormalities were observed. The LD50 of the gel exceeds 2000 mg/kg as no fatal outcome was observed at this cutoff dose, which is the highest dose that can be applied topically (Sari *et al.,* 2016).

Psoriasis induction. The animal is then placed on a bent piece of wood attached so that the legs do not touch the ground. This configuration keeps the animal immobile during subsequent UV irradiation. Wrap the entire animal in sunscreen film except for a 1.5 × 2.5 cm patch of epilated skin. Then, an open area measuring 1.5 × 2.5 cm was irradiated with a UV lamp for 45 minutes at a vertical distance of 20 cm from the skin (Wadher *et al.,* 2021). Dosing was started 12 hours after irradiation. And it went on for 3 days. Follow the schedule of applying twice a day 12 hours apart. (Vijayalakshmi and Madhira 2014).

Procedure: Clip the hair on one side of the skin with scissors and then shave carefully to avoid damaging the skin. The animal is then placed on top of a bent block of wood and tied to the block so that its legs do not touch the ground. This arrangement did not allow animals to move during subsequent UV irradiation. The entire animal was covered with a sunscreen film except for the epilated skin area of 1.5 × 2.5 cm (Selvakumar *et al.,* 2018). Then, an exposed area of 1.5 × 2.5 cm was irradiated with a UV lamp at a distance of 20 cm vertically from the skin for 45 minutes. Administration was started at 12 hours after irradiation and continued for 3 days. Follow the twice-daily application schedule 12 hours apart. On day 3, 2 h after the last treatment, the animals are anesthetized with ether and the exposed skin is removed via surgical incision. The incised skin was fixed in 10% buffered formalin and progressively dehydrated with increasingly stronger alcohol (80% to absolute alcohol). The skin was then immersed in kerosene and sectioned at 4 µm thickness using a microtome. The sections were transferred to glass slides and stained with hematoxylin and eosin (Lin *et al.,* 2021)

Psoriasis UV-C Induced Photo dermatitis Model:

Table 4: Epidermal Thickness.

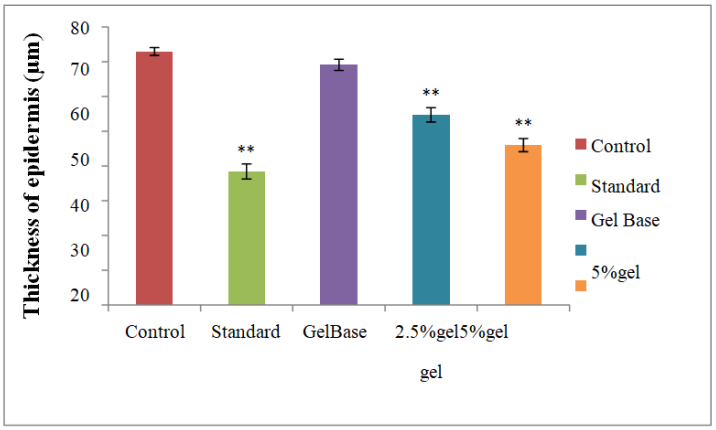
Sr. No.	Groups(n=5)	Thickness of Epidermis
1.	Control	72.932±1.12
2.	Standard	38.372±2.15**
3.	Gelbase	69.102±1.62
4.	*Hylocereus undatus* gel 2.5%	54.654±2.07**
5.	*Hylocereus undatus* gel 5%	45.984±1.87**

Values are expressed as Mean ± SEM; statistically analyzed by One Way ANOVA followed by Dunnett test.*p<0.05, **p<0.01 when all groups compared with the Control group. ##p<0.01

Control: Animal exposed t o Ultra violet-C

Standard: Animal exposed with Ultraviolet-C+ Standard gel Gel base: Animal exposed with Ultraviolet-C + Gelbase 2.5% ML gel: Animal exposed with Ultraviolet-C+2.5% *Hylocereus undatus* gel

5% ML gel: Animal exposed with Ultraviolet-C+5% *Hylocereus undatus* gel



Graph 1: Epidermal Thickness.

Values are expressed as Mean ± SEM; statistically analyzed by One Way ANOVA followed by Dunnett test.*p<0.05, **p<0.01 when all groups compared with the Control group. ##p<0.01**.**
Control: Animal exposed to Ultraviolet-C
Standard: Animal exposed with Ultraviolet-C + Standard gel
Gel base: Animal exposed with Ultraviolet-C + Gel base
2.5% ML gel: Animal exposed with Ultraviolet-C + 2.5% *Hylocereus undatus* gel
5% ML gel: Animal exposed with Ultraviolet-C + 5% *Hylocereus undatus* gel.

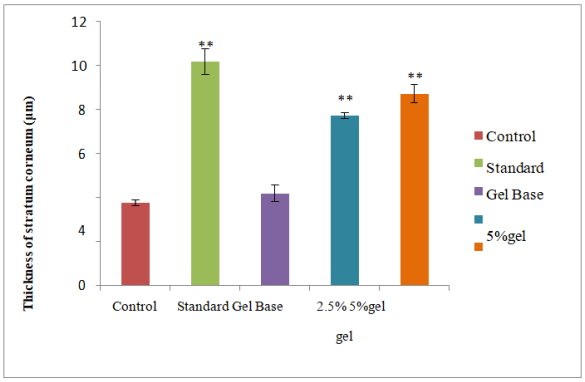
Drug Effects on Epidermal Thickness of UV-C Photosensitive Psoriasis Dermatitis Model:

Animals exposed to UVC light. Increase in the thickness of the epidermis due to the proliferation of keratinocytes. Animals treated with standard gel, 5% pitaya gel and 2. 5% pitaya gel had significantly reduced epidermal layer thickness (p<0.01) compared to the control group.

Table 5: Thickness of Stratum corneum.

Sr. No.	Groups(n=5)	Thickness of Stratum Corneum
1.	Control	3.74±0.13
2.	Standard	10.182±0.58**
3.	GelBase	4.174±0.38
4.	*Hylocereus undatus*ge l2.5%	7.716±0.14**
5.	*Hylocereus undatus*ge l5%	8.71±0.42**

Values are expressed as Mean ± SEM; statistically analyzed by One Way ANOVA followed by Dunnett test. *p<0.05, **p<0.01 when all groups compared with the Control group. ##p<0.01.
Control: Animal exposedt o Ultra violet-C
Standard: Animal exposed with Ultra violet-C+Standard gel Gelbase: Animal exposed with Ultraviolet-C+Gel base
2.5%ML gel: Animal exposed with Ultrav iolet-C+2.5% *Hylocereus undatus* gel 5% ML gel: Animal exposed with Ultra violet-C+5% *Hylocereus undatus* gel



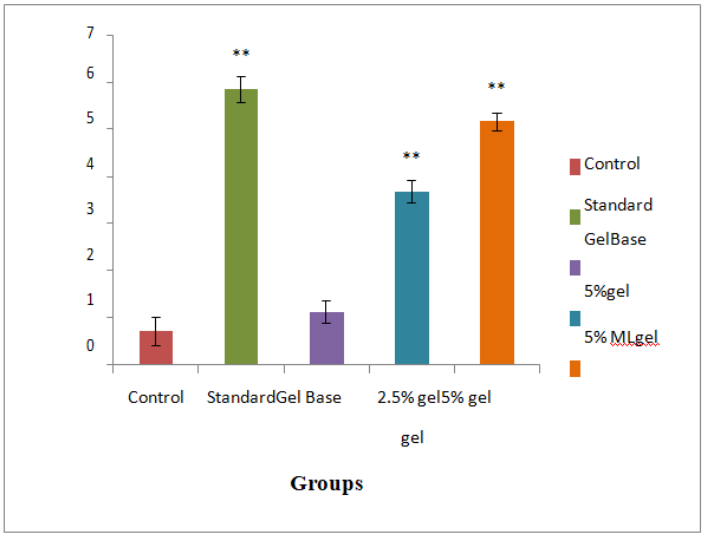
Graph 2: Thickness of Stratum corneum.

Values are expressed as Mean ± SEM; statistically analyzed by One Way ANOVA followed by Dunnett test.*p<0.05, **p<0.01 when all groups compared with the Control group. ##p<0.01.
Control: Animal exposed to Ultraviolet-C
Standard: Animal exposed with Ultraviolet-C+Standard gel
Gelbase: Animal exposed with Ultraviolet-C+Gelbase
2.5% ML gel: Animal exposed with Ultraviolet-C+2.5% *Hylocereus undatus* gel
5% ML gel: Animal exposed with Ultraviolet-C+5% *Hylocereus undatus* gel
Effect of drug on the thickness of stratum corneum layer of UV-C induced photo dermatitis model for psoriasis.
Animals treated with standard gel, *Hylocereus undatus* gel 5% & *Hylocereus undatus* gel 2.5% showed significant increases (p<0.01) in stratum corneum thickness as compared to a control group.

Table 6: Thickness of stratum granulosu.m

Sr. No.	Group(n=5)	Thickness of stratum Granulosum
1.	Control	0.7±0.1
2.	Standard	5.85±0.27**
3.	Gel Base	1.1±013
4.	2.5% *Hylocereus undatus* gel	3.67±0.24**
5.	5% *Hylocereus undatus* gel	5.16±0.19**

Values are expressed as Mean ± SEM; statistically analyzed by One Way ANOVA followed by Dunnett test.*p<0.05, **p<0.01 when all groups compared with the Control group. ##p<0.01.
Control: Animal exposed to Ultraviolet-C
Standard: Animal exposed with Ultraviolet-C + Standard gel
Gel base: Animal exposed with Ultraviolet-C + Gel base
2.5% ML gel: Animal exposed with Ultraviolet-C + 2.5% *Hylocereus undatus* gel
5% ML gel: Animal exposed with Ultraviolet-C + 5% *Hylocereus undatus* gel



Graph 3: Thickness of stratum granulosum.

Values are expressed as Mean ± SEM; statistically analyzed by One Way ANOVA followed by Dunnett test. *p<0.05, **p<0.01 when all groups compared with the Control group. ##p<0.01.
Control: Animal exposed to Ultraviolet-C
Standard: Animal exposed with Ultraviolet-C + Standard gel
Gel base: Animal exposed with Ultraviolet-C + Gel base
2.5% ML gel: Animal exposed with Ultraviolet-C + 2.5% *Hylocereus undatus* gel
5% ML gel: Animal exposed with Ultraviolet-C + 5% *Hylocereus undatus* gel.
Effect of drug on the thickness of stratum granulosum layer of UV-C induced photodermatitis model for psoriasis:
The stratum granulosum layer is absent or reduced when the animal is exposed to a UV-C lamp. Animals treated with standard gel, *Hylocereus undatus* gel 5% & *Hylocereus undatus* 2.5% showed significant

increases (p<0.01) in stratum granulosum thickness as compared to a control group.

Perry's Micetail model

Orthokeratosis(%):

Table 7: Orthokeratosis (%).

Sr. No.	Treatments	Orthokeratosis(%)
1.	Control	25.2±2.7
2.	Standard	64.7±4.2
3.	GelBase	29.9±1.6
4.	2.5%gel	37.3±2.5
5.	5%gel	51±2.7

Values are expressed as Mean ± SEM; statistically analyzed by One Way ANOVA followed by Dunnett test.*p<0.05, **p<0.01 when all groups compared with the Control group. ##p<0.01.

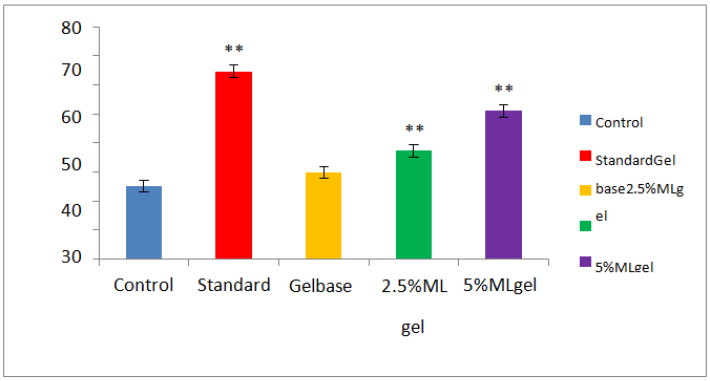
Control: The animal has received saline

Standard: The animal has received Standard gel

Gel base: Animal has received a Gel base

2.5% gel: The animal has received 2.5% *Hylocereus undatus* gel

5% gel: The animal has received 5% *Hylocereus undatus* gel



Graph 4: Orthokeratosis (%).

Values are expressed as Mean ± SEM; statistically analyzed by One Way ANOVA followed by Dunnett test.*p<0.05, **p<0.01 when all groups compared with the Control group. ##p<0.01.

Control: The animal has received saline

Standard: The animal has received Standard gel

Gel base: Animal has received a Gel base

2.5% ML gel: The animal has received 2.5% *Hylocereus undatus* gel

5% ML gel: The animal has received 5% *Hylocereus undatus* gel.

Effect of drug on Orthokeratosis % of Perry's Mice tail model for psoriasis

Animals treated with standard gel, *Hylocereus undatus* gel 5% & *Hylocereus undatus* gel 2.5% showed significant orthokeratosis (%) (p<0.01) when compared with the control group.

Drug activity(%):

Table 8: Drug Activity(%).

Sr. No.	Treatments	Activity(%)
1.	Control	-
2.	Standard	51**
3.	Gelbase	-
4.	2.5% *Hylocereus undatus* gel	16*
5.	5%*Hylocereus undatus* gel	29**

Values are expressed as Mean ± SEM; statistically analyzed by One Way ANOVA followed by Dunnett test.*p<0.05, **p<0.01 when all groups compared with the Control group. ##p<0.01.

Control: The animal has received saline.

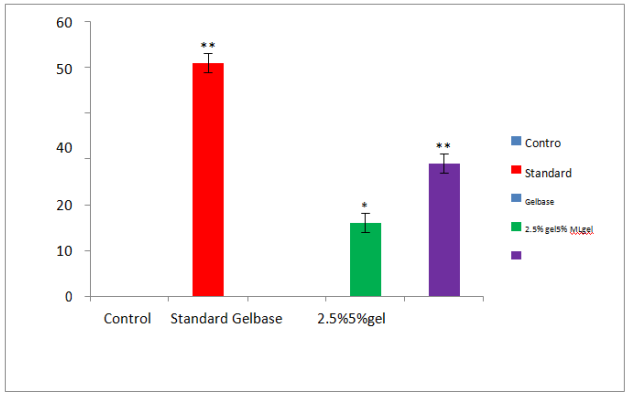
Standard: The animal has received Standard gel.

Gel base: Animal has received a Gel base.

2.5% ML gel: The animal has received 2.5% *Hylocereus undatus* gel.

5% ML gel: The animal has received 5% *Hylocereus undatus* gel

Values are expressed as Mean ± SEM; statistically analyzed by One Way ANOVA followed by Dunnett test. *p<0.05, **p<0.01when all groups compared with the Control group. ##p<0.01.



Graph 5.

RESULTS AND DISCUSSION

Phytochemical components present in the extract of *Hylocereus undatus* peel are found to have alkaloids, flavonoids, tannins, phenols, carbohydrates, coumarins, and proteins. The methanolic extract was found to have oils and terpenoids, saponins, and extracts. Steroids, anthraquinones, and amino. Alkaloids like donepezil, tacrine, rivastigmine and quinacrine are cholinesterase inhibitors that are used in Alzheimer's disease (Safira *et al.,* 2021). Another set of alkaloids that belong to steroidal, triterpenoids, and lycopodium classes like sarcodine, sarcosine, epipachysamine-D, examine B and C, confession, lycorine have anticholinesterase activity (Konrath *et al.,* 2013). The aqueous and methanolic extract was found to contain flavonoids. Flavonoids are the secondary metabolites present in plants (Cazarolli *et al.,* 2008). Flavonoids such as isoflavones, quercetin, isoflavonoids, naringenin, kaempferol, chalcones, flavans, and many other flavonoids have been found to have anti-microbial activities. Flavonoids such as kaempferol, quercetin, and isorhamnetin were found to be present in higher amounts in the pee (Mierziak *et al.,* 2014). Common models used for psoriasis are Ultraviolet C induced psoriasis, and Perry's mice tail model (Wadher *et al.,* 2021). With the help of these models, it can be possible to confirm the drug has anti-psoriatic activity. In the "ultraviolet ray photodermatitis model for psoriasis'', the exposure of the rat's skin to UV radiation using a UV-B bulb (wavelength 280-315 nm) induced a pro-inflammatory reaction in the skin that resembles the one observed in psoriasis. The extraction of *Hylocereus undatus* (5% & 2.5% ML gel) produced a significant reduction in the thickness of the epidermal layer as well as the presence of the stratum granulosum layer as compared to the control group. The thickness of the epidermal layer increases in a psoriatic condition where as the Stratum granulosum layer is greatly reduced or absent in psoriatic lesions. From the above findings, it might be shown that anti-psoriasis activity. In Perry's scientific mice tail model, the parakeratotic condition is seen in the adult mouse tail which is the hallmark of psoriasis. Induction of the orthokeratosis in adult mouse tails is the basis behind the mouse tail test. In this model extraction of *Hylocereus undatus* (5% & 2.5% ML gel) showed significant orthokeratosis (%) when compared with the control group, it also showed significant changes in epidermal thickness compared with the control group. Hence from the above findings, *Hylocereus undatus* shows anti-psoriatic activity.

CONCLUSIONS

On the basis of the results obtained in our study, it is observed that the seed contains high amounts of phytochemicals which have various activities like anti-cancerous activities, anti-microbial activities, antioxidant activities, etc. Various approaches are available for topical drug delivery system which fulfills pharmacotherapeutic aspects as well as patient compliance. Herbal formulations have growing demand in the world market as natural products are more acceptable in the belief that they are safer than synthetic one. However, for treatment of topical gel shows better results as compare to cream and ointment. The gel were prepared and evaluated for various physicochemical and performance characteristics and compared with marketed gel. *Hylocereus undatus* shows anti-psoriatic activity by reducing the thickness of epidermal layer, presence of stratum granulosum layer in ultraviolet-C induced psoriasis whereas orthokeratosis in Perry's mice tail model compared with control group. In Ultraviolet-C induced psoriasis model *Hylocereus undatus* shows significant reduction of epidermal layer thickness & presence of Stratum Granulosum. In Perry's scientific mice tail model *Hylocereus undatus* shows significant orthokeratosis % which is absent in mice.

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