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Research Article

Formulation and Evaluation of Piperine and Psoralea Corylifolea Anti Vitiligo Cream

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ABSTRACT

Another name of Vitiligo is leukoderma, a pigmentation disorder of the skin in which the formation of melanocytes destroys the skin. As a result, white patches appear on the skin on different parts of the body, which affects even the psychology & social status of the patient. It affects 1% - 2% of the population worldwide. In recent years, it has been proved that Piperine from Black Pepper& Psoralea Corylifolia from Babchi oil has the depigmenting capacity. The use of Piperine & Psoralea Corylifolia in Vitiligo reduces UV radiation and prevents side effects. The present work is about the extraction of Piperine from black pepper & Psoralea Corylifolia, its evaluation followed by formulation & evaluation of cream.

Keywords: *Vitiligo, leukoderma, Melanocytes, Psoralea Corylifolia, UV Radiation.*

INTRODUCTION

Vitiligo is an unacceptable and unpleasant disorder of the time. It harms human skin pigmentation due to the destruction of cutaneous melanocytes and affects 1%–2% of the population worldwide. The disease is acquisitions, idiopathic, circumscribed depigmentation of the skin characterized by separate white lesions, often connected with a positive family history. Milky-white macules or patches have been presented commonly in the hands and face. According to research & reviews that is Vitiligo occurs equally in Male & females. However, it is also presented in children's & adults. Considerable recent progress has been made in our understanding of the pathogenesis of Vitiligo, and it's now clearly classified as an autoimmune disorder related to genetic and environmental factors (Samidha et al., 2017, Ezzedinea, 2020, Hussain et al., & El-Halima et al., 2019).

The family of Psoralea corylifolia is Leguminosae, which is growing annually. It is also increasing in India. It is a famous herb that is commonly known as babchi.

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Babchi has been used in traditional medicine since ancient times for its magical effects to cure various skin diseases such as psoriasis, Leucoderma, and leprosy. It is the oldest remedy for Leucoderma; it has been tried considerably not only by the practitioners of Indian medicine but also by the followers of the Western system. The essential aspect of this plant is that every part of this plant is valuable. Steam, Roots, Seeds, leaves, and whatever blooms it has all are used to treat various skin problems, such as Leukoderma, skin rashes, infections, and others. Psoralen isolated from the seeds is considered therapeutically active constituents. Nature of origin is dry & hot and prescribed both topically & orally for external application in ointment or paste (Lecturer, 2019, Faulkner & Uthayakumar, 2021, & Purkayastha, & Dahiya, 2012).

Piperine is isolated & extracted from Black pepper by two methods. They are reflux method & another is soxhlation method in which we were found highest %yield is obtained for soxhlation method. The different evaluation works have been carried out for Piperine like PH, melting point, Solubility, UV Spectral analysis, TLC, chemical tests, Partition coefficient, Particle size. A 5%Piperine cream was prepared using beeswax as the base. TLC performed its evaluation, Partition coefficient, Scanning microscopic studies, pH, Moisture absorption studies, consistency, Irritancy test, Drug content uniformity, Organoleptic characters, etc (Kokate, 2019 & Rangari, 2007).

MATERIALS AND METHODS

Black Pepper was procured from Kota College of Pharmacy, while Psoralea corylifolia was procured from the local market. Other chemicals were to be found from the college lab.

Extraction of Piperine from Black Pepper:

The Piperine was extracted by the Soxhlation method by using 95% ethanol as solvent. The solution was filtered & concentrated under vacuum in a water bath at 600C. 50ml alcoholic potassium hydroxide was added to the concentrate & the solution was stirred continuously for 30min. The obtained solution was heated, and water was added dropwise until a yellow precipitate was formed. Water was added until no more residue appeared to develop, and this was allowed to settle overnight. The other day we found the sharp needles of Piperine which we have to separate.

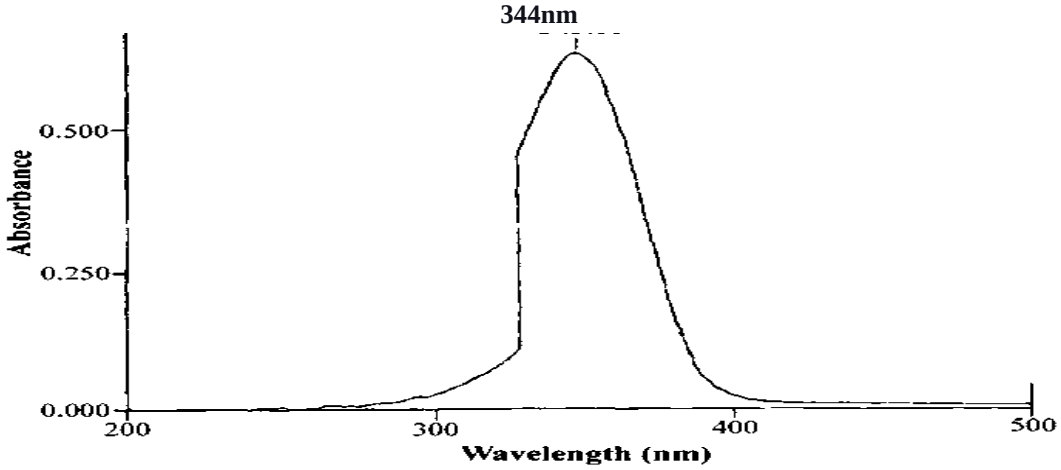
The needle was collected and washed with cold ether 2-3 times. It was recrystallized by using acetone. After this, dissolve needles in acetone and clear it out to do away with extraneous matter & maintain the filtrate apart for 24hrs so that crystals of Piperine are formed. Yellow-colored rod-shaped crystals were recrystallized after 24hrs. (Vinod et al., 2011).

Acquisition of oil of Psoralea corylifolia:

As per reviews information, it was prepared by steam distillation. By the rotary evaporator machine, the oil was further distilled. Babchi oil was dissolved in methanol (0.3 ml oil/ 2 ml methanol). The oil was transferred into a sterile container and stored at 20 ^{0}C until needed.

Analytical works on Piperine:

The λmax of standard Piperine is 342nm, and the λmax of isolated Piperine was 344nm. The drug concentration in each phase was determined by measuring the absorbance using a UV spectrophotometer at344nm.



The particle size of Piperine using a microscope: Some amount of powdered drug was spread on the slide & mounted using glycerine. With the help of an eyepiece micrometer, the diameter of 215 particles was determined. The Particle size distribution is expressed by histogram (Subrahmanyam & Jain, 2017 & Mohanta & manna, 2006).

The particle size of babchi using microscope: The particle size of babchi was found to be 20.33.

Determination of pH: A small amount of Piperine become dissolved in ethanol, and its pH becomes checked via way of means of the usage of pH meter & it becomes determined to be 7.9. (Ayurvedic pharmacopeia, 1999).

pH of Psoralea Corylifolia: A small amount of babchi was dissolved in methanol & its pH was checked by using a pH meter & which was found to be 6.8.

Chemical tests: The Chemical Tests for alkaloids were found positive, and especially with concentrated H_2SO_4 blood-red color was obtained. The brownish ppt was found with Dragendroff's Reagent.

Melting point of Piperine: 131^{o}C -132^{o}C

Melting Point of Psoralea Corylifolea: 120^{0}C

Isolation and purification by Column Chromatography (Piperine):

Preparation of sample: Preparation of sample of Glacial acetic acid extract (GE) and chromatography was done by adsorption of GE on activated Silica Gel plate (60-120) (105^{o}C, 30 minutes) with ratio 1:10, respectively. It was held on for drying in a hot air oven till extravagant material was formed.

Column Identification and Solvent system: The dried prepared sample was bringing to column chromatography, which used a glass column with a diameter of 36 X 4.5 cm. The glass column was filled with silica gel 80 (mesh size: 60–120#) in ethyl acetate: toluene (3:7). A prepared sample of the Glacial

acetic acid extract was added to the free volume at the top of the column. After settling down the material, Fractions (20ml, eight drops/minute) were collected, and the solvent was removed to reduce the volume of where collected. The solvent was removed to reduce the volume of the fraction by evaporation in a vacuum at 35^0C.

Analysis of fractions by Thin Layer Chromatography (TLC): By the Thin layer Chromatography (TLC pre-coated plate), we have performed a study of an isolated fraction of GE p (Silica gel GF250 plates 10cmX20cm, Mark) with solvent system ethyl acetate: toluene (3:7). A separation pattern of GE on TLC was observed by placing TLC plate in iodine chamber and with concentrated H_2SO_4 solution. Rf values were calculated for each spot-on TLC plate. The standard sample was also used for its comparison of Rf value with isolated piperine Fraction no. 3 (Fig. 2A), Fraction no. 4 (Fig. 2B) we were found the standard Piperine (Fig. 2C) were almost similar to the Rf value.

Table 1: Analysis of Fractions by TLC

Fraction no.	No of spot	Chemical test withconc.H_2SO_4	Rf Value
Authentic	**Single spot**	**Blood Red**	**0.55**
1	**Trail**	**-**	**0.61**
2	**Two Spot**	**Brick Red**	**0.56, 0.43**
3	**Single Spot**	**Brick Red**	**0.52**
4	**Single Spot**	**Brick Red**	**0.53**
5	**Single Spot**	**-**	**0.45**
6-10	**-**	**-**	**-**

TLC of Psoralea Corylifolia:

1 mg of Psoralea mixed in 1ml mg methanol then Mark the spots on Silica gel-G plates are eluted in benzene: chloroform then dried plate observed under UV light Psoralea shows the blue fluorescent spot in UV light. The RF value of Psoralea was found 0.2.

Formulation of Cream:

Beeswax (10gm), Lanolin (10gm) & Stearic acid (10gm) were taken in one beaker in another beaker. Piperine (2gm) & Babchi oil (2ml) ware dissolve in methanol by Sonication & introduced into glycerin, water, triethanolamine (1ml) both the beaker was maintained at 600C. All ingredients were melted. Then oily phase is added to the aqueous phase & stirred continuously. When the temperature moves down, then we were added peppermint oil & mixed well up to the

required consistency was to be found & Kohli, 1991).

Evaluation Parameter of Cream: Evaluation of cream Organoleptic characters were studied by visual appearance, color, and odour. By using an eyepiece micrometer, the diameters of 215 particles were determined randomly Paye et al.

Clarity test: Clarity tests were successfully done; no foreign particles were observed.

Partition coefficient of cream: The Partition coefficient of drug between phosphate buffer solution. (pH 6.6) n-hexane was determined at 370± 20. An excess amount i.e., 40mg of cream, was taken in a separating funnel containing a 1:1 ratio of buffer 6.6 & hexane & placed in a water bath for 24hr. The solution was shaken occasionally. Then, both of them were separated & filtered through a two µ filter & the amount solubilized in each phase was determined by measuring the absorbance using a UV spectrophotometer at 344nm. The formulated cream was kept intact in a closed container at room temp. (25- 30°C not exposed to light.

Irritancy test: Mark, an area (1sq.cm) on the left-hand dorsal surface of Rabbit. The cream was applied to the required space, and time was noted. Irritancy, redness, rashes, was checked; after 24hr we were checked, no irritancy, redness & rashes were seen Seth, 1991).

Rheological studies: The non-Newtonian flow was to be found in formulated In vitiligo cream. Take a fixed quantity of 5gms of cream in a 5ml beaker. Keep it impact for 1 hr. The beaker was half-mast to one side, then see the cream consistency that cream is liquefied. Beaker is shaken to and fro for continuous 5mnts and checked whether the consistency has changed or not. The beaker was again tilted and measure for the pourability of the cream. In which formulation did not show any thixotropic effects/characters. (Sinko, 2007).

RESULT & DISCUSSION

Black pepper& Psoralea Corylifolia, obtained from the local source, was subjected to standardization, and the results were found to obey the standard values. Piperine was extracted from black pepper by using the Soxhlet method, and the Babchi oil was found by the Distillation method of Babchi seeds. % Yield was found to be maximum in the Soxhlet method, i.e., 88.66%, whereas with reflux method, it is 85%. Yellow-colored rod-shaped crystals were found & a Blue florescent of Babchi was seen in UV light. Melting point determination of Piperine & babchi oil was performed twice by melting point apparatus, and the average was found to be 1280C& 1200C, which was compared with the standard value 1300C&1050C-1270C.

Piperine appears violet-colored under UV Radiation in Thin layer Chromatography. Blue florescent of Babchi was found in UV light. The Rf value of test babchi oil was seen at 0.25, similar to the standard value. The Rf value of the test (extracted Piperine) is found to be 0.27, which corresponds to the quality value of Piperine (0.25), and just one spot was obtained, indicating that it is pure.

From UV Spectra analysis, maximum absorption was found to be 344nm, almost the same as the standard. Hager's test did test for alkaloids, Dragendroff's test, Wagner's Hager's test Mayer's test, and it has given positive reaction for all the reagents make sure the presence of alkaloids. The same test was also performed with Psoralea Corylifolia; we measured some traces of alkaloids substances. Solubility of babchi oil was found to be in order-

Ethanol=Acetone>Methanol>Chloroform but insoluble in water. Solubility of Piperine was found to be in the order-chloroform>ethanol>Acetone but insoluble in water. Melting point and Rf values of the test(cream) and standard (Piperine, Babchi oil) were comparable, which indicates there is no change in the physical and chemical nature of the Piperine & Babchi oil.

This also measures the drug is compatible with other excipients like beeswax, Lanolin, etc. The particle size of Babchi oil was found to be 20.33±2. 12µm. The particle size of Piperine was found to be 21.5 µ. According to an expectation of the topical

formulation, the drug should penetrate the stratum corneum and get into the dermis but should not get into the bloodstream. The % of Babchi oil for cream retains in the skin, the site of action was found to be 58.06%. The Piperine cream retained in the skin, the location of the action was found 68.76% drug released into the buffer was found to be 17.78%.

The final preparation was found to be smooth texture and consistency and free from courageous nature with light yellow-brown color and Aromatic & peppermint odor. Accelerated stability studies were done, which shows no significant change within the concentration of the drug, which offers the stability of the formulation. Moisture absorption studies showed no significant absorption of moisture. The pH of the cream was found to be 6.8. In addition, an irritancy test was also performed on rabbits and found no redness, Swelling, Inflammation, and irritation.

CONCLUSION

The formulation of Piperine & Psoralea Corylifolia cream intended for Vitiligo was successfully done and evaluated. Drug targeting at the skin where the dark pigmentation dispersion is intended was achieved 69.06%. The topical formulation (In vitiligo cream) was no change throughout the shelf life. Other novel drug delivery formulations are highly recommended to increase the percentage of drug targeting.

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