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Formulation and Evaluation of a Psoralen-Containing Herbal Cream for Vitiligo Disease

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ABSTRACT
The objective is to formulate and evaluate herbal cream using psoralen, neem extract, and tulsi extract for vitiligo disease.The cream was prepared by using cream base i.e., liquid paraffin, beeswax, sodium benzoate, borax, distilled water, psoralen, neem, and tulsi extract. The cream was prepared by the extemporaneous method to provide a smooth texture and for mixing all ingredients properly. Psoralen was extracted from bakuchi seeds by Distillation method, by using 50% Ethyl alcohol followed by filtration, distillation, to obtain yellow crystals of psoralen. Neem extract was prepared using propylene glycol and ethanol as solvents as neem has anti-bacterial properties. Tulsi extract was also prepared using DMSO as solvent, as Tulsi leaves promote melanin pigmentation in skin. Different batches of cream were formulated. All these batches were evaluated for different parameters like physical evaluation, PH, irritancy, viscosity, phase separation, and Spreadablity.
RESULT: Among all Formulations F3H (third batch) shows a good appearance, almost neutral PH, no erythema, edema, and rashes on the skin during the irritancy study, is easily washable, has good spreadablity, and no separation. This batch was stable at room temperature and can be safely used on the skin.
CONCLUSION: The Formulation can be safely used over skin Hence study suggests that composition of extract and formulation F3H is more stable. The formulation of psoralen-containing cream intended for vitiligo was successfully done and evaluated.
KEYWORDS: Psoralen, Neem, Tulsi, Vitiligo disease, Herbal cream.

I. INTRODUCTION

[1] The skin gradually becomes less pigmented when vitiligo is present. Without predilection for a particular skin tone or gender, it affects roughly 0.5% of the world's population. The condition often develops before the age of 20, however vitiligo can also develop later in life. Patients experience social isolation as a result of depigmented lesions because they are cosmetically unattractive.

[2] Patients with vitiligo have endured the same mental mistreatment as lepers since ancient times. Indeed, the term "White leprosy" or "Sweta Kustha" was used to describe vitiligo. Because of the stark contrast, vitiligo disfigures persons of all ethnicities, especially dark-skinned people more so than others.

[3] Melanocytes, the skin's pigment-producing cells, are destroyed in vitiligo, a pigmentation condition. As a result, the skin on various body areas develops white patches. The mucous membranes (tissues that line the inside of the mouth and nose) and the retina both exhibit comparable patches (inner layer of the eyeball). The hair that develops on Vitiligo-affected areas occasionally turns white. Although though vitiligo is not lethal, it is a chronic and advancing condition. The disease's social and psychological effects are likely its most significant side effect since patients may feel traumatised by their altered appearance. There are numerous therapies for vitiligo;however some of them have undesirable side effects, such as Cushing's syndrome, skin cancer, GI problems, etc. By minimising the effects of UV radiation and preventing adverse effects, piperine is utilised as an anti-vitiligo drug.

[4] Patients with the condition have a lower quality of life and find it challenging to function in public settings, particularly if the lesions are visible, particularly on the face or in the distal portions of the upper limbs. The illness has a serious impact on sufferers' wellbeing.

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[5] Depigmented or hypopigmented macules or patches that can range in size from a few millimetres to several centimetres and are surrounded by healthy skin are the most typical appearance of vitiligo. Vitiligo lesions that have fully lost their pigment have well defined borders and can take on a variety of shapes, including oval, circular, linear, and irregular. The hair may turn white if areas with hair are involved.

[6] Mucosal: Mouth and/or vaginal mucous membranes may be affected by mucosal vitiligo.

[7] A multifactorial condition called vitiligo is characterised by the disappearance of functioning melanocytes. There are numerous theories as to how melanocytes are destroyed in vitiligo. Among them include hereditary factors, autoimmune reactions, oxidative stress, the production of inflammatory mediators, and processes for melanocyte separation. It appears that both the innate and adaptive immune systems are at work. Although there is no agreement on the autoimmune nature of vitiligo, none of these proposed theories are sufficient in and of themselves to explain the various vitiligo phenotypes. The overall contribution of each of these processes is also still up for debate. Several mechanisms, including immunological attack or cell ageing and detachment, could be at play in the gradual loss of melanocytes. According to the "convergence hypothesis" or "integrated theory," several mechanisms might cooperate.

[8] According to studies on the vitiligo pathogenesis, melanocyte loss may start as a result of oxidative stress. In fact, it has been discovered that melanocytes from vitiligo sufferers are more sensitive to oxidative stress than those from unaffected people and are more challenging to culture ex vivo than those from healthy controls.

[9] The use of a Wood's lamp, a portable UV irradiation equipment that generates UVA, may aid in the diagnosis of vitiligo. Particularly in cases of light skin, it aids in the identification of focal melanocyte loss and the detection of areas of depigmentation that may not be visible to the human eye. The vitiligo lesions glow brightly blue-white under the Wood's light and have distinct borders. 13 One of the most challenging dermatological problems is still treating vitiligo.The first and most important step in managing vitiligo is realizing that it is more than just a cosmetic disease and that there are safe and effective treatments available. These therapies, which also include surgery, topical and systemic immunosuppressants, and phototherapy, may all work together to slow the progression of the condition and stabilise it.

BAKUCHI SEEDS

[10] Indian and Chinese traditional medicine both use it. Since time immemorial, Indian Traditional Medicine, or Ayurveda, has used this plant and its many compounds to treat a wide range of diseases. Plants have always been a great resource for treating a variety of human illnesses whenever there is a need for new treatments. Together with psoralen, bakuchi seeds contain a range of coumarins. Together with psoralen, bakuchi seeds contain a range of coumarins. The important plant bakuchi is used for its therapeutic effects. The kidney-shaped Bakuchi seeds have an offensive odour and a harsh flavour. Nonetheless, due to this plant's therapeutic qualities, every component is beneficial.

[11] Use: Skin conditions. It works wonders for shvitra (Vitiligo).

[12] Bakuchi exhibits observable changes in skin tone and colour. By assisting in the reduction of the white area, the darker skin area gradually covers all white skin patches.

[13,14] Skin conditions can be treated using bakuchi seed oil. From a very long time ago, bakuchi seed extracts have been used to treat skin conditions such dermatitis, eczema, boils, skin eruptions, vitiligo, scabies, leukoderma, and ringworm. Bakuchi has been shown to have anti-inflammatory, anti-oxidant, and antibacterial qualities, which serve to maintain and normalise individual skin pigmentation as well as minimise and control all skin-related problems.

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II. INGREDIENTS

SR.NO	INGREDIENTS	QUANTITY	CATEGORY/ ROLES
1	Psoralen	1.42 ml	Melanocyte proliferation
2	Tulsi extract (Eugenol)	1 ml	Anti- bacterial
3	Neem Extract (Nimbidine)	0.28 ml	Promote wound healing, relieves skin dryness, itching and redness
4	Beeswax	4.97 g	Emulsifying agent
5	Liquid paraffin	21.78 ml	Lubricating agent
6	Borax	0.056 g	Alkaline agent
7	Sodium Benzoate	0.02 g	Preservative
8	Rose oil	q. s	Fragrance
9	Distilled Water	q. s	Vehicle

III. FORMULATIONOF CREAM

INGREDIENTS	F1	F2	F3
psoralen	3 ml	1.42 ml	1.42 ml
Tulsi extract	2.5 ml	1.42 ml	1ml
Neem extract	1.5 ml	0.28 ml	0.28 ml
Beeswax	6.78 g	4.97 g	4.97 g
Liquid paraffin	16 ml	21.78 ml	21.78 ml
Borax	0.2 g	0.056 g	0.056 g
Distilled water	q. s	q. s	q. s
Rose oil	q. s	q. s	q. s
Sodium Benzoate	0.02 g	0.02 g	0.02 g

EXTRACTION PROCEDURE OF PSORALEN FROM BAKUCHI SEEDS

[15] Take Crushed seeds of bakuchi and soke with 50% ethyl alcohol followed by filtration and then perform distillation to remove alcohol. Slurry sediment is obtained which is dissolved in methyl alcohol then keep it overnight, the obtained solution is then filtered and yellow crystals are obtained which are subjected to recrystallization to obtain white needle like crystals of psoralen. Recrystallizationis performed for purification by dissolving yellow crystals in ethanol in a beaker and keep that beaker in ice bath and filtered the obtain mixture to obtain white crystals of psoralen.

PROCEDURE FOR NEEM EXTRACTION

[16] Take fresh leaves ofneem and wash with cold water and then again rinse with distilled water and blend the leaves in mixer without water and put the obtained crushed leaves of neem in volumetric flask add distilled water and propylene glycol and ethanol in same flask and mix it properly. Add the obtained mixture in a air tight container and stir the contains of container once a day for 6 subsequent days then filter the obtained

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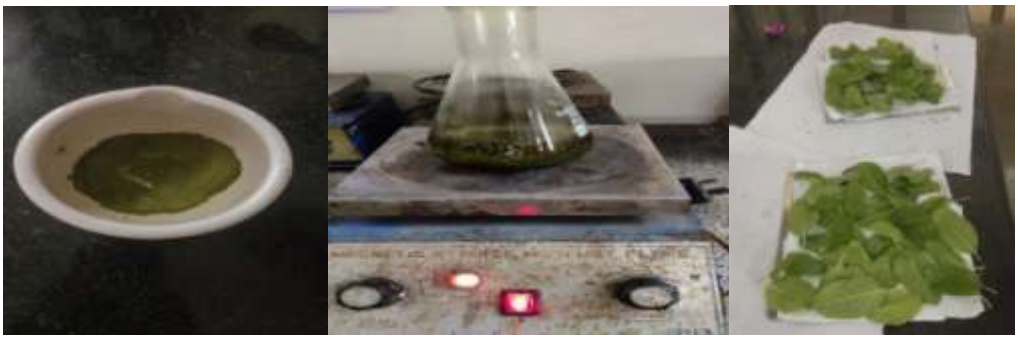
solution for further use.Check the ph. of the obtained solution if ph. 6 then solution is good.

PROCEDURE FOR EXTRACTION OF TULSI

Wash fresh tulsi leaves with distilled water and dry them in a hot air oven. Take 1gm of tulsi leaf powder to add 10 ml of DMSO (Dimethyl Sulfoxide) to it in a volumetric flask and shake the mixture for 1 hr. on a magnetic stirrer then heat that solution on water bath at 800C to 1000C and then filter the mixture for further use.



METHOD OF PREPARATION OF PSORALEN CREAM

[17] This cream is water in oil type of emulsion.

Heat liquid paraffin and beeswax in beaker at 75°C while maintaing temperature then dissolve borax, sodium benzoate and psoralen in another beaker and heat at 75°C to dissolve.Slowly add aqueous phase to heated oil phase by continuous stirring and add neem extract, tulsi extract and rose oil individually drop by drop with vigorous stirring to obtained uniform smooth texture of cream.

IV. INGREDIENTS KEY FEATURE

1.Psoralen

A naturally occurring furocoumarin, psoralen is used in the treatment of vitiligo and psoriasis. It is present in the seeds of Psoralea corylifolia and other plants. Epidermal cells avidly absorb psoralen, which intercalates into DNA. Psoralen damages and kills cells by forming DNA crosslinks when exposed to ultraviolet (UV) light. Psoralens are used topically and orally in combination with ultraviolet radiation to treat vitiligo. They have photosensitizing action.

2.Tulsi extract (Eugenol)

[18] The botanical name of the Tulsi plant is Ocimum sanctum, and it is a member of the Lamiaceae, a tiny family. Tulsi leaves exhibit anti-bacterial and preventive effects against skin conditions.

[19] Eugenol, a phenolic molecule, is one of the significant bioactivecompounds found in Ocimum sanctum Linn, ranging from 40 to 70% depending on the plant.

[20] The free radical scavenging activity of eugenol, inhibition of reactive oxygen species (ROS) and reactive nitrogen species (RNS) formation, protection of DNA and protein damage, and enhancement of cellular anti-oxidant potency are its generally acknowledged functional features.

3.Neemextract (Nimbidine)

[21] A member of the Meliaceae family, neem (Azadirachta indica) has medicinal

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implications for the prevention and treatment of illnesses. As a high source of antioxidants and other beneficial active substances like azadirachtin, nimbolinin, nimbin, nimbidin, nimbidol, salannin, and quercetin, it is thought that Azadirachta indica has medicinal properties.

[22] Neem can help calm inflamed skin because of its antibacterial and anti-inflammatory qualities. Neem is advantageous for treating skin irritation since it has the benefit of cooling the skin. Neem also has a calming effect on dry or parched skin. Neem's abundance in antioxidants contributes to its ability to scavenge free radicals. In the following order: nimbolide > azadirachtin > ascorbate, nimbolide and azadirachtin both exhibited concentration-dependent antiradical scavenging activity and reductive potential. The neem component plays a beneficial role in the control of skin itching. Neem also functions as an anti-inflammatory by controlling the activity of proinflammatory enzymes including cyclooxygenase and lipoxygenase.

4. Beeswax

[23] Beeswax is a component in creams, ointments, and lotions used in the cosmetic industry. It's antimicrobial in nature.

[24] The base is made of beeswax. More than 300 different compounds, including hydrocarbons, free fatty acids, esters of fatty acids and fatty alcohol, diesters, and foreign materials, make up beeswax. Because of the distinctive properties of beeswax, which lend solidity to emulsified solutions and boost the water-holding capacity of ointments and creams, beeswax is a crucial component of creams, ointments, and lotions. Moreover, it gives the cream thickness.

V. EVALUATION

EVALUATION OF PSORALEN OBTAINED FROM BAKUCHI SEEDS

[25] Perform melting point of psoralen using Thiele tube method taking small amount of compound in capillary tube and observe the compound carefully till it melts and report the melting point and compare it against standard value to confirm the presence of psoralen. Standard melting point ofpsoralen is 158° C. For TLC apply small spot of obtained psoralen test sample on the silica gel plate on the mark 5 cm above from base and keep it in mobile phase and observe the rise in the mobile phase and remove once it reaches the 1/3rd of the plateand dry and observe the raise spot and calculate the RF value using the formula. Compare the obtained RF value against the standard value and confirm the presence of psoralen. Standard RF value of psoralen is 0.62.

EVALUATION OF EUGENOL IN TULSI EXTRACT

[26] Perform evaluation process by TLC method using Toulene: Ethyl acetate as mobile phase in the ratio of 2:1.

EVALUATION OF NIMBIDINE IN NEEM EXTRACT

[27] Take extract of neem and add conc. sulphuric acid to it and observe the change if yellow colour at the lower layer than test confirms the presence of Nimbidine in neem.

[16]EVALUATION OF CREAM

1. **PHYSICAL EVALUATION:** Cream was observed for colour, texture, odour, etc.
2. **IRRITANCY:** Mark 1cm2 area on the left-hand dorsal surface. Cream is applied to that area and note that time. After interval up to 24 hours it is checked for irritancy , erythema and edema if any reported
3. **WASH ABILITY:** Apply A small amount of cream on the hand and wash with the tap water.
4. **PH:** 0.5 g cream was taken and dispersed in 50 ml distilled water and PH was measured by digital PH meter.
5. **VISCOSITY:** Measured by Brooke field viscometer at room temperature using spindle no.63 at 2.5 RPM.
6. **PHASE SEPARATION:**cream is kept in close container away from light at 25-100°C for one month and observed for phase separation.
7. **SPREADIBILITY:** Spreadablity is carried out for all formulations. The less time taken for separation of both slides better the spreadablity.
8. **GREASINESS:** The cream is applied in the form of smear on the surface of skin and observed if smear is oily or grease like.

VI. RESULT AND DISCUSSION:

EVALUATION OF PSORALEN OBTAINED FROM BAKUCHI SEEDS

White-coloured crystals were found after recrystallization.

MP determination of psoralen was found to be 159° C, TLC was also performed and got an Rf value that is 0.61 [mobile phase -n-hexane: acetone: formic acid (2:1:0.025 v/v)]

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EVALUATION OF EUGENOL IN TULSI EXTRACT

The performed TLC showed the RF value of 0.54 which confirms the presence of eugenol in tulsi extract.

EVALUATION OF NIMBIDINE IN NEEM EXTRACT

The test performed using extract of neem added with conc. sulphuric acid showed yellow colour at lower layer which confirms the presence of nimbidin in the extract.

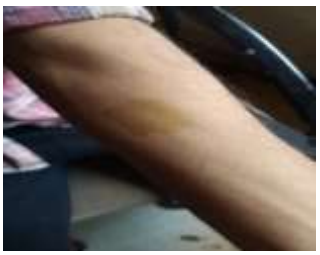
EVALUATION OF CREAM

PHYSICAL EVALUATION:

SR.NO	PARAMETERS	OBSERVATION
1	Colour	Greenish colour,
2	Odour	Pleasant
3	Texture	Smooth
4	State	Semi-solid

IRRITANCY

Mark the area ($1cm^2$) on the left-hand dorsal surface. After that, the cream was administered there, and the duration was recorded. Then it is checked for irritancy, erythema, and edema if any for an interval up to 24 h and reported. According to the results, the formulation is showing no sign of irritancy, erythema, and edema.



WASHABILITY

Applying a small quantity of cream to the hand and then washing it with tap water served as the wash ability test.

VISCOSITY

The viscosity of cream was done by using a Brooke field viscometer at a temperature of 25 ℃ using spindle No. 63 at 2.5 RPM. According to the results, showed adequate viscosity that is 26080 cps Viscosity.

Phase separation

The prepared cream was kept in a closed container at a temperature of 25-100 ℃ away from light. The phase separation was then monitored for24 hour for 30 days. Any change in the phase separation was observed/checked. According to the results, no phase separation was observed in the formulation.

SPREADIBILITY

The spreadablity of the formulation was carried out and the time taken by the 2 slides to separate is less so as said in the description of the evaluation test lesser the time taken for separation of the two slides better the spreadablity so according to this state better spreadablity.

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GREASINESS

Here the cream was applied on the skin surface in the form of a smear and checked if the smear was oily or grease-like. According to the results, we can say that all three formulations were non-greasy.

pH

According to the results, the pHformulation was found to be 7.14 so it can be safely used on the skin.

VII. CONCLUSION

The formulation was stable at room temperature. By using psoralen, neem extract, and tulsi extract all show a good effect on the skin. Stability parameters like visual appearance, viscosity, and PH of formulation showed that there was no significant variation during the study period. The formulation of psoralen-containing cream intended for vitiligo disease was successfully done and evaluated.

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