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Development and Evaluation of Polyherbal Cream for Treatment of Vitiligo Patches

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Abstract:- Vitiligo is an autoimmune disorder or inflammatory condition associated with itchy, scaly skin & disfiguring skin lesion. Numerous studies on natural products with anti-psoriatic action have been conducted as a result of the lack of treatment options and accompanying drawbacks in allopathic therapy. Nigella sativa (Black cumin), Ocimum sanctum (Tulsileaves), GlycyrrhizaGlabra (Liquorice), Curcuma longa (Turmericrhizomes) was found to be efficacious and cheap anti-inflammatory and immuno modulator drugs with least in comparison to synthetic medications used to cure vitiligo, negative effects. These medications were chosen for the current investigation since the objective was to produce a herbal cream for use in vitiligo skin repigmentation therapy. A polyherbal cream design and development attempt was produced taking into account the aforementioned justification. Different crude drugs extracts were taken &polyherbal cream was prepared. Accelerated stability testing of three sample has been conducted in the environmental chamber with temperature 25 ± 10^0C and humidity 60 ± 10% RH. The "F1" formulation were found to be stable with no sign of phase separation and no change in the colour. The sensitivity test patch has also been used, and there has been no indication of skin irritability or allergy symptoms.

Keywords:- *Vitiligo, Polyherbal Cream, Synthetic chemical, Cosmeceutical, Repigmentation*

I. INTRODUCTION

Vitiligo is a relatively common autoimmune depigmenting disorder of the skin is marked by the selective death of melanocytes, which result in the pigment dilution in the skin's affected areas infrequently the eyes and the hairs may also lose colour [1]. Although vitiligo patches can form anywhere on the body, they generally occur around orifices, genitalia, or sun-exposed areas like that of the hands and face. A completely amelanotic, nonscaly, chalky-white macule with clear lines is the hallmark lesion. Our understanding of the etiology of vitiligo has advanced significantly in recent years, and it is now officially recognized as an autoimmune disease which is affected by genetic and environmental variables in addition to problems in metabolism, oxidative stress, and cell detachment. Vitiligo should not be ignored as a cosmetic or minor disease, as its effects can be psychologically distressing, and frequently have a significant negative impact on daily life Several inflammatory mediators take involvement in melanogenesis regulation in melanocytes: Interleukin 1 (IL-1), interleukin 4, interleukin 6, interleukin 17, and tumor necrosis factor can suppress melanogenesis, whereas interleukin 18 (IL-18), interleukin 33, granulocyte macrophage colony stimulating factor, interferon, and prostaglandin E2 have the stimulating effects [2].

II. TYPES OF VITILIGO

A. Segmental Vitiligo:

Segmental vitiligo (SV) form exhibits a quick stabilization, a typical unilateral distribution, and a less connection with autoimmune disorders [3]. Localized skin conditions, particularly linear morphea, have frequently been seen in segmental vitiligo patients. Segmental vitiligo has several unique characteristics compared to non-segmental vitiligo. It often only affects one section of the body and has a clear boundary along the body's midline. Various sign and symptoms of segmental vitiligo like pale vitiligo areas or patches that gradually turn white, segmental vitiligo patients also experience hair follicle damage that results in leukotrichia, as a result, those who have segmental vitiligo could grow white hair, brows, eyelashes, beards, etc. and vitiligo patches with asymmetrical shapes.

- **Non-Segmental Vitiligo:** Non-segmental vitiligo (NSV) is distinguished by its symmetrical distribution, irregular course, and connection to autoimmune disorders. Although the pathophysiology of nonsegmental vitiligo is still unknown, autoimmune stress and oxidative stress are presently thought to collaborate to cause melanocytes to undergo apoptosis, or cell death. Nonsegmental vitiligo can occur due to both cellular and humoral immunity. Overall, it was found that the root of the nose was the Centre of the upper face patches (forehead, periorbital, and malar). Around fissures such the palpebral fissure, nares, oral fissure, and external ear, many patches often developed [4].
- **Mixed Vitiligo:** Segmental vitiligo and non-segmental vitiligo coexist to form mixed vitiligo (MV). In mixed vitiligo (MV) after a recognizable segmental involvement, bilateral vitiligo patches usually develop in a subsequent phase.The risk factors for the development of segmental vitiligo from SV to MV at the time of first diagnosis include leukotrichia and halo nevi.

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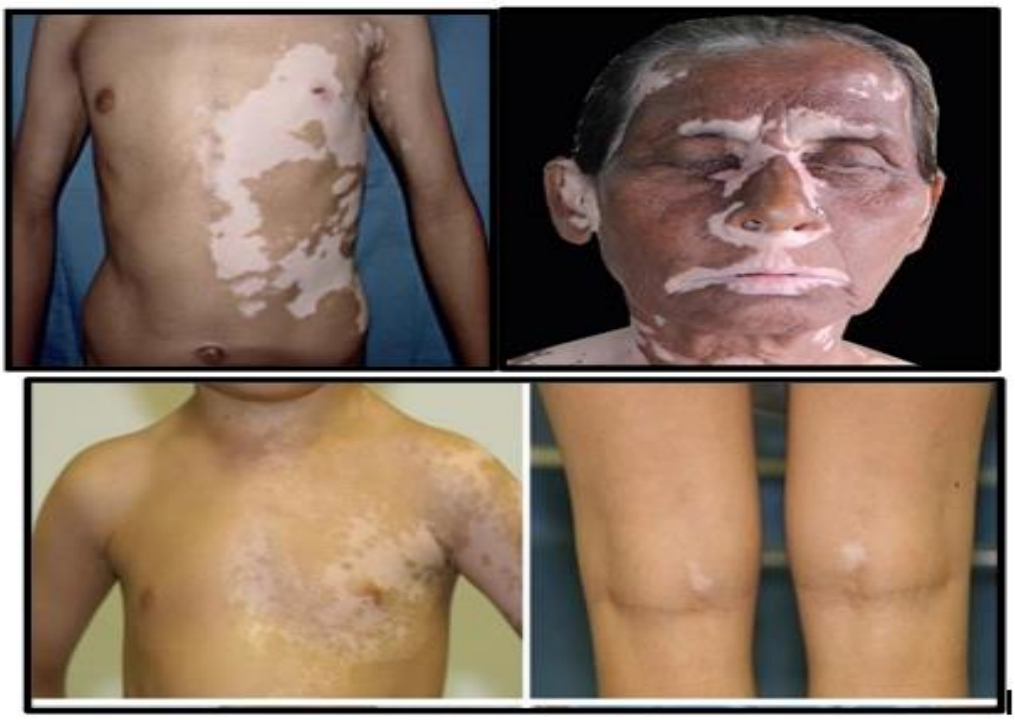

Fig. 1: Various types of Vitiligo

III. HISTORY

The first records of vitiligo and its treatment date back 3,500 years. These historical documents outline the symptoms of vitiligo, underline the shame that society placed on the condition and list the therapies which were available about 1550 BC., the Ebers Papyrus described two types of depigmentation that might have been leprosy or vitiligo-like depigmentation [5]. By 1400 BC, white leprosy lesions were referred to in the Atharva Veda as Sveta khushtha,while depigmentation of the Amarakosa was mentioned in Japanese Shinto rituals in 1200 BC. The Ashtanagahridaya provided an explanation of depigmentation prognostic variables around 600 BC. Kilăsa was first mentioned in writing around 2200 B.C., during the Aushooryan period. this is a social issue that is still widespread in some, but not all, parts of the world According to studies from India, the prevalence of vitiligo among dermatological outpatients ranges between 0.25 and 4 %, and in Gujarat and Rajasthan, it can reach 8.8 % Vitiligo is also very prevalence in Mexico and Japan. It has been discovered that these individuals had a significant prevalence of vitiligo, with cases ranging from 7.7% to more than 50%. A favorable family history also results in an earlier mean age of onset [6].

IV. TREATMENT OF VITILIGO

One of the most challenging skin problems is still treating vitiligo. The first step in treating vitiligo is realizing that it's more than simply a cosmetic issue and that there are safe and efficient treatments available. These treatments which combine surgery procedures, topical and systemic immunosuppressant, and phototherapy, may aid to halt the disease, stabilize lesions that have lost their pigment, and encourage repigmentationthe subtype of the disease, its extent, distribution, and activity, alongside the patient's age, skin type, impact on quality of life, and desire for therapy, all influence the choice of treatment. The face, neck, trunk, and mid-extremities react to treatment the best, whereas the lips and distal appendages are more resistant. Repigmentation first develops around the edges of the lesions or in a perifollicular pattern. To assess a treatment's effectiveness, it must be administered for at least two to three months. The most popular treatment for vitiligo, UV light-based therapy, is connected with a better result when paired with another therapy [7]. Vitiligo patient management necessitates ample time for a thorough initial evaluation. When examining individuals with vitiligo, a complete history and skin examination are necessary to assess the severity of the disorder and identify any specific prognostic factors. The clinical examination items that may be helpful for evaluation are included in an assessment form developed by the Vitiligo European Task Force. It also includes information on personal and family history. Patients should routinely be questioned on their family history of thyroid disease, other autoimmune diseases, premature hair greying, vitiligo and other autoimmune diseases [8].

Both the Old treatment and Buddhist literature have provided descriptions of the emergence of White patches on the skin. Several myths surround vitiligo, particularly in nations where leprosy and other infectious disorders are linked to the whiteness of the skin. The social stigmata that follow cause vitiligo patients to endure a great deal of psychological distress; in fact, certain nations still carry these stigmas to this day. Recently, new impact assessments for vitiligo have been created, like The Vitiligo Affect Patient Scale may provide a more realistic representation of the hardship associated with vitiligo. The significant prevalence of depressed symptoms in vitiligo makes the psychological impact of the condition obvious. According to

a recent meta-analysis, depression has a pooled odds ratio of 5.05 when compared to controls. Without necessarily meeting all criteria for clinical depression, more than one-third of vitiligo patients reported having had some sort of depressed symptom. It is significant that numerous autoimmune conditions, including thyroid disorders, alopecia areata (hair loss), dermatitis, diabetes mellitus, and rheumatoid arthritis, have been related to non-segmental vitiligo. [9].

V. AYURVEDIC TREATMENT METHOD FOR VITILIGO SKIN PATCHES

Switra and Kilasa have identical Ayurveda descriptions. *Vitiligo is the term for switra in biomedicine*. The skin functions as a vital sensory organ in Ayurveda. Fundamental energy concepts like "mobile natured energy" (vata) and one of the five metabolic-like heat-producing activities (bhrajaka pitta) reside in the skin. The skin, or bhrajaka pitta, should be kept in good condition and require ongoing care because it covers the entire body. As a result, Ayurveda possibly offers a variety of therapies for dermatoses and skin care. Some authors consider these two as subtypes. Kilasa is a type of the disease that mainly affects the skin, according to Sushruta. Madhava and Vagbhata agree with him that Kilasa is steady (nirdista) because it remains at skin level (called the twakgata condition in Ayurveda). According to Charaka, Switrasolidifies when the fundamental energy principles (dosha) permeate the various layers of the fundamental body tissues (dhatu), as shown by a change in the tone of the lesions.One of the successful treatments that has helped treat a vitiligo lesion is the use of herbal drugs [10] Various ayurvedic drugs used in the treatment of vitiligo disease like Turmeric, Bakuchi (P. corylifolia), Licorice, Nigella sativa, Holy basil etc. Leaf of holy basil used in the treatment of vitiligo disease. Also, Bakuchi is the most commonly used ayurvedic medication for reducing the vitiligo. Hence, Ayurveda play an important role in the cure or treatment of vitiligo.

In this context, numerous investigations on various herbal treatments have been carried out globally to both regulate and treat vitiligo. Nigella Sativa is one of herbal plant to treat the vitiligo disease. Many natural bioactive substances, such as alkaloids, saponins, alpha-hederin, and thymoquinone, are present in Nigella sativa [11] Thymoquinone is a major component of the seeds of Nigella Sativa plant i.e. that strengthens the immune system and has anti-inflammatory, antibacterial, antioxidant, and anticancer properties [10] Nigela Sativa seeds used topically for the treatment of vitiligo.

One of the natural medications that is used to cure vitiligo is turmeric. Turmeric is frequently used in dermatology. The anti-inflammatory, antioxidant, and anti-cell proliferation properties of turmeric have been the subject of extensive research, and it is an effective and affordable treatment for many ailments [12]. The primary active ingredients of the rhizome are the nonvolatile curcuminoids and the volatile oils. The best home remedies for the cure of vitiligo disease is the paste of turmeric powder and castor oil.

VI. NEED OF RESEARCH

As compared to the chemical based antivitiligo cream, which may cause skin irritation and other skin related allergic condition so, natural herbal cream is preferred. From the traditional way there are many herbal products, which are found beneficial and have good results. The natural drug or home remedies are to be apply on pigmented skin may effective in repigmentation, from the literature review it revealed that not a single successful medication therapy which can give total repigmentation on skin patches and the phototherapy have number of disadvantages. So, the development of polyherbal cream is important.

VII. PLANT PROFILE

A. Nigella Sativa:

Fig. 2: Nigella Sativa flower, leaves, seeds

- **Synonyms:** Black cumin, Nigella, Kalonji, charnushka
- **Biological source:**Dried nigella sativa seeds
- **Family:** Ranunculaceae
- **Chemical composition:** Thymoquinone
- **Uses:** Acne, Vulgaris, Burn, Wounds, Injury Treatment

B. Turmeric

Fig. 3: Dried Rhizome of Curcuma longa

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- **Biological source:**Dried Rhizome of Curcuma longa
- **Family:** Zingiberaceae
- **Chemical composition:** curcumin, curcuminoids, demethoxycurcumin, bisdemethoxycurcumin
- **Uses:** Antiseptic, Antibacterial

C. Liquorice

Fig. 4: Dried Rhizome of Glycyrrhiza Glabra

- **Synonyms:** Jesthamadh, Black sugar, Glycyrrhizaglabra
- **Biological source:** Dried Rhizome of Glycyrrhiza Glabra
- **Family:** Leguminoceae
- **Chemical Composition:** Glycyrrhizin, Glycyrrhetic acid, Isoliquiritin, Isoflavones,
- **Uses:** Antimicrobial, Antifungal, Wound cleaning

D. Ocimum Sanctum

Fig. 5: Leaves of Ocimum Sanctum

- **Synonyms:** Holy basil, Tulsi, Tamole, damole
- **Biological source:**Leaves of ocimum sanctum
- **Family:**Lamiaceae
- **Chemical composition:**Oleanolic acid, Ursolic acid, Rosmarinic acid, Eugenol, Carvacrol, Linalool, and β-caryophyllene
- **Uses:**Anti-aging, Anti-inflammatory, Antibacterial, Astringent

VIII. MATERIAL AND METHODS

A. Collection of plant material

The herbal ingredient like Black cumin (Nigella sativa) are purchased from Abbumiya Ayurvedic Shop (Shop no. 10 Pritam apartment Kashmir galli, indorachowk, Nagpur), the drug like holy basil (Osimumsantum) is collected from Nagpur (Hingana) and the other drug like Turmeric (curcuma longa) and Liquorice (Glycyrrhizaglabra) drugs and different solvents and chemicals have been obtained from a college lab.

B. Extraction Procedure: ***[13]***

➢ *Hydroalcoholic Extraction (Maceration)*

By using the maceration procedure of crude drug Holy basil, liquorice, and turmuric may all be extracted using the appropriate solvent water in methanol as 7:3 ratio. For these steps, we haveto weight accurately 25 gm of dried crushed drug into glass stoppered conical flask with a label. The conical flask was filled with the appropriate amount of solvent; the solvent's volume should be three times greater than the space occupied by the drug. Also, to prevent microbial growth add 1 to 2 ml of chloroform in the solvent while mixing the drug. Place this conical flask in a cool and dark place away from sunlight for 3 to 4 days, shaking at regular intervals time to prevent saturation.

Fig. 6: The prepared conical flask liquorice, holy basil, turmeric with label

After the 3 to 4 days shift to the filtration process and transferred in tared flat-bottomed dish (china dish). This dish should be labelled with the filtrate name then this lebelledchina dish introduced on steam water bath for evaporation at constant temperature 110°C. This process is continued until solid residue is left in the dish. After complete evaporation place china dish immediately in the dessicator and leave for 30 minutes in order to cool and keep the moisture content low. After the sample has cooled, weigh it accurately.

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Fig. 7: Filtration process of Curcuma Longa, Holy basil, Liquorice

Fig. 8: Evaporation of solvent from extract of sample on steam water bath at 110°c

Methanolic Extract by using Soxhlet Extraction Process

Soxhlet extraction method used after detailed literature review, we observed that the percentage extractive yields of *curcuma longa* and *nigella sativa* are high in methanol solvent for extraction; Place around 50 gm of the drug in the mortal and pestal for size reduction. Drug particles should size range from 0.5 to 0.8 mm. The reduced particle can be sieved to eliminate tiny particles that could block the siphon tube during extraction [14].

The methanolic extract is done by using Soxhlet extractor. Powdered dried crude drug (50 gm.) are extracted in 250 ml of solvent. The solvent is heated at temperature 50^0 C to reflux. The liquid enters the chamber holding the thimble of solid after ascending a distillation arm. Any solvent vapour is made to cool and drip back down into the chamber containing the solid substance by the condenser. Warm solvent progressively fills the compartment containing the solid substance. In the heated solvent, some of the desired chemical dissolves. The Soxhlet chamber is drained by the syphon when it is almost full. The distillation flask receives the solvent back. The thimble makes sure that no solids are transported to the still pot by the solvent's rapid velocity. You might let this cycle continue for many hours or days. During each cycle, a portion of a non-volatile component dissolves into the solvent.After numerous cycles, the desired component gets concentrated in a distillation flask.One advantage of this technique is that heated solvent is recycled in a single batch, as opposed to numerous portions being passed through the sample. Once extraction is complete, the solvent is removed and placed in a flat, round-bottom dish (a “china dish”) where it is placed for evaporation, usually using a steam bath. This produces the solid extracted residue. The dry solid residue was immediately placed in a dessicator to provide cooling and prevent moisture content. Then precisely weigh it [15].

Fig. 9: A) Soxhlet extraction assembly for extraction curcumin from Turmeric and B) Soxhlet extraction assembly for Nigella sativa

*C. Preliminary Phytochemical Screening of Drug Extract **[16]***

➢ *Test for alkaloids*

- **Mayer's test**- After adding 1% hydrochloric acid HCl and transferring 5 mg of extract into the test tube, the resulting solution was gently warmed. Because potassium mercuric iodine is present in Mayer's reagent, the presence of red indicates the presence of alkaloids.
- **Wagner's test**- In this experiment, a test tube containing 5 mg of the extract was filled with 0.5 mg of the Wagner reagent before being thoroughly shaken. The presence of alkaloids is indicated by the coloration, which is reddish brown. Shade of reddish brown Iodine causes the formation of an insoluble compound that is brownish-reddish in colour.
- **Dragendorff test**-I took a tube of a 5 mg extract. And after that, the test tube received one drop of the dragendroff reagent.Alkaloids can be seen from the orange-red coloration. Because of the chemicals used to make the Dragendroff reagent—bismuth nitrate, nitric acid, iodine, and water—it produces an orange-red colour when alkaloids are present. [74].

➢ *Test for flavonoids:*

- **Shinoda test**: In this test5mg of the extract were added to the test tube initially, then small amount of magnesium along with a few drops of concentrated hydrochloric acid. The pink tint associated with the flavonoids should be indicated. Flavones, flavonoids, and flavanones were each identified by a different colour, ranging from orange to red, red to crimson, and magenta to crimson. Catechins produce a reddish-pink coloration when combined with a vanillin solution in hydrochloric acid.
- **Lead acetate test for flavonoids**- After adding 1 ml of lead ethanoate solution, 5 mg aqueous extract was put to the test tube. If alkaloids are present, the solution turns buff in colour.
- **Sodium hydroxide test for flavonoids**- After using 5 mg of the extract and 1 ml of diluted hydrochloric acid, 10% sodium hydroxide was added to the mixture to give it a yellow colour. When two milliliters of diluted hydrochloric acid are added, the colour should shift from yellow to colorless in the presence of alkaloids.
- **Alkaline reagent test for flavonoids**- 5 mg of extract was added to the test tube, stirred, and 2 ml of a 2% solution of sodium hydroxide was added if yellow coloration appeared. This colour disappeared after a few drops of diluted acetic acid were added. It implies that the holy basil contains alkaloids.
- **Ferric chloride test**- A ferric chloride test was run to determine whether flavonoids were present in the aqueous extract. First, 0.5 ml of a diluted ammonia solution was added to the mixture of 5 mg extract and 1 ml of distilled water. A few drops of strong sulfuric acid were combined after the addition of weak ammonia. Flavonoids aid in the yellowish formation.

➢ *Test for glycoside*

- **Liebermann's test-**In this test, which used Liebermann's method to determine whether glycosides were contained in an aqueous extract, 5 mg of extract was appropriately combined with 2ml of chloroform before being added to with 2ml of acetic acid. After that, the solution was chilled in ice. After cooling, one milliliter of sulfuric acid was added. The extract's alkaloids will turn its colour from violet to green.
- **Salkowski's test**- The glycoside was analyzed using 1 ml of extract and 2 ml of chloroform. After that, 2 ml of concentrated sulfuric acid was added, and it was gently shaken. A reddish-brown colour indicated the presence of glycoside.
- **Keller-kilani test for cardiac glycosides-** To verify the extract's glycoside content, 5 mg of the extract and 1 ml of glacial acetic acid was added to test tubes. A few drops of 2% ferric chloride solution were added to it. The mixture was then added 1 ml pure sulfuric acid. The presence of cardiac glycosides will result in the formation of a brown colour ring at the edge.

➢ *Test for tannins*

- **Ferric chloride test-** 5 mg of aqueous extract were mixed with 0.5 ml of ferric chloride solution. Blackish precipitate forms when tannin is present.
- **Gelatine test**- A gelatine test was performed to see if tannin was present in the extract. In this experiment, gelatine was mixed with 5 mg of extract and 1 ml of water. There should be white precipitate.
- **Lead acetate**- Using a lead acetate test, 5 mg of test samples were collected and placed in test tubes to evaluate the presence of tannin. When a few drops of basic lead acetate are added to the sample solution, tannin will be present if a brown, thick precipitate appears.

➢ *Test for saponins*

- For the purpose of identifying saponin in the aqueous extract, a foam test was carried out after dissolving 1ml of the extract in 5ml of distilled water. It was shaken for good mixing after the addition of the distilled water until foam was visible. Two drops of olive oil and a small amount of foam were added, and it was vigorously mixed. The saponins should be used to create the emulsion.

➢ *Test for oil*

- **Stain test:**The presence of oil on the filter paper will show that there is oil in the small amount of aqueous extract that was placed over the paper.
- **Saponification test**- The extract was added to the test tube along with a few drops of alcoholic potassium hydroxide, and everything was thoroughly mixed. The mix solution contained 1–4 drops of phenolphthalein. It was heated for one hour in a water bath. Alkali formation with partial neutralization is an indication of the presence of fats and oils.

➢ *Test for carbohydrates*

- **Benedict's test**-The decision was made to examine the carbohydrates using Benedict's reagent. After mixing the 5 mg extract with a few drops of Benedict's reagent and letting it boil, the reddish-brown precipitate is seen along with the absence of carbohydrates.
- **Molisch's test**- Initially, 1 ml of Molisch's reagent was applied to a test tube containing 5 mg of extract. The mixture was thoroughly shaken. Then, 2 ml of concentrated sulfuric acid was gently poured along the test tube's side. The presence of carbohydrates was revealed by the appearance of a violet ring at the contact.
- **Test for steroids**: The combination of 1 ml chloroform and 5 mg extract was added with a few drops of strong sulfuric and acetic acid. The greenish colour indicates the existence of steroids.
- **Salkowski's test**-To the 5 mg extract, 3 drops of pure sulfuric acid were applied. Red coloration suggests the presence of steroids when it forms.

➢ *Test for proteins*

- **Biuret'stest**- A few drops of the biuret's reagent and 5 mg of extract were added. The resultant mixture was well mixed and warmed for 1 to 5 minutes. Protein content was detected by the presence of red or violet colour.
- **Million's test**- 2ml of Million's reagent was combined with 5 mg of extract. When the solution was heated up for five minutes, red pigment precipitated and turned crimson, showing that protein is present.
- **Ninhydrin test**- Aqueous extract was combined with 2 ml of a 0.2% solution of Ninhydrin and heated for 2 minutes on a water bath to check for the presence of amino acids and proteins. If violet colour emerged, the amino acids and proteins were present.

Fig. 10: Preliminary Test of Crude Drug Extract Showing In Front Of White Background.

D. Determination of Ash Value: ***[17]***

Any organic material's non-volatile inorganic components make up its ashControlled incineration of plant drugs results in an ash residue, which is composed of an inorganic mixture of metallic salts and silica. When the proportion of ash weight varies significantly across samples of a medicine, it suggests a change in the drug's quality. Certain undesirable components of medications, like the cork on liquorice and the sclereides in the undesired pericarp of colocynth, might have a character that increases the ash value, it is not necessary for the drug's powder form. The ash value can quickly identify more overt contamination, like sand or earth.

➢ *Total ash:*

Ashing involves an oxidation of the component of the product. A high ash value indicates improper preparation of the unfinished drug for marketing, such as falsification, contamination, substitution, or carelessness. The carbonates, phosphates, silicates, and silica that make up the total ash typically contain both physiologic ash, which comes from the plant tissue itself, and non-physiologic ash, which is the byproduct of the material that adheres to the surface of the plant. E.g. sand and soil. A tared silica dish that had been dried out and weighed contained 2 g of powdered medication. It was burned until the carbon was removed in a furnace. The collected ash was weighted

➢ *Acid insoluble ash:*

It is the substance left behind after burning the residual insoluble material and boiling the complete ash in diluted hydrochloric acid. This gauges the silica content, particularly in sand and siliceous earth.

25 ml of diluted hydrochloric acid was added to the crucible containing the complete ash, covered with a watch glass, and slowly heated for 5 minutes. The ash-free filter paper was used to capture the insoluble material, and hot water was used to wash it off until the filtrate was neutral. On a hot plate, it was dried and burned to a steady weight. The residue was allowed to cool in a suitable dessicator for 30 minutes, and then weighedimmediately without delay.

➢ *Water-soluble ash:*

It is the portion of the total ash that is water soluble. It is a strong indicator of either improper preparation or earlier extraction of the drug's water-soluble salts. It is expressed as a minimum value. To the crucible containing the total ash, 25 ml of water was added and boiled for 5 minutes,after gathering the insoluble material on ash-free filter paper, hot water was used to wash it, and it was set on fire in a crucible for 5 minutes. This residue's weight was deducted from the total ash value's weight.

E. Thin layer chromatography Method

➢ *TLC Analysis for Nigella Sativa*

Seed powder was dissolved in 1 ml of methanol. Benzene and glacial acetic acid were used in the mobile phase in a preliminary study. Two mobile phase solvent solutions were used in a comparison study for the analysis, (A) consisted of benzene: glacial acetic acid (1: 1) and (B) consisted of carbon tetrachloride/acetone/glacial acetic acid (15.2: 3: 1). Analyzed samples were methanolic and water extract as well as commercially available black cumin oil. Start line was drawn with pencil on the pre-coated TLC plate (Figure 17). Analyzed samples were thrown as starting points. In the chamber, the TLC plate was created with an appropriate mobile phase. A pencil was used to mark the completion line after plate development of the spots in order to determine retention factors (RF) was done under UV lamp [18].

➢ *TLC analysis of curcuma longa [**19**]*

Thin Layer Chromatography Silica gel plates with aluminum backs were used as the stationary phase, including ultraviolet light-sensitive The TLC plates were prepared by establishing a 1 cm distance from the starting point, an 8 cm solvent travel distance, and a 1 cm solvent front distance. Capillary glass tubes were used to spot roughly 10 L of the samples with a 1 cm separation between each band on the TLC plates. The plates were then produced using mobile phase elution in glass chambers. Using a UV such as Multiband UV - 254-366 nm, the compounds produced by the ingredients that could not be seen in the visible zone were visualized.

➢ *TLC Analysis for liquorice **[20]***

First and second bands were used as reference standards when the extract was placed band-wise onto asilica gel 60F254 TLC plate. Thermolabile phase was a mixture of butanol: acetic acid: water (6:1:3 v/v).Before being used, the plates undergone development, drying, and treatment with anisaldehyde sulphuric acid reagent. The plate was heated and then examined with a UV light.

➢ *TLC Analysis for Holy Basil**[21]***

The O.sanctumleafextracts were applied in spot forms, using glass capillaries, on silica gel TLC plates, with a developing distance of 1.5 cm, among four tracks. The plate was produced in a developing chamber using a solvent solution of hexane and ethyl acetate (8:2 v/v). After taking the TLC plate out of the developing chamber, it was allowed to air dry until the solvent had migrated a predefined 15 cm away from the point of origin. In visible light, the colour components found on the TLC plate get captured on camera. After that, the TLC plate was analyzed in both visible and UV light (365 nm) in an iodine chamber.

F. Method for preparation of Polyherbal Cream[22]

Add necessaryquantity of Borax in sufficient amount of water in 100 ml beaker and prepare a aqueous phase then heat it on water bath.

- In the above beaker, add required quantity of nigella sativa, turmeric, liquorice and holy basil extract correctly measured by using weighing balance and make a phase 1 which is known as aqueous phase.
- Then, weigh accurately mustard oil, liquid paraffine, almond oil and add into beeswax in separate 100 ml beaker, melt on water bath to prepare phase 2 which is known as oil phase.
- After adequate temperature aqueous phase poured into mortar pestle then mixed oil phage properly by continuous triturating while clicking sound is produced.

After setting completely, the Polyherbal Cream formulation was left aside for roughly an hour in a cool, dry area away from direct sunlight. It was then used 48 hours later after being stored at room temperature for stability and analytical testing.

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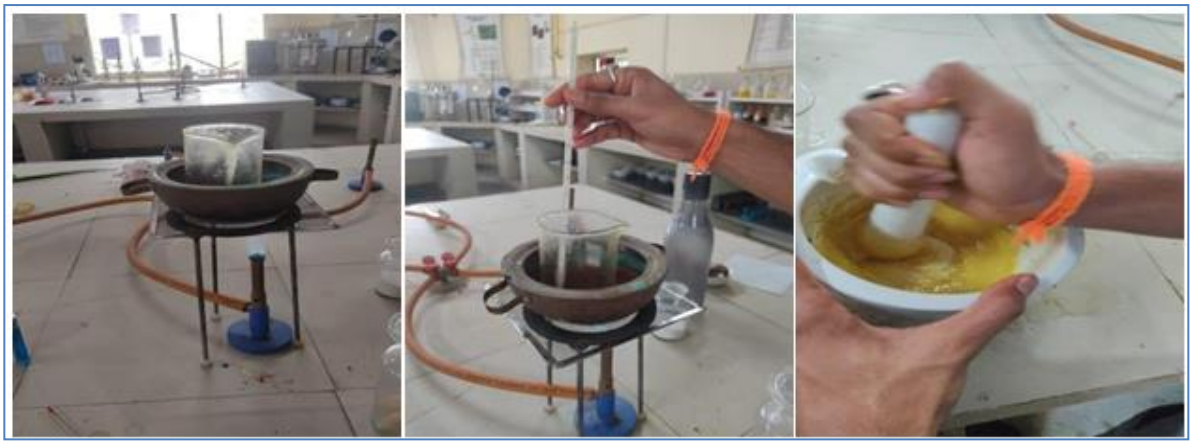

Fig. 11 (A): Process for the formulation of polyherbal cream

Fig. 11 (B): Formulation (F1, F2 & F3) of Polyherbal cream

IX. FORMULATION TABLE

Table 1: Formulation Table

Ingredient	Formulation 1	Formulation 2	Formulation 3
Nigella sativa	0.5 g	0.4 g	0.45 g
Turmeric	0.4 g	0.5 g	0.3 g
Liquorice	0.35 g	0.3 g	0.4 g
Holy Basil	0.3 g	0.5 g	0.45 g
Mustard oil	01 ml	1.5 ml	01 ml
Vitamin E	0.2 ml	0.2 ml	0.2 ml
Bees wax	4.0 g	4.5 g	3.5 g
Liquid Paraffin	3 ml	4.5 ml	4 ml
Perfume	q.s.	q.s.	q.s.
Water	q.s.	q.s.	q.s.

X. RESULT AND DISCUSSION

A. *Evaluation of Polyherbal Cream*

- **Physical evaluation:** Colour, odour, consistency, and formulation state were additional physical criteria used to assess the prepared herbal cream.
- **Colour:** A visual inspection was done to determine the cream's colour.
- **Odour:** Cream's odour was discovered to have distinctive qualities.
- **Consistency:** The formulation was tested by manually rubbing cream on the hand. The cream is of a fluid viscosity. After application, cream didn't leave oily residue on the skin's surface.
- **State:** The state of cream was visually inspected. Having a semisolid condition.
- **PH:** Using a digital pH meter, the pH of the obtained herbal cream was measured after it was mixed with 100 ml of distilled water and set aside for two hours.
- **Spredability:** The sample was sandwiched between two slides to achieve uniform thickness before being crushed to test the spreadability of the cream formulation. Spreadability was determined by how long it took to separate the two slides.

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- **Wash ability:** The simplicity of water washing was assessed after the skin had been treated with the prepared Polyherbal cream.
- **Non-irritancy Test:** The formulation of an herbal cream was assessed for the non-irritancy test. The sites were observed for 02 hours.
- **Phase Separation:** Transferring the produced cream into a suitable wide mouth container. After 24 hours of storage, the separation of the oil phase and aqueous phase could be seen.
- **Evaluation parameter:** We performed evaluation test of our prepared formulation F1, F2 and F3 O/W type cream with the standard parameter and marketed preparation. By the observation all parameter we have selected formulation number 01 (F1) and their evaluation parameters are given in table number table 02.

Table 2: Formulation table

Sr. no.	Evaluation	Result
1.	Physical evaluation	-
	Colour	Yellowish
	Odour	Sandalwood fragrance
	Consistency	Smooth
	State	Semisolid
	pH	6.5-7.0
2.	Separability	Easily spreadable
3.	Wash ability	Easily washable
4.	Non-irritancy test	Non- irritant
5.	Phase separation	No phase separation

- **Extraction:** The following are the extract values from crude drug *Nigella sativa, Curcuma longa, Ocimumsantum and Glycyrrhizaglabra* % extractive yield is shown in Table 01.

✓ *% Extractive Yield*

Table 3: %Extractive Yield

Druge Name	Total weight of crude drug	Extract weight	% Etractiveyeild
Nigella sativa	35 gm	4 gm	11.42 %
Curcuma longa	30 gm	3.65 gm	12.16 %
Ocimumsantum	30 gm	4.50 gm	15.60 %
Glycyrrhizaglabra	30 gm	3.0 gm	10.00 %

B. Preliminary Screening of Crude Drug extract:

Protein was detected by the phytochemical screening of the examined extracts of Nigella sativa, Curcuma longa, Ocimumsantum, and Glycyrrhiza glabra, flavonoids, phenolic compounds, tannins, and vitamin extracts as shown in Table 04.

Table 4: Preliminary phytochemical Test Result of *Nigella sativa, Curcuma longa, Ocimumsantum and Glycyrrhizaglabra*

Test	Nigella Sativa	Turmeric	Liquorice	Holy Basil
Alkaloids	+	+	+	+
Phenols	+	+	-	+
Steroids	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	-	+	+
Carbohydrates	+	+	-	+
Glycosides	-	+	+	+
Proteins and Amino acids	+	+	-	+
Tannins	-	-	+	+

➢ *Ash Value Result*

Table 5: Result of Ash Value

Drug	Total ash value % w/w	Acid-insoluble ash % w/w	Water-soluble ash % w/w
Nigella Sativa	4.06	0.059	10.33
Turmeric	16.6	2.8	3.93
Liquorice	3.75	1.93	3.51
Holy Basil	8.0	0.2	3.2

C. TLC Result

The separation of the crude extract by TLC using different solvent given in (Table 06-10). TLC plate is visualized under visible light and long UV wave (365nm).

- The spots are small & clearly defined
- A Higher the Rf value indicates that the compound has travelled for up the plate & is less polar.
- A lower Rf value indicates that compound has not travelled far & is more polar.

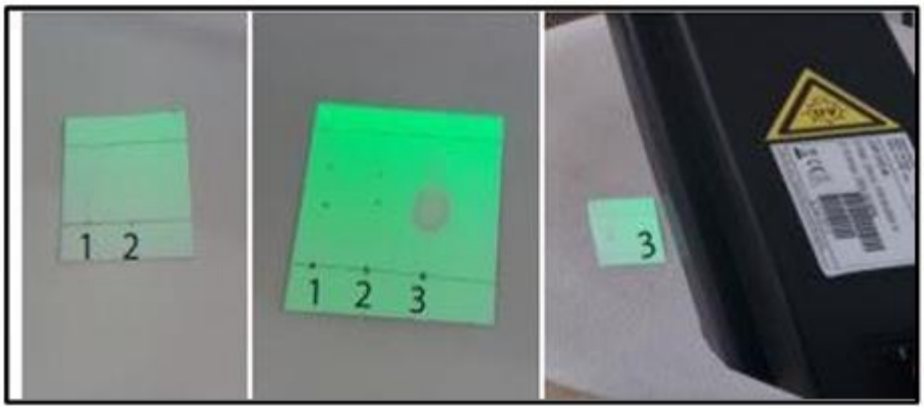

Fig. 12: TLC chromatograms of extracted Nigella Sativa seed in methanol (1), water (2), and oil (3).

Table No. 6: Result of TLC plate and calculating nigella sativa RF values

No	Sample	Type of mobile phase (v/v)	Rf (thymoquinone)	Rf (dithymoquinone)
1	Methanolic extract	Benzene: glacial acetic acid (1: 1)	< 0.3	< 0.3
2	Water extract	Carbon tetrachloride/acetone glacial acetic acid (15.2 : 3 : 1)	0.6	0.5
3	Oil	Chloroform	0.62	0.45

D. TLC method result of curcuma longa

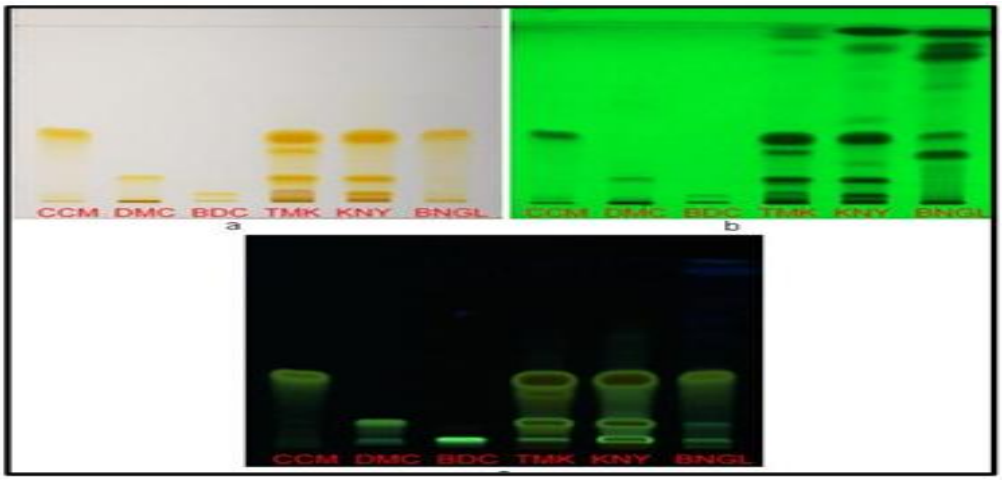

Fig. 13: TLC fingerprints of turmeric with visualization on visible light (a), UV 254 nm (b), and UV 366 nm (c) Note: CCM = Curcumin; DMC = Demethoxycurcumin; BDC =Bisdemethoxycurcumin; TMK = Java turmeric; KNY = Turmeric; BNGL = Cassumunar ginger

Table 7: Result of TLC plate and calculating RF values of Curcuma Longa

Identity	Standard	Height (cm)	R_F(cm)
BH	DDT	5.52-7.04	0.69-0.88
C	Curcumin	5.52-7.04	0.69-0.88
DMC	Demethoxy curucumin	4.48-5.52	0.56-0.69
BDMC	Bisdemethoxy curucumin	3.04-4.48	0.38-0.56
LAC	Lactose	1.70	0.21
MAL	Maltose	2.30	0.29
GLU	Glucose	3.0	0.38
VO	Turmeric volatile oil	4.48-5.52	0.56-0.69

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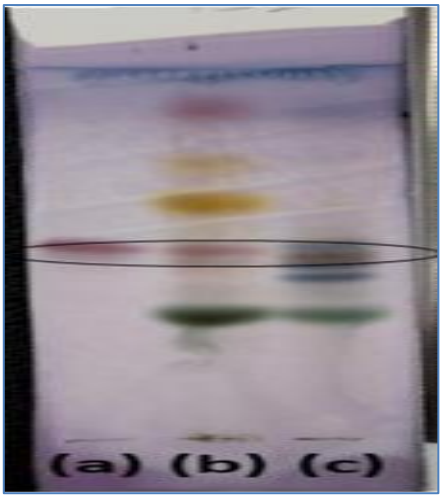

Fig. 14: TLC of glycyrrhizin in plant Samples

Table 8: Result of TLC plate and calculating RF values of Liquorice

No.	Sample	Mobile phase	Rf value
1.	Methanolic extract	Butanol:Glacial acetic acid:Water (6:1:3)	0.5

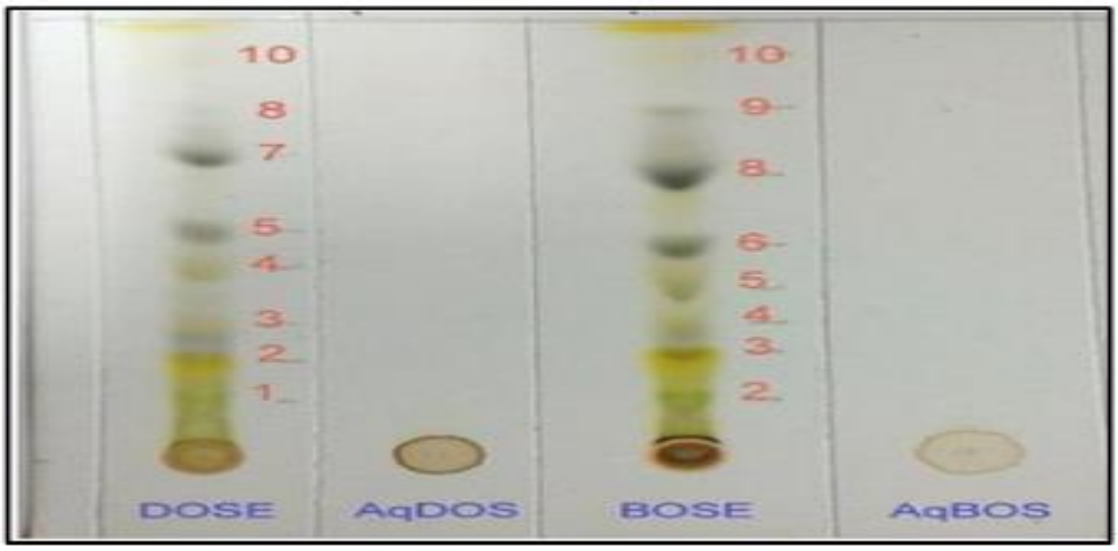

Fig. 15: TLC chromatogram of Ocimum sanctum leaf extracts in methanol solvent system. A: spots detected in visible light.

Table 9: Result of TLC plate and calculating RF values of *ocimum sanctum*

No	Sample	Rf value
1.	Methanol extract	0.82
2.	Water extract	0.29- 0.84
3	Ethanol extract	0.06-0.94

XI. SUMMARY

The present study is directed to a polyherbal cream composition for repigmentation of skin white patches occurring in vitiligo. Nigella sativa is used as main ingredient in formulation which shows immunomodulator, anti-inflammatory and anti-cancer activities along with high repigmentation rate and the other drugs like Curcuma longa and O. santum gives anti-oxidant properties which can help to remove the impurities from skin. Glycyrrhizin Glabra involved in formulation shows immune modulator activity. When combined with these other components, the other herbs show the existence of major phytoconstituents that genuinely nourish the skin pigmentation. From the above observation it has been concluded that because of its lower moisture content, the formulation made of naturally occurring dried herbal components has less chances of deterioration. In order to monitor changes in colour, order, texture, and appearance that indicated good stability, the formulation was kept at room temperature. It can be easily stored and used by any person. The natural herbal formulation is nontoxic, easily usable, enhances smoothing of skin and causes repigmentation in faded areas. Therefore, it can be said that it may give good patient complies and can be a novel formulation it was free from the ill effects of synthetic

chemicals and steroids. This leads to an increase shelf life with stable ingredients.

XII. CONCLUSION

As we know the population in developed and developing country is widely using herbal drugs due to its negligible side effects. The present study proves that the prepared polyherbal cream for inhibition of vitiligo patches comprising a mixture of plant extract have in build Immuno modulator, anti-oxidant, anti-inflammatory, anti-bacterial, anti-microbial activities. According to DPPH assay for anti-oxidant test and Thin Layer Chromatography, it confirm that all the required active chemical composition are present in our extract. This formulation contains methanol soluble herbal extracts which was highly environment friendly. In this research, we can conclude that the herbal constituents used in our formulation give repigmentation rate with no side effects.

CONFLICT OF INTEREST

The author declare that they have no conflict of interest

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