PROJECT REPORT

Formulation and evaluation of vitiligo herbal gel

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ATMIYA UNIVERSITY

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Certificate

This is to certify that Ms. **Daraniya Mansi Vinodbhai** *with Enrollment no.* ***200501011*** *has satisfactorily completed project on "* ***FORMULATION AND EVALUATION OF VITILIGO HERBAL GEL****" as a part of curriculum of B.Pharm Semester-VIII in the SSIP project during the academic year 2023-2024.*

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DECLARATION

We, all hereby declare the Work is presented in the project report entitled Preparation and Evaluation of herbal vitiligo gel .

It is an authentic record of work carried out by us during the studying period of semester 8 at and underthe guidance of Atmiya University, Rajkot, and is being submitted for partial fulfillment of the requirement for the award of a bachelor's degree in B.pharm. This is not submitted anywhere else for the award of any other degree/diploma.

Daraniya Mansi

Dhruve Ishita

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Padia Binal

Title	Preparation and evaluation of herbal vitiligo gel
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AIM AND OBJECTIVES

Aim:- To prepare herbal gel formulation to treat vitiligo containing Psoralea and Tamarind seed extracts.

Objectives:- To examine and assess the pharmacological effects of psoralea corylifolia seed extracts, such as their antibacterial, antiviral, antioxidant, and other properties.
To compare the pharmacological effects of extracts from psoralea seeds to those of other herbal medications.

FORMULATION AND EVALUATION OF VITILIGO HERBAL GEL

ABSTRACT

Features of vitiligo are Reduction in number of melanocytes in the epidermis and mucous membranes, a chronic multifactorial condition impacted by oxidative stress, toxins, and heredity. The pathophysiology of this old religious literature is now more understood. This paper examines surgical, herbal, and Ayurvedic treatments for vitiligo, with a focus on Psoralea corylifolia because of its long history of usage and many health advantages. Examining its 163 chemical constituents, which possess anti-inflammatory, antibacterial, and antioxidant characteristics, indicates possible treatment paths. Targets for drug development includeregulatory T cells, interleukin-17, tumor necrosis factor-alpha, and keratinocyte modulators. The study recommends investigating new herbal remedies for the treatment of vitiligo.
Key words: ***psoralea corylifolia,*** Vitiligo, Specific autoimmune commorbidities, Antioxidants, Reactive oxygen species, oxidative stress.

INTRODUCTION

1.1 Vitiligo disorder:

Vitiligo is a pigmentary disorder affecting the skin and mucous membranes. It puts a great deal of stress on the affected individual due to its cosmetic and psychological effects. The worst case scenario is when white patches create malformations in the exposed body area (Panda, 2005).
It is typified by symmetrical white macules and patches that get bigger over time due to the degeneration of melanocytes (Lotti and D'Erme, 2014). The patient's quality of life is significantly diminished whenever vitiligo manifests. In addition to skin adverse effects, immune-related problems might arise from it (Taieb et al., 2013) (Alikhan et al., 2011).
Thomas Addison discovered a link between vitiligo and adrenal insufficiency in 1855 (Lotti et al.,2004) (Hann and Nordlund, 2000) (zelissen et al., 1995). VASI and VETF provide more accurate therapeutic evaluation criteria and vitiligo severity indicators for better assessment, outperforming clinical photography in this regard (Taieb et al., 2007).

Table 1: Vitiligo stage scale assessed on each body part (Kawakami and Hashimoto, 2011).

Srno.	Vitiligo stage scale	Vitligo disease Condition
1.	Stage-0 Stage-1	No skin depigmentation.
2.		Insufficient or lighter depigmentation.
3.	Stage-2	Complete depigmentation, which can involve up to 30% hair whitening.
4.	Stage-3	Complete depigmentation along with significant white hairs (>30%)

The most prevalent type of infection that affects people of all ages is skin disease. Because of their unsightliness andaccompanying difficulties, skin disorders are among the most difficult conditions to adjust to, particularly when they affect an area of the body like the face that is challenging to hide, even with makeup. The majority of skin infection treatments take a while to start working. If the condition does not improve with therapy for skin disorders, the issue becomes more concerning. Although precise statistics regarding the prevalence of skin illnesses in this nation are lacking, it is generally believed that 10–20% of patients seeking medical attention have skin diseases.

1.1.1 Classification of vitiligo disease:

Based on the location and extent of the lesions, Vitiligo can be classed as either a localized or generalized type according to the categorization criteria. The several vitiligo classes are shown here (Lee et al., 2007).
Table-2: Classification of vitiligo on the basis of location of leisons (Oh and Hann, 2018).

Srno.	Localized leisons	Generalized leisons
1.	Focal	Acrofacial
2.	Segmental	Vulagaris
3.	Mucosal	(a) Universal, (b) Mixed

1.2 Epidemiology of vitiligo:

47,033 people in a sample region of Denmark showed a 0.38 percent vitiligo prevalence. Both men and women were making an equal effect. There were no discernible variations in the distribution of 179 vitiligo patients among five municipalities or between urban and rural regions (Howitz et al., 1977).
No particular ethnic group of skin type is more prone to vitiligo than others.

1.3 Pathogenesis of vitiligo:

The absence of functional melanocytes is a characteristic of vitiligo, a multifactorial disorder (Ezzedine et al., 2012) (Le Poole et al., 1993) (Rodrigues et al., 2017). Many pathways have been linked to melanocyte destruction in vitiligo. These include vitiligo genetics, autoimmune responses, oxidative stress, the synthesis of inflammatory mediators, and melanocyte separation procedures. It seems that the immune systems—innate and adaptive—are cooperating
(Bergqvist et al., 2020). Following shows some major causes of vitiligo..

1.3.1 Vitiligo genetics:

Epidemiological research indicate that vitiligo typically runs in families (Alkhateeb et al., 2003) (Majumder etal., 1993) (Nath et al., 1994). Vitiligo is a genetic ailment; twenty percent of patients have a first-degree relative with the illness. First-degree relatives are seven to ten times more likely to be affected. Monozygotic twins' 23% concordance highlights the impact of the environment (Nath et al., 1994). Genome-wide studies in European and Chinese populations have identified 50 genetic loci that confer an increased risk of vitiligo, suggesting a range of genetic characteristics that are specific to certain ethnic groups (Spritz et al., 2017) (Jin et al., 2012).
impacted melanogenesis, apoptosis, and immunological regulation; linked to autoimmune, autoinflammatory, and pigmentary illnesses (Spritz et al. 2017) (Shen et al., 2016) affected apoptosis, immunological modulation, and melanogenesis; associated with autoimmune, autoinflammatory, and pigmentary diseases (Baharav et al., 1996) (Kemp et al., 1997) (Rezaei et al., 2007). NSV may infect white Europeans through TYR, which is seldom seen in melanoma (Jin et al., 2010).

1.3.2 Oxidative stresses:

Etiology studies suggests that oxidative stress may be the first cause of melanocyte loss (Dell'Anna et al., 2001) (Speeckaert et al., 2018). Melanocytes from vitiligo patients exhibit greater susceptibility to oxidative stress in ex vivo culture as compared to those from healthy persons (Puri et al., 1987). Reactive oxygen species (ROS), which disrupt the antioxidant system, are released by stressed melanocytes. ROS, superoxide dismutase, and malondialdehyde levels are out of equilibrium. Antioxidant systems are significantly absent from skin and blood (Dell'Anna et al., 2006) (Maresca et al., 1997) (MORRONE et al., 1992) (Bulut et al., 2011).

Melanocytes with vitiligo are more vulnerable to pro-oxidants when there is an imbalance in antioxidants (Maresca etal., 1997) (Jimbow et al., 2001) (Dell'Anna et al., 2007). By oxidizing proteins, breaking apart DNA, and peroxiding lipids, the production and accumulation of ROS impairs cellular function (Dell'Anna et al., 2007) (Bickers et al., 2006).
The disruption of mitochondrial function is the main source of ROS in vitiligo (Dell'Anna et al., 2003). Oxidative stress alters proteins and lipids due to changes in the electron transport chain and increased activity of mitochondrial malate dehydrogenase, which impairs cellular function (Jimbow et al., 2001) (Dell'Anna et al., 2007).

Oxidative stress causes an increase in calcium flow, which in turn causes melanocyte death through the transient receptor potential cation channel subfamily M member 2 (Kang et al., 2018). Monobenzone is the most widely used depigmenting agent (AlGhamdiet al., 2011).

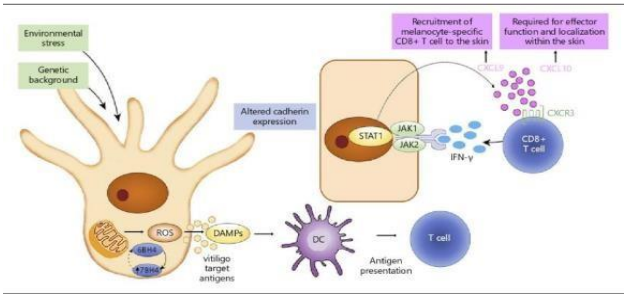

Figure-1: Pathogenesis of vitiligo (Bergqvist et al., 2020)

1.4 Etiology:

The pigment produced by the cutaneous melanocyte protects the skin against many environmental dangers as well as potential cellular damage that might lead to cancer and aging of the skin (Boissy et al., 2004) (Gilchrest, 1989). Melanin and melanosomes are primarily found above the keratinocyte nucleus (Boissy, 2003). DNA is protected when melanin is positioned optimally to act as a potent free radical scavenger and efficiently absorb UV radiation (Mason et al., 1960) (Sarna, 1992).
Absence of epidermal melanin is an indication of aging skin and increases the risk of skin cancer (Boissy et al., 2004) (Gilchrest et al., 1979). According to Boissy et al. (2004) and Lerner et al. (1971), the etiology of vitiligo is uncertain. Melanocytes that are more delicate and prone to apoptosis might initiate this process. Precipitating variables that enhance melanin synthesis include sunburn, pregnancy, stress, and exposure to cytotoxins, especially after UV exposure (Scott et al., 2002)(TSATMALI et al., 2000). Estrogens over the course of pregnancy (Grimes, 1995)(Kippenberger et al., 1998), cytokines in circumstances of stress and trauma (e.g., endorphins, neurotrophins, ACTH, and nerve growth factor etc.) (Peacocke et al., 1988) (Kauser et al., 2003) canall cause melanocytes to upregulate the production of melanin. Quinones, indoles, and melanin intermediates may pose a risk to melanocytes(Hochstein and Cohen, 1963) (Riley, 1998).
It is obvious that vitiligo has a complicated inherited component that predisposes individuals to the illness (Nath et al., 1994) (Hafez et al., 1983) (Majumder et al., 1988) (Majumderet al., 1993).
According to one study, people who had vitiligo had seven times more direct family members than those who were expected to get the illness (Nath et al., 1994). A simple Mendelian inheritance process does not cause vitiligo (Lacour and Ortonne, 1995). On the other hand, inheritance patterns have a more intricate expression (Kim et al., 1998) (Shah et al., 1975) (Shah et al., 1977) (Bhatia et al., 1992) (Alkhateeb et al., 2003). While depigmentation is initiated by an external cause, vitiligo is mostly a hereditary condition. The majority of the time, there is no identified trigger, and the cases are classified as idiopathic. One distinct type of vitiligo is contact or occupational vitiligo (Boissy et al., 2004) (Lerner et al.,, 1971). This kind is unique in that the onset of the illness corresponds with exposure to specific chemicals that induce chemical leukoderma (Boissy et al., 2004).

REVIEW OF LITERATURE

2.1 Herbal compounds for treating vitiligo (other than psoralea)

For the treatment of vitiligo, herbal remedies of various kinds and effects have been utilized since antiquity (Gianfaldoni et al., 2018).

2.1.1. Ginkgo Biloba:

One of the oldest trees on Earth, ginkgo biloba has been extensively utilized in medicine for a very longtime.It has been demonstrated that ginkgo extracts are useful in treating a variety of illnesses, includingallergies, varicose veins, premenstrual syndrome, headache, and vertigo (Fleming, 1998). Though its exact mechanismof action is still unknown, ginkgo biloba, which has anti-inflammatory and antioxidantqualities, efficiently heals vitiligo, especially when paired with conventional medicines like corticosteroids and phototherapies(Cohen et al., 2015)(Grimes and Nashawati, 2017).

2.1.2. Cucumis melo:

Superoxide dismutase (SOD) activity preventing melanocytes development because of oxidative stresses in early vitiligo stages is naturally present in cucumis melo extract (Naini et al., 2012) (Yuksel et al., 2009). In each trial, the skin lesions were treated with the gel formulation and either artificial or natural narrow band UVB radiation. Despite the drug's shown safety, there was no difference in the rate of repigmentation when compared to those who received just phototherapy. The use of a new topical mixture combining phenylalanine, Cucumis melo extract, and acetyl cysteine is more intriguing and encouraging.(Buggiani et al.,2012).

2.2 Psoralea corylifolia (Bakuchi):

Potent source of chemicals and alkaloids for the Leguminosae family is P. corylifolia (Bakuchi) (Belge and Jeurkar, 2023). Thirty species total, with the majority occurring in South Africa, Australia, North and South America, and a few in Asia and Europe (Li et al., 2016). P is an acronym meaning "afflicted with itch or leprosy," which is derived from the Greek word "Psoraleos." Also known as "Kushtanashini," a leprosy treatment, and "Babchi" (Bakuchi), a widely distributed shrub species (Belge and Jeurkar, 2023). The plant's whole body possesses skin-healing properties. It is recognized by both Sanskrit and regional names, and it relieves rashes, infections, and leucoderma. Bakuchi and Babchi are two instances of names (Belge and Jeurkar,2023). Marathi: Bavanchi, Baachi, Bavachya,etc (Khushboo et al., 2010)

Figure-2: Psoralea Corylifolia plant and seeds (Chen et al., 2023)

2.2.1 Pharmacological effect of compounds present in psoralea corylifolia:

Table-3: Pharmacological effect of various chemical compounds present in psoralea corylifolia (Chen et al., 2023).

Srno.	Pharmacologic alaction	Active compound/ Extract	Mechanism
1.	Anti inflammato ry	Psoralen (In vivo study)	decreased inflammatory cell infiltration andinflammatory factor release.
		Isopsoralen (In vitro and in vivo study)	MIF was the goal, and the release of inflammatory factors was decreased.
		Bavachinin (In vitro study)	Reduced phosphorylation of JNK and p38, as well as reduced inflammatory factor production and release in macrophages.
		Bakuchiol (In vitro study)	SIRT1 signaling pathway is involved.
2.	Anti bacterial	Psoralen (In vitro study)	Prevented the development of biofilms.
		Isopsoralen (In vitro study)	Prevented the development of biofilms
		Isobavachalcone (In vitro study)	Increased the cell wall permeability, altered the bacterial shape, destroyed the cell membrane, and raised the quantity of soluble protein leaks in the cell.
3.	Anti oxidant	Bavachinin A (In vitro study)	Increased antioxidant enzyme activity and mitochondrial membrane potential.
		Corylisoflavone A (In vitro study)	ROS levels were decreased via mitochondrial and non-mitochondrial pathways and the Nef2 signalling pathway was controlled.
		Bakuchiol	The SIRT3-SOD2 signalling pathway was activated.
		(In vivo study)	Controlled the PPAR- γ/Wnt pathway.
		Isopsoralen (In vivo study)	

2.3 Tamarind seeds:

Tropical Africa is home to the Tamarind (Tamarindus indica), a leguminous tree of the Fabaceae family. The edible, pod-like fruits of the tamarind tree are widely utilized in international cuisines. Traditionally, fevers, intestinal diseases, and diabetes have been treated using tamarind seeds. Additionally, the bark and seeds are therapeutic.
Tamarind leaves offer a wide range of ethnobotanical uses because of their antibacterial, antifungal, and antiseptic properties also they are used as antidiarrheal drug and as laxative for constipation.
Scientific name = Tamarindus Indica (Gupta et al., 2014).
Tannins, flavonoids etc as biomolecules and phenolic compounds contained in Tamarind seeds shows strong anti-inflammatory activity (Komakech et al., 2019).

Figure-3:-The primary characteristics of Tamarindus indica's botanical morphology, (a) Eastern Ugandian Tamarindus indica trees, (b) T. indica's stem bark is grayish, (c) T. indica leaf with complex, opposing leaflets that is alternate, (d) Tamarindus Indica flowers, (e) Tamarindus Indica fruits,(f) rusty-brown T. indica pulp,
(g) T. indica seeds that are smooth, lustrous, and irregularly shaped, with extremely hard testa (Komakech et al., 2019).

3.1Materials and methods:-

3.1.1 Chemicals/materials and equipments required:

(i) Chemicals :

Psoralea corylifolia seed extracts and Tamrind seed extract were purchased from market and other gelingredients as mentioned below were used from laboratory chemicals.

Table-4:- List of chemicals/materials used along with their purpose of using.

Ingredients/materials	Uses
Carbopol-940	As a gelling agent
NaOH	Humectant, Ph adjuster and thickening agent
Distilled water	As aqueous gel base.
Propyl paraben	Preservative

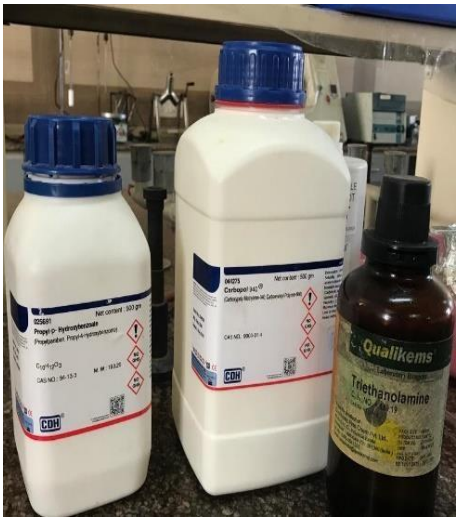

Figure-4:- Laboratory chemicals used in preparation of gel.

3.1.2 Formulations:

(1) F1 batch(trial batch)= 0.4gm carbopol + 1 gm propylene glycol + 1 drop triethanolamine + 15ml hot distilledwater.
Result observed= Gel was highly viscous than desired also hard lumps were observed.

(2) F2- batch = 0,3gm carbopol + 1 gm propylene gylcol + 1 drop triethanolamine + 15ml hot water.Result= Hard lumps found.

(3) F3 batch= 0.2gm carbopol +1gm propylene glycol + 2 drop triethanolamine + 15ml hot water.

(4) F4 batch= 0.2gm carbopol + 1 gm propylene glycol + 1 drop trethanolamine+ 15ml hot water.

(5) F5 batch= 0.2gm carbopol + 1 gm propylene glycol + 1 drop trethanolamine+ 15ml hot water.

(6)F6 batch = 2gm carbopol +2.6ml both drugs + 7 drops NaOH + Triethanolamine 1drop + 200ml cold water.

(7) F7 batch = 2gm carbopol + 2.6ml both drugs+ NaOh 7 drops + Triethanolamine 1drop + 200ml cold water.Result= Got desired viscosity and ph.

(8) F8 **(**Final batch) = 2gm carbopol+ 200ml distilled water + 2.6ml drugs (each)+ NaOH (q.s) + Propyl paraben (q.s).


Figure-5:- Final batch gel made.

3.1.3 Method of preparation of gel:

(1) Prepare gel base = (i) Weigh **2gm** carbopol-940 and dissolve in **200ml** distilled water.
(Note= Shake gently, do not stirr.)
(ii)Keep it aside for min. 24 hours for proper soaking of carbopol with water.

(2) Add psoralia and Tamarind seed extracts into it. (Add each 2.6ml)

(3) Add NaOH (q.s) and then finally add prservative (q.s).

3.1.4 Evaluation of gel:-

(1) Physical appearance **(2) Ph**
(3) Skin irritation **(4) Spreadabilty & washability**
(5) Extrudability **(6) TLC method**

(1) Physical appearance and viscosity:- For better patient compliance its physical appearance is very important as it is topically applied. So, it needs to be clear with suitable colour or transparent and having good texture.

(2) Ph :- As it is topical medication its ph it should be between 6-7.

(3) Skin irritation:- Gel was applied on the specific area of skin and its time of application was noted. At specific time intervals if it causes irritation or other skin reaction or not was observed.

(4) Spreadability:- (i) It was determined by wooden block and glass slide. Weigh 1gm gel and sandwiched it between two glass slides, compress to form uniform distribution, remove excess with filter paper. Weight of 80gm was placed on another side of the equipment.
Allow to separate two slides by keeping it in vertical direction. Note down the time required to separate theslides and calculte the spreadability by following equation (Laxmi et al., 2013):

S= ML/T
M= Weight placed on slide.
L= Length of glass slide.
T= Time required to separate two slides completely from each other..

(ii) This test was also determined by applying some gel on hand by gentle application.

Washability:- After applying gel on hand , they easily washout by the tap water.

(5) Extrudability:- It is an empirical test to measure the force required to exclude the material from the collapsible tube. Place sufficient material on the collapsiblw tube so that atleast 0.5cm should be excluded in10 sec. Measure extrudability by following equation (Pattanayek et al., 2018):

Extrudability = Applied weight(gm) to extrude gel from tube/ Area (cm^2).

(6) TLC Method:- TLC was performed in which mobile phase was prepared. The pre-coated silica plate was usedand the spot was observed under UV-chamber. Following materials were used to perform TLC:-

Mobile phase= Ethylacetate: methanol: water (10:1.35:1)Spraying reagent= 10% methanolic KOH Colour observed = violet colour indicates presence to psoralen (Laxmi et al., 2013).

RESULT FOR PREPARED GEL BATCHES:-

Batchno.	Physical appearance and viscosityfound	Ph	Spreadability &Washability	Extrudability	Skin irritation	TLC
F1	Lumpy & viscous					
F2	Hard lumps	Much acidic and unstable ph	N/A	N/A	N/A	N/A
F3	Some lumps with much visible particles					
F4	Less lumpy than previous still with some particles					
F5 F6 F7 F8 (Final batch)	**Viscosity:** 29603 cps **Tork :** 97.2 %. According to (Shiehzadeh et al., 2023) viscosities lower than 4,000 cps have been considered suitable for topical gel products.	Desired ph i.e 6 after mfg. and 24 hours also.	Gel was spreadedd nicely by gently applying. Spreadability value was Gel was washed out within 10 sec. after keeping hand under tap water.	Extrudability of gel was found to be 21.93 gm/ cm^2 which was compared to values mentioned in reference.	It didn't caused irritation to skin when applied.	Rf value = 0.4 was obtained And violet coloured spot was observed.

Calculation:-

1) **Viscosity:-**

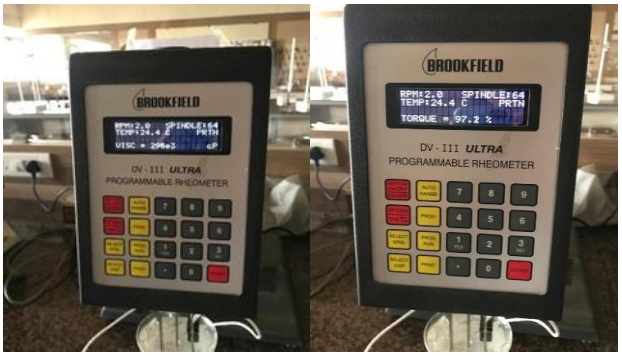

Figure-6:- Viscosity and torque obtained of final batch.

2) PH & Skin Irritation:-

PH was obtained between 6 to 7.

After applying gel on the skin, they do not produce a irritation.

Figure-7:- (A) PH test (B) Skin irritation test.

3) Spredability:-

S= ML/T

=80×7.5/14

=42.85 gm.cm/sec.

4) Extrudability:-

Extrudability= Applied weight(gm)to extrude gel from tube/ Area (cm^2).

= 428.47/1.86×10.5

= 21.93 g/cm^2.

5) TLC Method:-

Figure-8:- TLC to determine presence of psoralen

DISCUSSION: -

F6, F7 and F8 batches passed all the above evaluation tests and were considered safe

for use as they all had suitable and stable ph matching with skin ph, their viscosity, spreadability, extrudability also were found suitable according to its route of application.

Present experiment indicates that the gel prepared when applied on skin is non-irritating and also is getting washed properly without leaving any of its residues after that.

CONCLUSION

Presently available cures are limited by genetic variables, and there is no universal surgical therapy. Research for new vitiligo medicines has been sparked by its widespread recognition.
Radiation therapy and surgery are available as treatment alternatives; Psoralea corylifolia has demonstrated historical efficacy in this regard. The use of traditional Chinese medications and herbal remedies such as Ginkgo Biloba and Cucumis melo are also investigated.
From this investigation it has been revealed that vitiligo herbal gel of Psoralea corylifolia seeds and Tamarind seed extracts can be formulated using excipients such as carbopol-940 as gelling agent along with other excipients also gel has shown satifactory result of all its evaluation parameters.
June 25 is World Vitiligo Day, a day to promote awarenessof the condition and the need for a long-term strategy and public-private cooperation to develop new, secure, reasonably priced, and effective natural treatments for vitiligo.

REFERENCES

1. Panda, A.K., 2005. The medico historical perspective of vitiligo. Bulletin of the Indian Institute of History of Medicine, 25, pp.41-46.
2. Lotti, T. and D'Erme, A.M., 2014. Vitiligo as a systemic disease. Clinics in dermatology, 32(3), pp.430-434.
3. Taieb, A.V.E.T.F., Alomar, A., Böhm, M., Dell'Anna, M.L., De Pase, A., Eleftheriadou, V., Ezzedine, K., Gauthier, Y., Gawkrodger, D.J., Jouary, T. and Leone, G., 2013. Guidelines for the management of vitiligo:the European Dermatology Forum consensus. British Journal of Dermatology, 168(1), pp.5-19.
4. Alikhan, A., Felsten, L.M., Daly, M. and Petronic-Rosic, V., 2011. Vitiligo: a comprehensive overview: part I. Introduction, epidemiology, quality of life, diagnosis, differential diagnosis, associations, histopathology, etiology, and work-up. Journal of the American Academy of Dermatology, 65(3), pp.473-491.
5. Lotti, T., Hautmann, G. and Hercogová, J., 2004. Vitiligo: disease or symptom? From the confusion of the past to current doubts. BASIC AND CLINICAL DERMATOLOGY, 29, pp.1-14.
6. Hann, S.K. and Nordlund, J.J., 2000. Vitiligo: A monograph on the basic and clinical science. In *Vitiligo: A monograph on the basic and clinical science* (pp. xiv-306).
7. Zelissen, P.M., Bast, E.J. and Croughs, R.J., 1995. Associated autoimmunity in Addison's disease. *Journal of autoimmunity*, *8*(1), pp.121-130.
8. Taieb, A., Picardo, M. and other VETF members, 2007. The definition and assessment of vitiligo: a consensus report of the Vitiligo European Task Force. *Pigment cell research*, *20*(1), pp.27-35.
9. Lee, S.J., Cho, S.B. and Hann, S.K., 2007. Classification of vitiligo. *Surgical management of vitiligo Blackwell Publishing Delhi*, *3*, pp.20-29.
10. Oh, S.H. and Hann, S.K., 2018. Classification and clinical features of vitiligo. *Vitiligo: Medical and Surgical Management*, pp.33-47.
11. Howitz, J., Brodthagen, H., Schwartz, M. and Thomsen, K., 1977. Prevalence of vitiligo: epidemiological survey on the Isle of Bornholm, Denmark. *Archives of dermatology*, *113*(1), pp.47-52.
12. Ezzedine, K., Lim, H.W., Suzuki, T., Katayama, I., Hamzavi, I., Lan, C.C., Goh, B.K., Anbar, T., Silva de Castro, C., Lee, A.Y. and Parsad, D., 2012. Vitiligo global issue consensus conference panelists. Revised classification/nomenclature of vitiligo and related issues: the vitiligo global issues consensus conference. *Pigment Cell Melanoma Res*, *25*(3), pp.E1-13.
13. Le Poole, I.C., Das, P.K., Van Den Wijngaard, R.M.J.G.J., Bos, J.D. and Westerhof, W., 1993. Review of the etiopathomechanism of vitiligo: a convergence theory. *Experimental dermatology*, *2*(4), pp.145-153.
14. Rodrigues, M., Ezzedine, K., Hamzavi, I., Pandya, A.G., Harris, J.E. and Vitiligo Working Group, 2017. New discoveries in the pathogenesis and classification of vitiligo. *Journal of the American Academy of Dermatology*, *77*(1), pp.1-13.
15. Bergqvist, C. and Ezzedine, K., 2020. Vitiligo: a review. *Dermatology*, *236*(6), pp.571-592.
16. Alkhateeb, A., Fain, P.R., Thody, A., Bennett, D.C. and Spritz, R.A., 2003. Epidemiology of vitiligo and associated autoimmune diseases in Caucasian probands and their families. *Pigment Cell Research*, *16*(3), pp.208-214.
17. Majumder, P.P., Nordlund, J.J. and Nath, S.K., 1993. Pattern of familial aggregation of vitiligo. *Archives of dermatology*, *129*(8), pp.994-998.
18. Nath, S.K., Majumder, P.P. and Nordlund, J.J., 1994. Genetic epidemiology of vitiligo: multilocus recessivity cross-validated. *American journal of human genetics*, *55*(5), p.981.
19. Spritz, R.A. and Andersen, G.H., 2017. Genetics of vitiligo. *Dermatologic clinics*, *35*(2), pp.245-255.
20. Jin, Y., Birlea, S.A., Fain, P.R., Ferrara, T.M., Ben, S., Riccardi, S.L., Cole, J.B., Gowan, K., Holland, P.J., Bennett, D.C. and Luiten, R.M., 2012. Genome-wide association analyses identify 13 new susceptibility loci for generalized vitiligo. *Nature genetics*, *44*(6), pp.676-680.
21. Shen, C., Gao, J., Sheng, Y., Dou, J., Zhou, F., Zheng, X., Ko, R., Tang, X., Zhu, C., Yin, X. and Sun, L., 2016. Genetic susceptibility to vitiligo: GWAS approaches for identifying vitiligo susceptibility genes andloci. *Frontiers in genetics*, *7*, p.3.

22. Baharav, E., Merimski, O., Shoenfeld, Y., Zigelman, R., Gilbrud, B., Yecheskel, G., Youinou, P. and Fishman, P., 1996. Tyrosinase as an autoantigen in patients with vitiligo. *Clinical & Experimental Immunology*, *105*(1), pp.84-88.
23. Kemp, E.H., Gawkrodger, D.J., Watson, P.F. and Weetman, A.P., 1997. Immunoprecipitation of melanogenic enzyme autoantigens with vitiligo sera: evidence for cross-reactive autoantibodies to tyrosinase and tyrosinase-related protein-2 (TRP-2). *Clinical & Experimental Immunology*, *109*(3), pp.495-500.
24. Rezaei, N., Gavalas, N.G., Weetman, A.P. and Kemp, E.H., 2007. Autoimmunity as an aetiological factor in vitiligo. *Journal of the European Academy of Dermatology and Venereology*, *21*(7), pp.865-876.
25. Jin, Y., Birlea, S.A., Fain, P.R., Gowan, K., Riccardi, S.L., Holland, P.J., Mailloux, C.M., Sufit, A.J., Hutton, S.M., Amadi-Myers, A. and Bennett, D.C., 2010. Variant of TYR and autoimmunity susceptibilityloci in generalized vitiligo. *New England Journal of Medicine*, *362*(18), pp.1686-1697.
26. Dell'Anna, M.L., Maresca, V., Briganti, S., Camera, E., Picardo, M. and Falchi, M., 2001. Mitochondrial impairment in peripheral blood mononuclear cells during the active phase of vitiligo. *Journal of investigative dermatology*, *117*(4), pp.908-913.
27. Speeckaert, R., Dugardin, J., Lambert, J., Lapeere, H., Verhaeghe, E., Speeckaert, M.M. and van Geel, N., 2018. Critical appraisal of the oxidative stress pathway in vitiligo: a systematic review and meta-analysis. *Journal of the European Academy of Dermatology and Venereology*, *32*(7), pp.1089-1098.
28. Puri, N., Mojamdar, M. and Ramaiah, A., 1987. In vitro growth characteristics of melanocytes obtained from adult normal and vitiligo subjects. *Journal of investigative dermatology*, *88*(4), pp.434-438.
29. Maresca, V., Roccella, M., Roccella, F., Camera, E., Del Porto, G., Passi, S., Grammatico, P. and Picardo, M., 1997. Increased sensitivity to peroxidative agents as a possible pathogenic factor of melanocyte damage in vitiligo. *Journal of investigative dermatology*, *109*(3), pp.310-313.
30. MORRONE, A., PICARDO, M., LUCA, C.D., TERMINALI, O., PASSI, S. and IPPOLITO, F., 1992. Catecholamines and vitiligo. *Pigment cell research*, *5*(2), pp.65-69.
31. Bulut, H., Pehlivan, M., Alper, S., Tomatır, A.G., Onay, H., Yüksel, S.E. and Özkinay, F., 2011. Lack of association between catalase gene polymorphism (T/C exon 9) and susceptibility to vitiligo in a Turkish population. *Genetics and Molecular Research*.
32. Jimbow, K., Chen, H., Park, J.S. and Thomas, P.D., 2001. Increased sensitivity of melanocytes to oxidative stress and abnormal expression of tyrosinase-related protein in vitiligo. *British Journal of Dermatology*, *144*(1), pp.55-65.
33. Dell'Anna, M.L., Ottaviani, M., Albanesi, V., Vidolin, A.P., Leone, G., Ferraro, C., Cossarizza, A., Rossi, L. and Picardo, M., 2007. Membrane lipid alterations as a possible basis for melanocyte degeneration in vitiligo. *Journal of Investigative Dermatology*, *127*(5), pp.1226-1233.
34. Bickers, D.R. and Athar, M., 2006. Oxidative stress in the pathogenesis of skin disease. *Journal of Investigative Dermatology*, *126*(12), pp.2565-2575.
35. Dell'Anna, M.L., Urbanelli, S., Mastrofrancesco, A., Camera, E., Iacovelli, P., Leone, G., Manini, P., D'ischia, M. and Picardo, M., 2003. Alterations of mitochondria in peripheral blood mononuclear cells ofvitiligo patients. *Pigment Cell Research*, *16*(5), pp.553-559.
36. AlGhamdi, K.M. and Kumar, A., 2011. Depigmentation therapies for normal skin in vitiligo universalis. *Journal of the European Academy of Dermatology and Venereology*, *25*(7), pp.749-757.
37. Boissy, R.E. and Manga, P., 2004. On the etiology of contact/occupational vitiligo. *Pigment Cell Research*, *17*(3), pp.208-214.
38. Gilchrest, B.A., 1989. Skin aging and photoaging: an overview. *Journal of the American Academy of Dermatology*, *21*(3), pp.610-613.
39. Boissy, R.E., 2003. Melanosome transfer to and translocation in the keratinocyte. *Experimental dermatology*, *12*, pp.5-12.
40. Mason, H.S., Ingram, D.J.E. and Allen, B., 1960. The free radical property of melanins. *Archives of Biochemistry and Biophysics*, *86*(2), pp.225-230.
41. Sarna, T., 1992. New trends in photobiology: properties and function of the ocular melanin—a photobiophysical view. *Journal of Photochemistry and Photobiology B: Biology*, *12*(3), pp.215-258.

42. Gilchrest, B.A., Blog, F.B. and Szabo, G., 1979. Effects of aging and chronic sun exposure on melanocytes in human skin. *Journal of Investigative Dermatology*, *73*(2), pp.141-143.
43. Lerner, A.B., 1971. On the etiology of vitiligo and gray hair. *The American Journal of Medicine*, *51*(2), pp.141-147.
44. Scott, M.C., Suzuki, I. and Abdel-Malek, Z.A., 2002. Regulation of the human melanocortin 1 receptor expresion in epidermal melanocytes by paracrine and endocrine factors and by ultraviolet radiati. On. *Pigment Cell Research*, *15*(6), pp.433-439.
45. TSATMALI, M., ANCANS, J., YUKITAKE, J. and THODY, A.J., 2000. Skin POMC peptides: their actions at the human MC-1 receptor and roles in the tanning response. *Pigment Cell Research*, *13*, pp.125-129.
46. Grimes, P.E., 1995. Melasma: etiologic and therapeutic considerations. *Archives of dermatology*, *131*(12), pp.1453-1457.
47. Kippenberger, S., Loitsch, S., Solano, F., Bernd, A. and Kaufmann, R., 1998. Quantification of tyrosinase, TRP-1, and Trp-2 transcripts in human melanocytes by reverse transcriptase-competitive multiplex PCR–regulation by steroid hormones. *Journal of investigative dermatology*, *110*(4), pp.364-367.
48. Peacocke, M., Yaar, M., Mansur, C.P., Chao, M.V. and Gilchrest, B.A., 1988. Induction of nerve growth factor receptors on cultured human melanocytes. *Proceedings of the National Academy of Sciences*, *85*(14),pp.5282-5286.
49. Kauser, S., Schallreuter, K.U., Thody, A.J., Tobin, D.J. and Gummer, C., 2003. Regulation of human epidermal melanocyte biology by β-endorphin. *Journal of investigative dermatology*, *120*(6), pp.1073-1080.
50. Hochstein, P. and Cohen, G., 1963. The cytotoxicity of melanin precursors. *Annals of the New York Academy of Sciences*, *100*(1), pp.876-886.
51. Riley, P.A., 1998. Mechanisms of inhibition of melanin pigmentation. *The Pigmentary System: Physiology and Pathophysiology*.
52. Nath, S.K., Majumder, P.P. and Nordlund, J.J., 1994. Genetic epidemiology of vitiligo: multilocus recessivity cross-validated. *American journal of human genetics*, *55*(5), p.981.
53. Hafez, M., L. Sharaf, and S. M. Abd el-Nabi. "The genetics of vitiligo." *Acta dermato-venereologica* 63, no. 3 (1983): 249-251.
54. Majumder, Partha P., S. K. Das, and C. C. Li. "A genetical model for vitiligo." *American journal of human genetics* 43, no. 2 (1988): 119.
55. Lacour, J.P. and Ortonne, J.P., 1995, January. Genetics of vitiligo. In *Annales de dermatologie et de vénéréologie* (Vol. 122, No. 4, pp. 167-171.
56. Kim, S.M., Chung, H.S. and Hann, S.K., 1998. The genetics of vitiligo in Korean patients. *International journal of dermatology*, *37*(12), pp.908-910.
57. Shah, V.C., Mojamdar, M.V. and Sharma, K.S., 1975. Some genetic, biochemical and physiological aspects of leucoderma vitiligo. *J Cytol Genet Congr*, *1*, pp.173-178.
58. Shah, V.C., Haribhakti, P.B., Mojamdar, M.V. and Sharma, K.S., 1977. Statistical study of 600 vitiligo cases in the city of Ahmedabad. *Gujarat Med J*, *42*, pp.51-59.
59. Bhatia, P.S., Mohan, L., Pandey, O.N., Singh, K.K., Arora, S.K. and Mukhija, R.D., 1992. Genetic nature of vitiligo. *Journal of dermatological science*, *4*(3), pp.180-184.
60. Gianfaldoni, Serena, Uwe Wollina, Michael Tirant, Georgi Tchernev, Jacopo Lotti, Francesca Satolli, Miriam Rovesti, Katlein França, and Torello Lotti. "Herbal compounds for the treatment of vitiligo: a review." *Open access Macedonian journal of medical sciences* 6, no. 1 (2018): 203.
61. Fleming, T., 1998. PDR for herbal medicines. Montvale, NJ: medical Economics Company.
62. Cohen, B.E., Elbuluk, N., Mu, E.W. and Orlow, S.J., 2015. Alternative systemic treatments for vitiligo: a review. *American journal of clinical dermatology*, *16*, pp.463-474.
63. Grimes, P.E. and Nashawati, R., 2017. The role of diet and supplements in vitiligo management. *Dermatologic clinics*, *35*(2), pp.235-243.
64. Naini, F.F., Shooshtari, A.V., Ebrahimi, B. and Molaei, R., 2012. The effect of pseudocatalase/superoxide dismutase in the treatment of vitiligo: A pilot study. *Journal of Research in Pharmacy Practice*, *1*(2), p.77.

65. Yuksel, Yuksel,E.P., Aydin, F., Senturk, N., Canturk, T. and Turanli, A.Y., 2009. Comparison of theefficacy of narrow band ultraviolet B and narrow band ultraviolet B plus topical catalase-superoxide dismutase treatment in vitiligo patients. *European Journal of Dermatology*, *19*(4), pp.341-344.
66. Buggiani, G., Tsampau, D., Hercogovà, J., Rossi, R., Brazzini, B. and Lotti, T., 2012. Clinical efficacy of anovel topical formulation for vitiligo: compared evaluation of different treatment modalities in 149 patients. *Dermatologic therapy*, *25*(5), pp.472-476.
67. Belge, D.A. and Jeurkar, M.M., 2023. MORPHOLOGICAL, PHARMACOLOGICAL ANDTOXICOLOGICAL PROFILE OF PSORALEA CORYLIFOLIA L: A REVIEW.
68. Li, C.C., Wang, T.L., Zhang, Z.Q., Yang, W.Q., Wang, Y.F., Chai, X., Wang, C.H. and Li, Z., 2016.Phytochemical and pharmacological studies on the genus psoralea: a mini review. *Evidence-Based Complementary and Alternative Medicine*, *2016*.
69. Khushboo, P.S., Jadhav, V.M., Kadam, V.J. and Sathe, N.S., 2010. Psoralea corylifolia Linn.—"Kushtanashini". *Pharmacognosy reviews*, *4*(7), p.69.
70. Chen, L., Chen, S., Sun, P., Liu, X., Zhan, Z. and Wang, J., 2023. Psoralea corylifolia L.: a comprehensivereview of its botany, traditional uses, phytochemistry, pharmacology, toxicology, quality control and pharmacokinetics. *Chinese Medicine*, *18*(1), pp.1-38.
71. Gupta, C., Prakash, D. and Gupta, S., 2014. Studies on the antimicrobial activity of Tamarind (Tamarindus indica) and its potential as food bio-preservative. *International Food Research Journal*, *21*(6), p.2437.
72. Komakech, R., Kim, Y.G., Matsabisa, G.M. and Kang, Y., 2019. Anti-inflammatory and analgesic potential of Tamarindus indica Linn.(Fabaceae): a narrative review. *Integrative Medicine Research*, *8*(3), pp.181-186.
73. Shiehzadeh, F., Mohebi, D., Chavoshian, O. and Daneshmand, S., 2023. Formulation, characterization, and optimization of a topical gel containing tranexamic acid to prevent superficial bleeding: In vivo and in vitro evaluations. *Turkish Journal of Pharmaceutical Sciences*, *20*(4), p.261.
74. Pattanayek, S. and Puranik, S., 2018. Formulation and evaluation of ketoprofen loaded nanoparticulate gel for topical delivery. *IJPPR*, *11*(3), pp.1-11.
75. Laxmi, R.J., Karthikeyan, R., Babu, P.S. and Babu, R.N., 2013. Formulation and evaluation of antipsoriaticgel using natural excipients. Journal of Acute Disease, 2(2), pp.115-121.