

A Randomized Single Blind Non-Inferiority Clinical Trial of Unani Polyherbal Formulations on Non-Segmental Vitiligo

Nadeem Ahmad Shah^{1*}, Mohammed Aleemuddin Quamri¹, Sabba Saltanat², Mariyam Ahad³

ABSTRACT

Background & Objectives

Vitiligo is a common depigmenting skin disorder that impairs a patient's quality of life. Although there is no definitive treatment for vitiligo, some formulations have been tried in Unani Medicine. This study aimed to compare the efficacy of two polyherbal Unani formulations - *Dawa-e-Baras* and *Safuf-e-Baras* in Non-Segmental Vitiligo.

Methods

A randomized-single blinded study, on 28 subjects with vitiligo treated with *Dawa-e-Baras* or *Safuf-e-Baras* 4.5 gm each once a day - orally as *Zulal* and locally the sediment for 60 days. Outcome measures were compared Vitiligo Extent Tensity Index score (VETI) and Dermatology Life Quality Index score (DLQI) at baseline, 15th, 30th, 45th and 60th days between the groups (Test group/Group A-*Dawa-e-baras* and Control group/Group B- *safuf-e-baras*) using Mann Whitney U test and Wilcoxon's signed rank test.

Results

The Mean $\pm$ standard deviation of VETI score at baseline and 60th day in the Group A was 0.908 ± 0.753 , and 0.663 ± 0.668 (d -0.245), and in Group B 1.019 ± 0.874 and 0.586 ± 0.699 (d -0.433), and intragroup comparison was found significant ($p < 0.0001$). Similarly, DLQI at baseline and 60th day in the Group A was 14.86 ± 4.294 and 10.36 ± 3.272 (d -4.5), and in the Group B 13.50 ± 4.703 and 7.64 ± 3.319 (d -5.8), and intragroup comparison was found significant ($p \leq 0.001$). The mean intergroup differences after the treatment for VETI and DLQI were 0.077 ($p = 0.511$) and 2.72 ($p = 0.044$), respectively.

Interpretation & Conclusion

It can be concluded that both formulations *Dawa-e-Baras* and *Safuf-e-Baras* are equally efficacious for *Baras* (Vitiligo).

Keywords

Unani Medicine; Vitiligo; *Dawa-e-Baras*; *Safuf-e-Baras*, VETI, DLQI

INTRODUCTION

Vitiligo is an acquired depigmenting skin condition characterized by the development of circumscribed white macules (patches) as a consequence of the loss of functional melanocytes from the epidermis.¹ It has an impact on the entire body, including the skin, hair, eyes, and also other areas such as the inside of the mouth. Vitiligo affects patients of all age groups and both the sexes.²⁻³ The most apparent sign or symptom of vitiligo is the depigmented macules and patches of different shapes and sizes on the skin initially occurs as small patches usually enlarged over time. The lesions typically and initially appear on the face, hand and wrist.⁴ The Vitiligo European Task Force (VETF) defines vitiligo vulgaris/ nonsegmental vitiligo as an acquired chronic pigmentation disorder characterized by white patches, often symmetrical, which usually increase in size with time, corresponding to a substantial loss of functioning epidermal and sometimes hair follicle melanocytes.¹ Vitiligo affects approximately 0.5%–1% of the world's population. The highest incidence has been reported in India, followed by Mexico and Japan⁵. In India it is found to be 0.25%–2.5%, and states like, Gujarat and Rajasthan have the highest prevalence.^{6,7} The prevalence of vitiligo worldwide varies from 0.1% to 8.0%,⁸ and in India it is between 0.46% to 8.8%.⁹ Vitiligo can present at any age but almost half of the cases

1. Department of Jild wa Taz'iniyyat, NIUM, Bangalore. *
2. Department of Kulliyate Tibb, NIUM, Bangalore.
3. Rehbar Ayurvedic and Unani Tibbi Medical College.

Correspondence

Nadeem Ahmad Shah Department of Jild wa Taz'iniyyat, NIUM, Bangalore, Email: drnadeemshah72@gmail.com

present before the age of 20 years and about 70%–80% before 30 years of age.¹⁰

Pathophysiology of Vitiligo: The skin serves as the locus for a multitude of oxidative reactions, which are counterbalanced by intricate exogenous or endogenous enzymatic and non-enzymatic systems, resulting in a complex yet highly efficient equilibrium. The genesis of reactive species in this context can be attributed primarily to interactions with the environment, a characteristic trait of the integumentary system, and the distinctive features of its cells, particularly the epidermal cells known for their robust metabolic and proliferative activity. The emergence of oxidative stress, characterized by elevated levels of reactive oxygen species (ROS), can initiate a cascade of molecular and organelle dysfunction, subsequently eliciting an immune response and, ultimately, melanocyte demise¹¹. Under conditions of oxidative stress, ROS may incite autophagy by activating the Nrf2(nuclear factor erythroid 2-related factor 2) antioxidant pathway in melanocytes¹². Studies have revealed that the APE1(Apurinic/aprimidinic endonuclease1) polymorphism (Asp148Glu) exacerbates oxidative stress in human melanocytes and increases serum 8-OHdG(8-hydroxydeoxyguanosine) levels in a dose-dependent manner, thereby elevating the risk of vitiligo¹³. Vitiligo is a complex condition influenced by multiple factors, both genetic and non-genetic. Despite various proposed theories, the exact cause remains elusive. Generally, the absence and decline of melanocytes, as evidenced by histological examination, are attributed to their gradual destruction over time. Several theories attempt to explain this destruction:

1. Autoimmune mechanisms: Dysfunction of the immune system leads to the formation of autoantibodies targeting melanocyte antigens, potentially inhibiting melanogenesis or causing melanocyte destruction⁴⁸. Conversely, melanocyte damage can trigger the release of antigenic substances, prompting antibody formation against melanocytes or melanin production¹⁴. Several studies have documented that incidence of vitiligo is higher in patients with autoimmune diseases as compared to the general population.¹⁵⁻²⁰
2. Intrinsic melanocyte defects: The intrinsic theory posits that in vitiligo, there is a depletion of melanocytes due to various abnormalities, resulting in heightened apoptosis²¹ and accelerated cell senescence²². Previous studies have revealed diverse abnormalities in melanocytes, including cytoplasmic vacuolization, DNA marginalization, dendrite loss, and detachment²³. Supporting evidence for increased apoptosis in vitiligo includes diminished expression levels of the antiapoptotic proteins Bcl-2 and FLIP in vitiliginous skin compared to normal skin. Conversely, a notable upsurge in the expression of proapoptotic factors such as Bax and p53, alongside various caspases, has also been observed.
3. Auto-cytotoxic mechanisms: The anticytotoxic theory suggests that heightened melanocyte activity can trigger self-destruction. Limited lymphocytic infiltration near melanocytes supports this theory²⁴. Additionally, inhibition of thioredoxin reductase and reduced catalase levels contribute to cell death. Certain melanin production molecules may also exert harmful effects on melanocytes²⁵.
4. Oxidant-antioxidant mechanisms: Oxidative stress plays a significant role in vitiligo pathophysiology. Accumulation of free radicals, detrimental to melanocytes, contributes to their destruction²⁶.
5. Neural mechanisms: Specific compounds released at peripheral nerve endings inhibit melanogenesis and exert toxic effects on melanocytes according to the neural theory. Given the neural crest origin of melanocytes, nerve dysfunction likely influences their functionality. Anatomical studies demonstrate contact between nerve fibers and melanocytes, suggesting a crucial role for nerve deterioration in vitiligo development²⁷⁻²⁹.

Genetics of vitiligo: Its inheritance is complex, involving over 50 genetic loci, many shared with other autoimmune disorders³⁰. Notably, the HLA-A2 gene shows a significant association, alongside other major histocompatibility complex genes. Other implicated genes include CTLA4, PTPN22, NLRP1, GZMB, IL2RA, FOXP1, FOXD3, XPB1, MC1R, and TYR, highlighting roles of innate and adaptive immunity, melanocyte stress, and apoptosis in vitiligo development³⁰.

Unani Pathophysiology: Based on the literary review of the classic Unani literature, it was observed that the majority of Unani physicians characterized the *Baraṣ*, as a white discoloration of the skin that can permeate all the way to the bones. Most of the Unani physicians

agreed that the disease occur mostly due to Du'f *Quwwat Mughayyira*, *Mushabbihā*, and *Dafi'a*, and this weakness might be produced by the accumulation of *Balgham Ghaleez*, which results in *Fasād- ud-Dam* and *Burūdat- ud-Dam*, the digestive faculty cannot metabolize the food properly, there is lackness of healthy blood formation, which is of cold & moist temperament (Phlegmatic temperament) that leads to develop Baras^{31,32}

The various precipitating factors associated with the development of vitiligo include emotional stress, sunburn, major illness, surgery, pregnancy, contraceptives, vitamin deficiency, parturition and physical trauma.^{33,34}

Although the disease may not directly result in bodily deterioration, it may have a significant impact on the psychological well-being of the subject. The disease load comprises of Anxiety, stigmatization, dejection, impairment of quality of life, depression, dearth of self-confidence, frequent embarrassment. Suicide attempts have also been recorded from subjects with vitiligo having a poorer QOL and who are always in a state of depression as a consequence of the disease. The patient's deplorable condition and its social consequences frequently have a negative impact on the lives of the patient's family members.^{35,36}

Although there is no definitive treatment for vitiligo, several treatment options have been associated with relative satisfactory outcomes.³⁷ Current treatments for vitiligo remain far from ideal, as they are not universally effective in all patients, they do not repigment all anatomic locations, and they can be quite difficult for patients to use. Furthermore, all current treatments provide only short-term benefit, as patients relapse and can be both time-consuming and expensive to implement. On the other hand, long term use of these medications has been linked to various local and systemic side effects. Therefore, it is essential to provide an alternative therapy that can provide not only effective, safe and economical treatment which also can act for a longer duration, improve quality of life and should be free from adverse drug reactions (ADRs). However, in the classical Unani literature there is a vast description on the management of *Baras*, initially with *Tanqiya-i -Badan* through *Mundij* and *Mushil-i-Balgham* followed by *Mushil* (Purges) and *Tabrīd* (Cooling agents/drugs).³⁸⁻⁴⁰

In classical Unani literature a number of drug

formulations for oral and topical use are mentioned in the management of vitiligo; *Dawa-e-baras* and *safuf-e-baras* are the formulations that have been used and found to be effective in vitiligo/ *Baras*. In the context of vitiligo, antioxidants play a key role in shielding melanocytes from the deleterious effects of free radicals and reactive oxygen species stemming from skin cellular metabolism. Herbs possessing antioxidant properties contribute to the safeguarding of melanocytes and the continuous production of melanin. It can be postulated that these medications expel *fasid akhlāt* (*Balgham*) from the body, particularly at the lesion site, while also stimulating melanocyte activity to induce pigmentation and camouflage vitiligo.

The constituents of our test drug (*Dawa-e-baras*) are given in table 1. In *P. granatum*, antioxidant properties are attributed to compounds such as ellagic acid, hydrolysable tannins, punicalagin, punicalic acid, and anthocyanins^{41,42}. Another component of *Dawa-e-Baras*, sulfur, exhibits antioxidative effects by reducing the production of reactive oxygen and nitrogen species⁴³. Studies conducted in the field of homeopathic medicine have assessed the efficacy of sulfur in the treatment of vitiligo, and these studies have reported positive results from the use of sulfur during their research^{44,45}. References also traced that in various skin diseases like Psoriasis, Scabies, vitiligo etc. Geru was used as topical medicine⁴⁶. Reportedly, the seeds of *P. corylifolia* contain psoralen, which serves as a pharmacologically active component used in the treatment of vitiligo⁴⁷. Psoralen has been discovered to insert itself between DNA strands, creating mono- and di-adducts when exposed to long wavelength UV light. This property is utilized in treating skin conditions with hypo-pigmented lesions, such as vitiligo⁴⁸. In a specific research study, a group of 49 patients received *Psoralea corylifolia* treatment for a duration of 6 months. Among these patients, 14% achieved a complete cure, while an additional 19% experienced a significant improvement in pigmentation, covering at least two-thirds of the affected skin^{49,50}.

Safuf-e-Baras, a standardized Unani formulation with a long history of use in treating vitiligo, has been selected as the standard medication. The ingredients of this formulation are Babchi (*Psoralea corylifolia*), Chaksu (*Cassia absus*), Panwar (*Cassia tora*) and Anjeer-khushk (*Ficus carica*) listed in table 2. The biochemical properties of Babchi are already mentioned above.

Multiple researches done on *Cassia absus*(chaksu) have documented a notable and statistically significant correlation between antioxidant elements such as phenols, polyphenols, and tannins, and both the reducing power and DPPH radical scavenging ability making it a beneficial drug for vitiligo⁵¹. The phenolic active component, alaternin and nor-rubrofusarin glucoside isolated from extract of *Cassia* species show a potent free radical scavenging activity. The methanolic and aqueous extract of the seed shows antioxidant effect on peroxidation of linoleic acid⁵². Reportedly anjeer has shown antioxidant, free radical scavenging and immunostimulant properties due to its phenolic components^{53,54}.

Hence, it is hypothesized that the intrinsic properties of the ingredients of *Dawa-e- Baras* may be effective in vitiligo, though these drugs have been used by Unani physicians for long time, but their efficacy has not been evaluated scientifically so far. Hence, the present study aims to assess the comparative efficacy of *Dawa-e-Baras* and *Safuf-e- Baras* in *Baras* (Vitiligo)

MATERIALS AND METHODS

Study design

This single blind, randomized, comparative clinical study on the efficacy of *Dawa-e-Baras* and *Safuf-e-Baras* in *Baras* (Vitiligo) has been carried out in the Department of Amraze Jild wa Tazeeniyat, National Institute of Unani Medicine Hospital, Bangalore (NIUM), India between August 2022 to January 2023. The study was approved by the Institutional ethical committee NIUM, Bengaluru under IEC No: (NIUM/ IEC/ 2020-21/028/JT/01) and the protocol was registered with the clinical trial registry of India with CTRI registration number (CTRI/2022/04/041571). All the participants were explained about the study and given a written informed consent form and after getting their signed written informed consent they were enrolled in the study. The CONSORT guideline was followed and the trial was conducted in accordance with the Declaration of Helsinki.

Sampling Method

The sample size was estimated considering the primary outcome VETI score. We used the mean and standard deviation of a previous similar study as a reference for calculating sample size, with α error 1.96 and β error of 0.84.⁵⁵ The formula⁵⁶ used is $N = (r + 1) (Z \alpha/2 + Z$

$1-\beta)^2 \delta^2 / rd^2$. The sample size calculated was 25, which was rounded up to 28 by taking 10% dropout into consideration. So, each group consists of 14 subjects.

Randomization: Randomization was conducted by using statistical software. Participants were randomly assigned by a computer-generated randomization code to test (Group A) and control group (Group B).

Study Population Subjects: The inclusion criteria for participation were (1) Subjects with Non-Segmental vitiligo (Acrofacial, focal and generalized) of any sex (2) Age 18 to 60 years (3) Subjects with Chronicity of less than 3 years (4) Subjects with $\leq 2\%$ Body surface Area (BSA) (5) Subjects fulfilling the Vitiligo Extent Tensity Index (VETI)⁵⁷ score up to grade of tensity +3 (6) Subjects who have not taken any treatment within the last 2 weeks. The Exclusion Criteria were (1) Patients aged <18 years or >60 years. (2) Patients with Segmental Vitiligo/ Lip-Tip/ Universal Vitiligo/ Mucosal Vitiligo. (3) Patients with a history of photosensitivity/ photo exaggerated dermatoses and Connective Tissue Diseases. (4) Pregnant or Lactating women (5) Known cases of Cardiovascular/Endocrine/ Pulmonary/ Hepato-renal Dysfunction. (6) Known cases of Immunocompromised states (HIV/ AIDS, etc.)/ Malignancies (cutaneous or internal) (7) Patients not willing to attend treatment schedule regularly. (8) Patients suffering from cutaneous disorders like alopecia areata, halo nevus, Pityriasis alba, Malignant melanoma and Morphea.

Data collection tools

All the findings were recorded in the Case Report Form (CRF), designed for the study. Updated Kuppaswamy's socio-economic scale 2021 was used to assess demographic details and socioeconomic status. Patients were clinically examined and required hematological and biochemical investigations were done. Objective Parameters included Vitiligo Extent Tensity Index (VETI),⁵⁷ Dermatological Life Quality Index (DLQI),⁵⁸ and change in a photograph of skin lesion.

Monitoring and follow up: Patients fulfilling the inclusion criteria were enrolled in the present clinical study. The patients were assessed clinically by the relevant history taking, general physical examination, local dermatological examination, and other required parameters. SES was assessed by Kuppaswamy's socioeconomic scale modified for 2021. Assessment of Mizaj was done as per the parameters mentioned

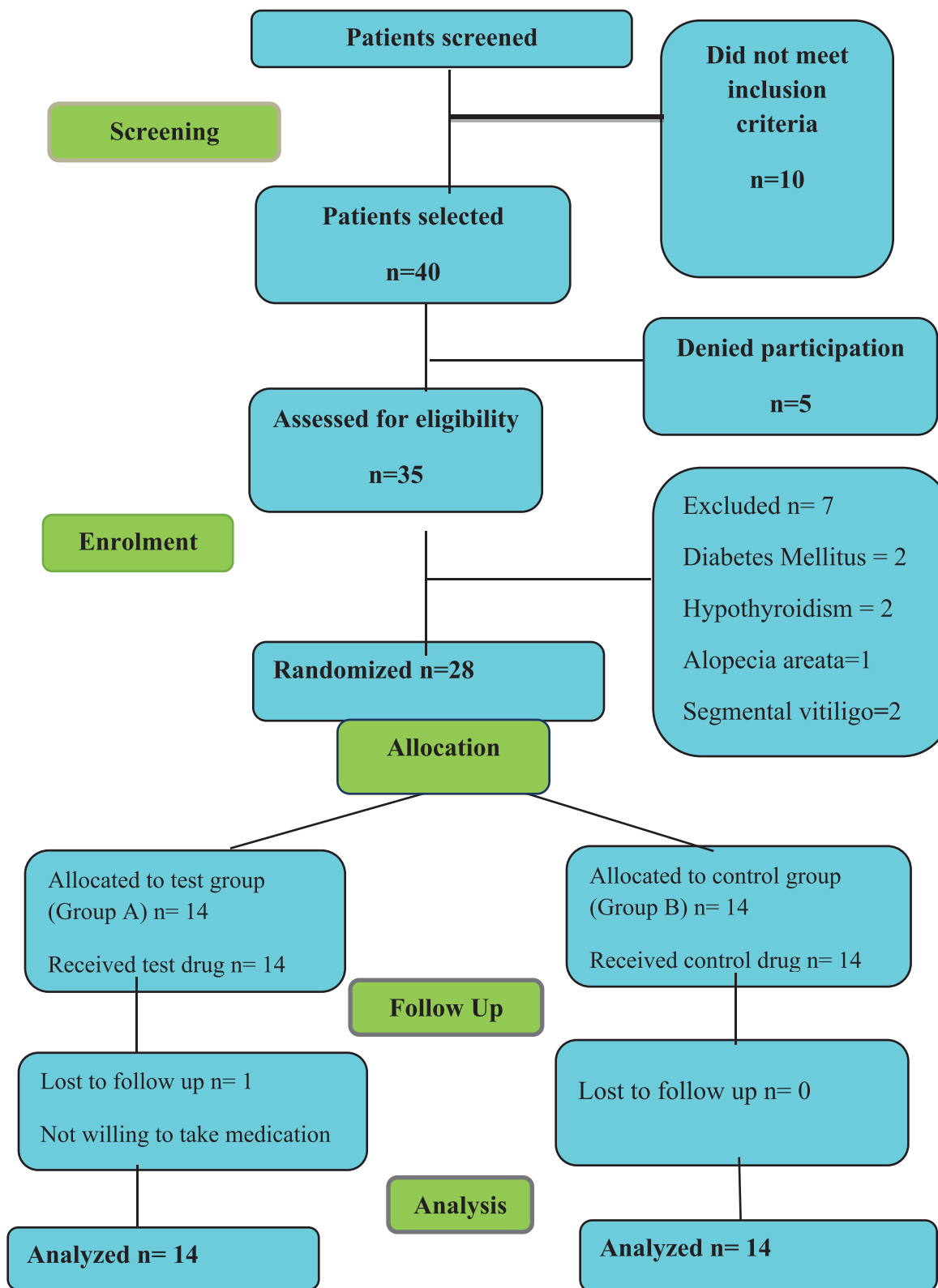


Figure 1: Consort diagram

in the classical Unani literature. All the information was recorded in the case record form designed for the study. After a thorough evaluation of the patients by history and clinical examination, diagnosed patients of vitiligo were advised for necessary lab investigations and were enrolled in the study after obtaining written voluntary informed consent. The treatment period was 60 days. Patients were asked to visit the hospital on every 15 days for a period of 60 days (Four visits after baseline i.e., 15th, 30th, 45th, and 60th day) and clinically assessed at every visit during the study. Any improvement/changes in objective parameters were recorded on every visit and laboratory investigations were done at baseline and after treatment. All adverse events were recorded at any time they happened, and all the patients were followed and clinically assessed fortnightly, and any suspicious events were closely monitored during the treatment phases of the trial. The participants were encouraged to report any symptoms and discomfort, even unusual ones. Failure to follow protocol therapy, non-compliance, and adverse reaction to the intervention were considered to be withdrawal criteria. Safety assessment was carried out by clinical examination and laboratory investigations.

Intervention: (Table 1 and 2)

These polyherbal Unani formulations were selected from Alqarabadeen,⁵⁹ Haziq⁶⁰, and Hamdard Pharmacopeia⁶¹. The ingredients of the Dawa-e- Baras include Gulnar (*Punica granatum* Linn), Babchi (*Psoralea corylifolia* Linn), Geru (*Bole rubra*), Gandhak (*Sulphur*), and ingredients of the Safuf-e-Baras include Anjir (*Ficus carica* Linn), Chaksu (*Cassia absus* Linn), Babchi (*Psoralea corylifolia* Linn), and Panwar (*Cassia tora* Linn). The formulations were prepared at institute pharmacy as per SOPs (Standard Operating Procedure) of Unani Formulary. The drug *Dawa-e- Baras* and *Safuf-e-Baras* was given to Group A and Group B respectively in a dose of 4.5gm orally once a day in the form of *Zulal* (decanted water) before breakfast and sediment was applied locally on affected parts in the form of paste once a day for 60 days. Participants were blinded regarding the assigned group.

Statistical methods for data analysis

The statistical software packages SPSS 28.0, were used for analysis of the data. Both Descriptive and inferential statistical analysis have been carried out in the present study. Results on continuous measurements were presented on Mean $\pm$ SD (Min-Max) and results on

categorical measurements were presented in Number (%). Significance was assessed at a 5 % level of significance. The normality of the data was assessed using Shapiro Wilk Test. Mann Whitney U test has been used to find the significance of study parameters between two groups (Intergroup analysis). Wilcoxon signed-rank test has been used to find the significance of study parameters within each group. The Chi-square/ Fisher Exact test has been used to find the significance of study parameters on a categorical scale between two groups. Independent sample t-test and paired t-tests have been used to find the significance of safety parameters on a continuous scale between two groups and within each group.

Table 1: Ingredients of the *Dawa-e- Baras*

1.	Gulnar	<i>Punica granatum</i> Linn	4.5gm
2.	Babchi	<i>Psoralea corylifolia</i> Linn	4.5gm
3.	Geru	<i>Bole rubra</i>	4.5gm
4.	Gandhak	Sulphur	4.5gm

Table 2: Ingredients of *Safuf-e-Baras*

1.	Anjir	<i>Ficus carica</i> Linn	100gm
2.	Chaksu	<i>Cassia absus</i> Linn	100gm
3.	Babchi	<i>Psoralea corylifolia</i> Linn	100gm
4.	Panwar	<i>Cassia tora</i> Linn	100gm

Table 3: Demographic Data

Characteristics	Group A(Dawa-e-baras)	Group B (Safuf-e-baras)	P Value
Age			
Mean $\pm$ SD	39.21 $\pm$ 13.63	38.21 $\pm$ 14.71	0.5473**
Gender			
F	6(42.9%)	5(35.7%)	0.699^
M	8(57.1%)	9(64.3%)	
Occupation			

Characteristics	Group A(Dawa-e-baras)	Group B (Safuf-e-baras)	P Value
Businessman	1(7.1%)	4(28.6%)	0.423**
Clerk	1(7.1%)	0(0.0%)	
Housewife	5(35.71%)	3(21.42%)	
Labour	4(28.6%)	4(28.6%)	
Student	3(21.4%)	2(14.3%)	
Teacher	0(0.0%)	1(7.1%)	
KSES			
Lower Middle	7(50.0%)	7(50.0%)	0.87**
Upper	1(7.1%)	0(0.0%)	
Upper Lower	5(35.7%)	5(35.7%)	
Upper Middle	1(7.1%)	2(14.3%)	
Mizaj			
Balghami (BAL)	12(85.7%)	12(85.7%)	0.419**
Damwi (DAM)	2(14.3%)	2(14.3%)	
Diet			
Mixed	9(64.3%)	10(71.4%)	0.686^
Vegetarian	5(35.7%)	4(28.6%)	
Family History			
Absent	12(85.7%)	12(85.7%)	0.59**
Present	2(14.3%)	2(14.3%)	

**Fishers exact test, ^Chi-square test

Table 4. Effect of Intervention on VETI SCORE

VETI SCORE	Group A (Dawa-e-baras)	Group B (Safuf-e-baras)	Mean Difference	P value^
	Mean ± SD	Mean ± SD		
Baseline	0.908±0.753	1.019±0.874	-0.003	0.91
15 th day	0.908±0.753	0.992±0.888	-0.084	0.982

VETI SCORE	Group A (Dawa-e-baras)	Group B (Safuf-e-baras)	Mean Difference	P value^
	Mean ± SD	Mean ± SD		
30 th day	0.872±0.753	0.902±0.816	-0.003	0.946
45 th day	0.713±0.671	0.768±0.738	-0.055	1
60 th day	0.663±0.668	0.586±0.699	0.077	0.511
Mean difference from baseline to 15th day				
	0	0.002		
P value#	1	0.317		
Mean difference from baseline to 30th day				
	0.036	0.117		
P value#	0.027*	0.012*		
Mean difference from baseline to 45th day				
	0.195	0.251		
P value#	0.008*	0.006*		
Mean difference from baseline to 60th day				
	0.245	0.433		0.031*
P value#	<0.0001*	<0.0001*	---	---

^Mann Whitney U test applied between the group comparisons

#Wilcoxon signed rank test applied within the group for pre and post comparisons.

Table 5. Effect of Intervention on DLQI SCORE

DLQI	Group A (Dawa-e-baras)	Group B (Safuf-e-baras)	Mean Difference	P value^
	Mean ± SD	Mean ± SD		
DLQI BT	14.86±4.294	13.50±4.703	1.36	0.511
DLQI AT	10.36±3.272	7.64±3.319	2.72	0.044*
Mean Difference	4.5	5.8	---	0.164
P value#	0.001*	<0.001*	---	---

^Mann Whitney U test applied between the group comparisons and

#Wilcoxon signed rank test applied within the group for pre and post comparisons.

Table 6. Statistical analysis of Safety Parameters

Assessment Variables	Group A (Dawa-e-baras) (n=14)	Group B (Safuf-e-baras) (n=14)	P* Value
SGOT(IU/L)			
Before Treatment	24.36±7.438	27.29±10.550	0.404
After Treatment	24.93±10.065	31.29±11.276	0.128
Difference	-0.571	-4	
P^ Value	0.825	0.113	
SGPT(IU/L)			
Before Treatment	21.00±10.160	31.50±18.007	0.069
After Treatment	25.00±10.038	34.93±21.302	0.127
Difference	-4	-3.43	
P^ Value	0.043	0.47	
Sr. Creatinine(mg/dl)			
Before Treatment	1.671±3.264	0.807±0.194	0.332
After Treatment	2.136±4.856	0.900±0.196	0.35
Difference	-0.464	-0.09	
P^ Value	0.296	0.084	
Blood Urea(mg/dl)			
Before Treatment	17.750±6.429	20.857±6.237	0.206
After Treatment	18.900±7.013	21.071±3.970	0.323
Difference	-1.15	-0.21	
P^ Value	0.081	0.873	

*Independent sample t test (intergroup analysis),

^Paired sample t test (Intragroup analysis)

Ethical clearance

The study was approved by the Institutional ethical committee NIUM, Bengaluru under IEC No: (NIUM/ IEC/ 2020-21/028/JT/01) and the protocol was registered with the clinical trial registry of India with CTRI registration number (CTRI/2022/04/041571).

RESULTS

A total of 50 patients were screened, out of them 22 did not meet the inclusion criteria and the remaining 28 were randomly allocated in a ratio of 1: 1 i.e., 14 each in Group A (*Dawa-e-baras*) and Group B (*safuf-e-baras*). Finally, 27 patients completed the course of treatment. Statistical analysis was carried out on Intention to Treat (ITT) basis on participants that received at least one dose of *Dawa-e-baras* or *Safuf-e-baras* and that underwent at least one assessment post baseline (Fig No. 1).

The study participants were similar in both groups in respect to baseline characteristics (Table 3). In Group A the mean VETI score before intervention was 0.908 ± 0.753 (Mean $\pm$ SD) and after intervention it was reduced to 0.663 ± 0.668 with a mean difference 0.245. In Group B the mean VETI score before intervention was 1.019 ± 0.874 (Mean $\pm$ SD) and after intervention it was reduced to 0.586 ± 0.699 respectively with mean difference 0.433. These findings show that both Group A and Group B show a highly significant reduction ($p < 0.0001$) in VETI score from baseline to 60th day by using the Wilcoxon signed rank test. Intergroup analysis showed that there was no significant difference in mean VETI score between the Group A and Group B before ($p = 0.910$) and after ($p = 0.511$) the treatment. On the analysis of data, it was found that the Group B had greater reduction than the Group A in mean difference from baseline after 60 days (control 0.433 v/s test 0.245) ($P = 0.031$) which was statistically significant using Mann Whitney U test. (Table 4)

In Group A the mean DLQI score before intervention was 14.86 ± 4.294 (Mean $\pm$ SD) and after intervention it was reduced to 10.36 ± 3.272 with the mean difference 4.5. In Group B the mean DLQI score before intervention was 13.50 ± 4.703 (Mean $\pm$ SD) and after intervention it was reduced to 7.64 ± 3.319 respectively with mean difference 5.8. These findings show that, in the Group A and Group B, a highly significant reduction ($p < 0.001$) was observed in DLQI score from baseline to 60th day by using Wilcoxon signed rank test. On the analysis of data, it was also found that the Group B had greater reduction than the Group A in mean difference (control 5.8 v/s test 4.5) from baseline to after 60 days which was not statistically significant ($p = 0.164$). In the present study, there was no significant difference in mean DLQI score between the Group A and Group B before treatment ($P = 0.511$) and there was

a significant difference in mean DLQI score after the treatment ($P=0.044$). (table 5)

The safety profile of both polyherbal Unani formulations were assessed before starting and after completion of treatment, with Hb%, TLC, DLC, SGOT (AST), SGPT (ALT), Blood Urea and Serum Creatinine. This study evidences that all safety parameters were found within the normal range and there was no significant difference in pre and post data (Table no.6). This indicates that the oral administration of both interventions is safe without recognizable adverse effects. Figure 2 and Figure 3 depict images illustrating the progression or changes observed in patients before and after undergoing treatment.



Figure 2: Photograph of patient showing a vitiligo lesion on the leg before and 60 days after treatment, showing repigmentation.

DISCUSSION

Our study assessed the efficacy of Dawa-e-Baras and Safuf-e-Baras in vitiligo patients. Results revealed significant improvements in VETI scores and DLQI from baseline to the 60th day within both groups ($p<0.0001$). Intergroup analysis showed a significant difference in DLQI ($p=0.044$) but not in VETI ($p=0.511$). On the analysis of data, it was found that Group B had greater reduction than Group A in mean difference from baseline after 60 days. These findings suggest a potential benefit of the treatments, particularly in enhancing patients' quality of life. Safety parameters

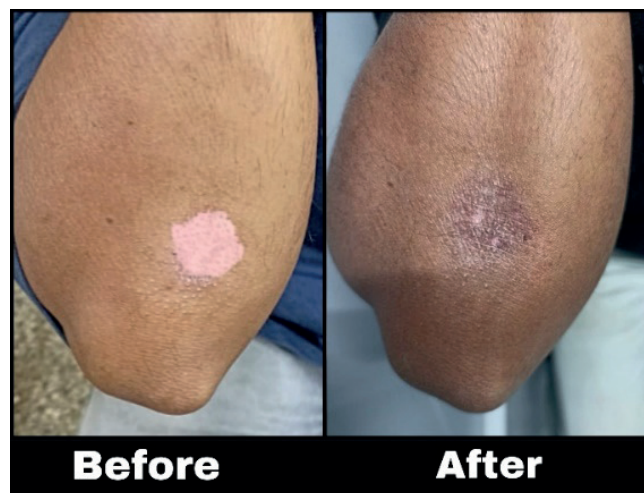


Figure 3: Photograph of patient showing a vitiligo lesion on the forearm near elbow before and after treatment, showing significant results.

remained within normal range for both groups, indicating the safety of the interventions. The Unani medicine has a collection of remedies known for their antioxidant properties, which assume a crucial role in addressing vitiligo, believed to be driven by oxidative stress.

One notable Unani remedy widely employed in the treatment of vitiligo is Dawa-e-Baras, which is provided in powdered form and was evaluated for its effectiveness in our clinical study. Safuf-e-Baras, a standardized medication utilized by Unani practitioners for vitiligo treatment, served as the control in our study. The constituents of dawa-e-baras, namely Gulnar (*Punica granatum* Linn), Babchi (*Psoralea corylifolia* Linn), Geru (*Bole rubra*), and Gandhak (Sulphur), contribute to its pharmacological activities. The significant effect of the interventions can be comprehended to the pharmacological action of the ingredients of Gandhak amla sar (blood purifying, anti-inflammatory, diuretic,⁶² Mujaffif, Jali, Muhallil, Jadhib- i- rutubat),^{63,64} Geeru (Qabid),⁶⁵ Gulnar (anti-inflammatory, antioxidant, diuretic,^{66,67} Qabid, Mujaffif, Radi).^{64,65} Babchi (Immunomodulator, Antioxidant, anti-inflammatory, hepatoprotective,⁶⁸ anti-leucodermic activity,⁶⁹ Musaffi-i-dam, Mohammir-i-jild, Amrad-e-fasad- i- khood).^{63,64,70} The benefits of these drugs in vitiligo may be due to their antioxidant property. Remarkably, many drugs used in vitiligo treatment possess antioxidant properties arising from the presence of polyphenols and flavonoids in them, likely operated

by donating electrons to neutralize free radicals⁷¹.

As previously mentioned, oxidative stress may serve as a catalyst for autoimmune destruction, ultimately leading to apoptosis, damage, and antigen exposure in melanocytes, thereby triggering vitiligo. So, recent research has placed greater emphasis on unraveling the mechanisms through which natural products combat oxidative stress in melanocytes. Through extensive exploration of targeted antioxidant therapy with Unani herbs, we predict a good approach to vitiligo treatment. While plants hold immense potential for skin care, additional research trials and clinical evidence are imperative, as the efficacy of many such extracts remains unverified. A cross-sectional study among pharmacy students showed strong belief and reliance on traditional medicine, indicating high acceptability of herbal therapies within future healthcare practice⁷². Reviews on the role of vitamins in dermatological conditions highlight that micronutrients such as vitamins A, C, D, E, B12 and folate influence melanocyte function, oxidative stress, and immune regulation mechanisms central to vitiligo, thereby supporting the biological plausibility of antioxidant- and immunomodulatory-rich Unani formulations⁷³. Experimental work on plant extracts, including studies demonstrating anti-inflammatory and wound-healing effects, further reinforces the therapeutic potential of botanicals similar to those used in Unani medicine⁷⁴. Additionally, reviews on herb–drug interactions emphasize the need for rigorous scientific evaluation and standardization of traditional therapies, underscoring the significance of conducting controlled trials such as the present study⁷⁵. Collectively, these findings align with and strengthen the rationale behind assessing Unani polyherbal interventions as safe, acceptable, and potentially effective options for managing non-segmental vitiligo.

This study aimed to compare the effectiveness of two traditional Unani medicines, namely Dawa-e-Baras and Safuf-e-Baras, in treating Baras (Vitiligo). Due to ethical reasons, placebo was not included in the study. The hypothesis is that the ingredients of Dawa-e-Baras may possess intrinsic properties that can prove to be

effective in treating Vitiligo. However, the results of the study suggest that both therapies are equally effective in reducing VETI scores and improving the quality of life of vitiligo patients.

The current study has certain constraints, such as a limited sample size, a relatively short study duration, and a restricted range of assessment parameters. Therefore, it is advisable for future clinical trials to consider larger sample sizes, extended study durations, and the incorporation of more comprehensive assessment scales.

CONCLUSION

The present findings shows that the *Dawa-e-Baras* was equally effective as *Safuf-e-Baras* in reducing VETI score in vitiligo patients and also improved the quality of life of these patients. It has also been observed that the safety parameters remained unchanged before and after the trial in both the Group A and Group B. Further studies are imperative to validate the outcome of this study and also to generate more evidences on the effectiveness of the interventions.

Source of fund: None

Conflict of Interest: None

Author contribution

Nadeem Ahmad Shah*: Conceptualization, study design, patient recruitment, clinical trial execution, data collection, statistical analysis, and drafting of the manuscript.
Mohammed Aleemuddin Quamri: Supervision, methodological guidance, critical review of the manuscript, and final approval of the version to be published.
Sabba Saltanat: Literature review, data analysis assistance, manuscript editing, and formatting in accordance with journal guidelines.
Mariyam Ahad: Clinical monitoring and contributed to the interpretation of results.

All authors have read and approved the final version of the manuscript.

REFERENCES

1. Taïeb A, Picardo M. The definition and assessment of Vitiligo: A consensus report of the Vitiligo European Task Force. *Pigment Cell Research*. 2007;**20**(1):27-35. doi:10.1111/j.1600-0749.2006.00355.x
2. Krüger C, Schallreuter KU. Cumulative life course impairment in vitiligo. In *Dermatological Diseases and Cumulative Life Course Impairment 2013*; **44**: pp. 102-117. Karger Publishers.
3. Lerner AB, Case JD. Part III: general considerations of skin pigmentation: pigment cell regulatory factors. *Journal of Investigative Dermatology*. 1959 Feb 1;**32**(2):211-21.
4. Lerner AB. On the etiology of vitiligo and gray hair. *The American Journal of Medicine*. 1971 Aug 1;**51**(2):141-7.
5. Sehgal VN, and Srivastava G. Vitiligo: Compendium of clinico-epidemiological features. *Indian J Dermatol Venereol Leprol* 2007;**73**(3):149-56.
6. Shajil EM, Agrawal D, Vagadia K, Marfatia YS, Begum R. Vitiligo: clinical profiles in Vadodara, Gujarat. *Indian journal of dermatology*. 2006 Apr 1;**51**(2):100.
7. Vora RV, Patel BB, Chaudhary AH, Mehta MJ, Pilani AP. A clinical study of vitiligo in a rural set up of Gujarat. *Indian journal of community medicine: official publication of Indian Association of Preventive & Social Medicine*. 2014 Jul;**39**(3):143.
8. Krüger C, Schallreuter KU. A review of the worldwide prevalence of vitiligo in children/adolescents and adults. *International journal of dermatology*. 2012 Oct;**51**(10):1206-12.
9. Shah H, Mehta A, Astik B. Clinical and sociodemographic study of vitiligo. *Indian journal of dermatology, venereology and leprology*. 2008 Nov 1; **74**:701.
10. Herane MI. Vitiligo and leukoderma in children. *Clinics in dermatology*. 2003 Jul 1;**21**(4):283-95.
11. Xuan Y, Yang Y, Xiang L, Zhang C. The role of oxidative stress in the pathogenesis of vitiligo: a culprit for melanocyte death. *Oxidative Medicine and Cellular Longevity*. 2022 Jan 22;2022.
12. Denat L, Kadekaro AL, Marrot L, Leachman SA, Abdel-Malek ZA. Melanocytes as instigators and victims of oxidative stress. *Journal of Investigative Dermatology*. 2014 Jun 1;**134**(6):1512-8.
13. Wei C, Jian Z, Wang L, Qiang H, Shi Q, Guo S, Li K, Huang Y, Liu L, Li Q, Luan Q. Genetic variants of the APE1 gene and the risk of vitiligo in a Chinese population: a genotype-phenotype correlation study. *Free Radical Biology and Medicine*. 2013 May 1; **58**:64-72.
14. Ramiah Abburi "VITILIGO". *Ind J Dermatol Venereol Leprol*. 1985; **51**: 247-255
15. Lerner A.B. "VITILIGO". *J Invest Dermatol*. 1959 ;**32**:285-310.
16. Bor S, Feiwel M, Chanarin I. Vitiligo and its aetiological relationship to organ-specific autoimmune disease. *British Journal of Dermatology*. 1969 Feb 1;**81**(2):83-8.
17. Lerner AB, Nordlund JJ. Vitiligo: What is it? Is it important? *Jama*. 1978 Mar 20;**239**(12):1183-7.
18. McGregor BC, Katz HI, Doe RP. Vitiligo and multiple glandular insufficiencies. *Jama*. 1972 Feb 7;**219**(6):724-5.
19. OCHI Y, DEGROOT LJ. Vitiligo in Graves' disease. *Annals of Internal Medicine*. 1969 Nov 1;**71**(5):935-40.
20. Hertz KC, Gazze LA, Kirkpatrick CH, Katz SI. Autoimmune vitiligo: detection of antibodies to melanin-producing cells. *New England Journal of Medicine*. 1977 Sep 22;**297**(12):634-7.
21. Lee AY, Youm YH, Kim NH, Yang H, Choi WI. Keratinocytes in the depigmented epidermis of vitiligo are more vulnerable to trauma (suction) than keratinocytes in the normally pigmented epidermis, resulting in their apoptosis. *The British Journal of Dermatology*. 2004;**151**:995-1003
22. Bondanza S, Maurelli R, Paterna P, Migliore E, Giacomo FD, Primavera G, et al. Keratinocyte cultures from involved skin in vitiligo patients show an impaired in vitro behaviour. *Pigment Cell Research*. 2007;**20**:288-300.
23. Boissy RE, Liu YY, Medrano EE, Nordlund JJ. Structural aberration of the rough endoplasmic reticulum and melanosome compartmentalization in long-term cultures of melanocytes from vitiligo patients. *The Journal of Investigative Dermatology*. 1991;**97**:395-404.
24. Moellmann G, Klein-Angerer S, Scollay DA, Nordlund JJ, Lerner AB. Extracellular granular material and degeneration of keratinocytes in the normally pigmented epidermis of patients with vitiligo. *Journal of Investigative Dermatology*. 1982 Nov 1;**79**(5):321-30.
25. RM H. Aberrations in T lymphocytes and natural killer cells in vitiligo. A flow cytometry study. *J Am Acad Dermatol*. 1986; **14**:733-7.
26. Ines D, Sonia B, Riadh BM, Amel el G, Slaheddine M, Hamida T, et al. A comparative study of oxidant-antioxidant status in stable and active vitiligo patients. *Archives of Dermatological Research*. 2006;**298**(4):147-152.
27. Nanja van Geel and Reinhart Speeckaert, Acquired Pigmentary Disorders, Christopher E. M. Griffiths, Jonathan Barker, Tanya Bleiker, Robert Chalmers, Daniel Creamer. *Rook's textbook of dermatology*. 9th edition. Oxford: *WileyBlackwell*; 2016;**88**:1-88.55
28. Morohashi M, Hashimoto K, Goodman TF, Newton DE, Rist T. Ultrastructural studies of vitiligo, Vogt-Koyanagi



- syndrome, and incontinentia pigmenti achromians. *Archives of Dermatology*. 1977 Jun 1; **113**(6):755-66.
29. Hara M, Toyoda M, Yaar M, Bhawan J, Avila EM, Penner IR, Gilchrist BA. Innervation of melanocytes in human skin. *The Journal of experimental medicine*. 1996 Oct 1; **184**(4):1385-95.
 30. Spritz, R. A., & Andersen, G. H. *Genetics of vitiligo*. *Dermatologic Clinics*, 2017; **35**: 245–255.
 31. Majūsi A. Kāmil us Sana'ah (Urdu Translation by Kantoori GH). New Delhi: *Idara Kitab us Shifa*; 2010.
 32. Tabri AASR. Firdous ul Hikmat (Urdu Translation Sambhali HMAS). New Delhi: *Idara Kitab us Shifa*; 2017
 33. Martis J, Bhat R, Nandakishore B, Shetty JN. A clinical study of vitiligo. *Indian journal of dermatology, venereology and leprology*. 2002 Mar 1; **68**:92.
 34. Kang S, Amagai M, Bruckner AL, Enk AH, Margolis DJ, McMichael AJ et al. Fitzpatrick's Dermatology. 9th ed. New York: *McGraw-Hill edu*; 2019.
 35. Parsad D, Dogra S, Kanwar AJ. Quality of life in patients with vitiligo. *Health and Quality of Life Outcomes*. 2003 Oct 23; **1**(1):1.
 36. Bin Saif GA, Al-Balbeesi AO, Binshabaib R, Alsaad D, Kwatra SG, Alzolibani AA, Yosipovitch G. Quality of life in family members of vitiligo patients: a questionnaire study in Saudi Arabia. *American journal of clinical dermatology*. 2013 Dec; **14**:489-95.
 37. Pazyar N, Hatami M, Yaghoobi R, Parvar SY, Radmanesh M, Hadibarhaghtalab M. The efficacy of adding topical 5-fluorouracil to micro-needling in the treatment of vitiligo: A randomized controlled trial. *Journal of Cosmetic Dermatology*. 2023 May; **22**(5):1513-20.
 38. Central Council for Research in Unani Medicine. *Monograph on Bars (vitiligo)*. Central Council for Research in Unani Medicine, Dept. of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy, Ministry of Health & Family Welfare, Govt. of India, 2006.
 39. Tabri AHABM. Al Moaljate Buqratiya (urdu translation by CCRUM) part 2nd. New Delhi: Central council for research in Unani medicine; 1997. p 199–200
 40. Rushd AWM. Kitabul kulliyat (urdu translation). 2nd ed. New Delhi: *Central council for research in Unani medicine*; 1987. 109-10 p
 41. Man G, Xu L, Wang Y, Liao X, Xu Z. Profiling phenolic composition in pomegranate peel from nine selected cultivars using UHPLC-QTOF-MS and UPLC-QQQ-MS. *Frontiers in Nutrition*. 2022 Jan 24; **8**:807447.
 42. Nuncio-Jáuregui N, Calín-Sánchez Á, Vázquez-Araújo L, Pérez-López AJ, Frutos-Fernández MJ, Carbonell-Barrachina ÁA. Processing pomegranates for juice and impact on bioactive components. In *Processing and Impact on Active Components in Food* 2015 Jan 1 (pp. 629-636). Academic Press.
 43. Beilke MA, Collins-Lech C, Sohnle PG. Effects of dimethyl sulfoxide on the oxidative function of human neutrophils. *The Journal of laboratory and clinical medicine*. 1987 Jul 1; **110**(1):91-6.
 44. Jha D, Debata L. A case of vitiligo treated by Sulphur. *Indian Journal of Research in Homoeopathy*. 2009; **3**(3):34-40.
 45. Vishwakarma A. A case report: Vitiligo treated by homoeopathy.
 46. Suryawanshi RM, Pawale SD. Therapeutic Potentials of Gairika with Special Reference to Charaka Samhita.ss
 47. Khare CP. Indian medicinal plants: an illustrated dictionary. *Springer Science & Business Media*; 2008 Apr 22.
 48. Vaidya AD. Reverse pharmacological correlates of ayurvedic drug actions. *Indian Journal of Pharmacology*. 2006 Sep 1; **38**(5):311.
 49. Bi S, Akhtar J, Bashir F, Alvi R. Pharmacological investigations of Babchi (*Psoralea corylifolia* Linn)–An important drug of Unani system of medicine. *International Journal of Unani and Integrative Medicine*. 2020; **4**(1):32-6.
 50. Sharma PC, Yelne MB, Dennis TJ. Database on Medicinal Plants used in Ayurveda, Central council for research in Ayurveda and Siddha, New Delhi, 2001; **2**:89-93
 51. Sebei K, Sbissi I, Zouhir A, Herchi W, Sakouhi F, Boukhchina S. Phylogenetic identification, phytochemical analysis and antioxidant activity of *Chamaecrista absus* var. *absus* seeds. *Journal of Plant Biology Research*. 2014; **3**(1):1-1.
 52. Sirappuselvi S, Chitra M. In vitro antioxidant activity of Cassia Tora Lin. *International research journal of Biological sciences*. 2012 Oct; **1**(6):57-61.
 53. Çalışkan O, Polat AA. Phytochemical and antioxidant properties of selected fig (*Ficus carica* L.) accessions from the eastern Mediterranean region of Turkey. *Scientia Horticulturae*. 2011 May 10; **128**(4):473-8
 54. Badgujar SB, Patel VV, Bandivdekar AH, Mahajan RT. Traditional uses, phytochemistry and pharmacology of *Ficus carica*: A review. *Pharmaceutical biology*. 2014 Nov 1; **52**(11):1487-503.
 55. Khemis A, Fontas E, Moulin S, Montaudie H, Lacour JP, Passeron T. Apremilast in combination with narrowband UVB in the treatment of vitiligo: a 52-week monocentric prospective randomized placebo-controlled study. *Journal of Investigative Dermatology*. 2020 Aug 1; **140**(8):1533-7.
 56. Suresh K, Chandrashekara S. Sample size estimation and Power Analysis for Clinical Research Studies. *Journal of Human Reproductive Sciences*. 2012; **5**(1):7. doi:10.4103/0974-

1208.97779

57. Feily A. Vitiligo Extent Tensity Index (VETI) score: a new definition, assessment and treatment evaluation criteria in vitiligo. *Dermatology practical & conceptual*. 2014 Oct;**4**(4):81.
58. Finlay AY and Khan GK. Dematology Life Quality Index (DLQI): a simple practical measure for routine clinical use. *Clin Exp Dermatol* 1994;**19**:210-216.
59. Kabīruddin HM. (2006). Al-Qarābādīn. CCRUM, New Delhi, *Taba 'Sāni*, pp. 761,1218- 19.
60. Khan MHA. Hāziq. Delhi: Jaseem Book Depo; 1983 p. 556
61. Said H. Hamdard Pharmacopiea of Eastern Medicine. Second (re. Said HM, editor. Delhi: Sri Satguru Publications, *Indian Book Centre*; 1997 ;p.153.
62. Mustehasan AM, Naushin S. Kibreet (Sulphur) A Unani Mineral Drug-Review. *IJSRBS*. 2020;**7**(3):121-6.
63. Kabeeruddin M. Makhzanul Mufradat. New Delhi: Idara Kitabul Shifa; 2007
64. Tariq N, Ahmad HD. Tajul Mufradat khwasul advia. Hussain SA, editor. New Delhi: *Idara kitab us shifa*; 2010.
65. Mohammad HA. Bustanul Mufradat. New Delhi: SH Offset press, *Idara kitab us shifa*; 2002.
66. Arun N, Singh D P Punica granatum: A review on pharmacological and therapeutic properties. Journal of Pharmaceutical Sciences and Research. 3. 1240-1245.
67. Fakudze NT, Aniogo EC, George BP, Abrahamse H. The Therapeutic Efficacy of Punica granatum and Its Bioactive Constituents with Special Reference to Photodynamic Therapy. *Plants*. 2022 Oct 24;**11**(21):2820.
68. Li CC, Wang TL, Zhang ZQ, Yang WQ, Wang YF, Chai X, et al. Phytochemical and pharmacological studies on the genus psoralea: a mini review. Evidence-Based *Complementary and Alternative Medicine*. 2016 Oct;2016.
69. Bi S, Akhtar J, Bashir F, Alvi R. Pharmacological investigations of Babchi (Psoralea corylifolia Linn)—An important drug of Unani system of medicine. *International Journal of Unani and Integrative Medicine*. 2020;**4**(1):32-6.
70. Anonymous. The Unani Pharmacopoeia of India. New Delhi: GOI (AYUSH); 2007
71. Reddy J, TV R, Hemachandran J. A Study on Phytochemical Analysis and Antioxidant Potentials of Plants used in the Treatment of Vitiligo. *European Journal of Biomedical and Pharmaceutical Sciences*. 2014;**1**(3):471-9.
72. Ashiq K, Bajwa MA, Khokhar R, Abid F, Tanveer S, Qayyum M, Ashiq S, Jameel S. A Cross-sectional analysis of knowledge, beliefs and practices regarding traditional medicine use among pharmacy students of different private and government universities of Lahore, Pakistan. *Bangladesh Journal of Medical Science*. 2022 Feb 25;**21**(2):323-34.
73. Iqbal S, Gul S. Report on Recent Advances in Treatment Options through Vitamins. *Bangladesh Journal of Medical Science*. 2021 Feb 1;**20**(2):234-40.
74. Shihab UA, Atiqah A, Ahmad NS, Isa NM. A Dose Optimization Study of Marantodes pumilum (Blume) Kuntze Extracts for Wound Healing Effect in Animal Model. *Bangladesh Journal of Medical Science*. 2022 May 21;**21**(3):659-68.
75. Glynn J, Bhikha R. Herbal products and conventional drugs—an uneasy alliance. *Bangladesh Journal of Medical Science*. 2019;**18**(1):24-9.