CellMed
Cellmed Orthocellular Medicine and Pharmaceutical Association

Original Article

Therapeutic Evaluation of a Topical Unani Formulation, *Tila-i Muhāsā* in *Buthūr Labaniyya* (Acne Vulgaris): A Randomized, Controlled Clinical Study

Mohd Azahar[1], Qamar Uddin[2*], Munawwar Husain Kazmi[3], Faiza Khatoon[1], Nazim Husain[1]

[1]MD Scholars, Department of Moalajat (Medicine), [2]Professor & HOD, Moalajat (Medicine), [3]Professor & HOD, Ilmul Advia (Pharmacology), National Research Institute of Unani Medicine for Skin Disorders (NRIUMSD), Hyderabad-500038, India

ABSTRACT

Introduction: *Buthūr Labaniyya* (Acne vulgaris) is a multifactorial disorder of the pilosebaceous units characterized by non-inflammatory and inflammatory clinical lesions. Several Unani medications have been used for centuries to treat acne.

Objectives: Evaluation of safety and efficacy of *Tila-i Muhāsā* in patients with acne vulgaris

Materials and Methods: This clinical study was conducted in patients with acne vulgaris. Patients applied *Tila-i Muhāsā* or 5% Benzoyl Peroxide (BPO) once daily for 6 weeks. Assessment of efficacy was carried out by Global Acne Grading System (GAGS) and Patient Global Assessment (PGA) scores. In addition, overall severity of acne was evaluated on a 5-point Cook's acne grading scale using photographic standards. Assessment of safety was performed through adverse drug reactions, local dermal tolerability, vital signs, and routine laboratory investigations.

Results: A total of 60 patients (30 in each group) completed 6 weeks of treatment. The mean percentage reduction in GAGS score at 6 weeks from baseline in Unani group (66.97%) and BPO group (59.09%) was statistically significant (P<0.0001). After 6 weeks of therapy, the mean percentage reduction in PGA score compared to baseline in Unani group (57.44%) and BPO group (50.23%) was statistically significant (P<0.0001). No serious adverse events were reported in both groups; however, mild adverse events occurred more frequently in BPO group (30%) compared to Unani group (10%).

Conclusion: *Tila-i Muhāsā* was found to be effective and safe in the treatment of acne vulgaris. However, further clinical studies with larger sample size and longer duration of therapy need to be conducted.

Keywords Acne vulgaris, *Buthūr Labaniyya*, Unani, *Tila-i Muhāsā*, Benzoyl peroxide

INTRODUCTION

Acne vulgaris (acne simplex or common acne) is a multifactorial disorder of the pilosebaceous units characterized by non-inflammatory and inflammatory clinical lesions. The non-inflammatory lesions are comedones, which may be either closed (whiteheads) or open (blackheads). The inflammatory lesions include papules, pustules, and nodules (Papadakis , 2019; Ralston *et al*, 2018; Bolognia *et al*, 2018; Goldsmith , 2012). The primary site of acne is the face (99%) and to a lesser degree the upper back (60%), and chest (15%) (Griffiths *et al*, 2016). These are sites of maximum density of sebaceous follicles (Patterson and Hosler, 2016). Post-inflammatory hyperpigmentation and scarring are common complications of acne, which may contribute to significant physical and psychosocial impact. Acne scarring can be minimized by early effective treatment (Griffiths *et al*, 2016; Patterson and Hosler, 2016; Goodman, 2001).

Acne affects approximately 633 million people globally, making it the 10th most prevalent disease worldwide as per the Global Burden of Disease 2015 (GBD 2015) (Vos *et al*, 2016). It is most common between the ages of 12 and 20 years, and affects over 90% of adolescents. It often begins around 10-13 years of age, lasts 5-10 years and usually resolves by age 20-25 (Ralston *et al*, 2018). The relationship between diet and acne remains a subject of controversy. However, various studies have shown that some dietary factors, including high glycaemic index (GI) foods, skimmed milk, nuts, chocolates, oily foods, whey protein supplements for body building, vitamin B12 supplementation, etc. may potentially trigger the development of acne (Bolognia *et al*, 2018; Griffiths *et al*, 2016).

Acne begins with the overproduction of sebum, often secondary to an increase in androgen levels (Zeind and Carvalho, 2018). The pathogenesis of acne is multifactorial, but four principal pathogenetic events identified are: (1) hyperkeratinization with occlusion of pilosebaceous duct due to retention of keratinous material in the follicle, (2) increased sebum production by sebaceous glands, (3) hypercolonization of pilosebaceous ducts by the gram-positive anaerobic diphtheroid *Propionibacterium acnes* (*P. acnes*), and (4) inflammation & release of inflammatory mediators to the skin (Papadakis *et al*, 2019; Ralston *et al*, 2018; Patterson and Hosler, 2016; Griffiths *et al*, 2016; Goldsmith *et al*, 2012; Williams *et al*, 2012). These various processes are interrelated and under hormonal and immune influence (Patterson and Hosler, 2016; Goldsmith *et al*, 2012).

Androgens stimulate sebaceous glands to produce more

*Correspondence: Qamar Uddin
E-mail: ccrumhqrsnd58@gmail.com
Received Apr 09, 2020; **Accepted** Apr 20, 2020; **Published** May 29, 2020
doi: http://dx.doi.org/10.5667/CellMed.2020.0015

sebum. The infundibulum of the sebaceous follicle is plugged due to hyperkeratinisation (retention hyperkeratosis), and this follicular plugging prevents drainage of sebum, leading to accumulation of keratin, sebum, and bacteria (acne bacillus) in the follicle. These impacted follicles are termed 'microcomedones'. Components of sebum, triglycerides and lipoperoxides may play a role in acne pathogenesis. There is overgrowth of *P. acnes*, normal flora of the pilosebaceous unit, which produce lipase, and this bacterial lipase converts triglycerides to free fatty acids and produce proinflammatory mediators (IL-I, TNF-α) that lead to an inflammatory response. These free fatty acids promote further bacterial clumping and colonization of *P. acnes*, incite inflammation, and may be comedogenic. Lipoperoxides also produce proinflammatory cytokines. *P. acnes* stimulate the release of hydrolases from neutrophils, which may damage the follicular wall. In addition, continued production of sebum and keratin, leads to rupture of the follicular wall, releasing the contents of the follicle (fatty acids, sebum, keratin, bacteria) into the dermis and initiating an inflammatory response. There is irritation by accumulated fatty acids, and foreign-body reaction to extra-follicular sebum. This inflammation in the dermis is responsible for the papules, pustules, and nodules. Intense inflammation leads to scars (Papadakis *et al*, 2019; Ralston *et al*, 2018; Griffiths *et al*, 2016; Patterson and Hosler, 2016; Wolff *et al*, 2013; Goldsmith *et al*, 2012; Sehgal, 2011).

Acne vulgaris is associated with significant psychological disability such as reduced self-esteem, anxiety, depression, and suicidal ideation (Barnes *et al*, 2012; Goodman, 2006). It is estimated that 30-50% of adolescents experience psychiatric disturbances due to acne (Goldsmith *et al*, 2012). The consequences can be devastating, leading to embarrassment, school avoidance, and life-long effects on ability to form friendships, attract partners, and acquire and keep employment. Even, mild acne can adversely affect the patients' quality of life (Ralston *et al*, 2018).

Acne treatment can reduce its severity and minimize scarring, but there is no known cure for acne. Therefore, the safe and most effective treatment for acne is needed. The goals of the treatment are to relieve discomfort, improve skin appearance, prevent scarring, and restore emotional well-being and self-esteem (Ralston *et al*, 2018; Zeind and Carvalho, 2018).

In Unani system of medicine *Buthūr Labaniyya* (acne vulgaris) is defined as an appearance of white papules on face and nose accompanied by discharge of viscous fluids from them. These are mostly due to the inflammation of sweat glands in young people (Anonymous, 2012). According to Ibn Sīna, acne lesions are small white eruptions on the nose and cheeks, which resemble a drop of condensed milk. Hence, in Unani system of medicine, acne vulgaris is termed as *Buthūr Labaniyya*, meaning eruptions of milk (Sīnā, 2010).

Renowned Unani physicians Zakariyya Rāzī, Ibn Sīnā, Ibn Hubal Baghdādī, Dā'ūd Antāki and Ḥakīm Akbar Arzānī have mentioned in their books, *Buthūr Labaniyya* (acne vulgaris) is a dermatological disorder of adolescents that present as whitish eruptions over the face caused by *Mādda Ṣadīdīyya* (purulent matter) or preponderance of *Ghalīz Mādda Balghamiyya* (thick phlegmatic matter), which enters the skin pores and is not resolved in the skin due to its viscosity (Rāzī, 1994; Sīnā, 2010; Baghdādī, YNM; Anṭākī, 2010; Arzānī, 2001).

In Unani System of Medicine, several polyherbal medications with *Jāli* (Detergent), *Muhalil* (Resolvent) and *Mujaffif* (Desiccative) properties have been used for centuries to treat *Buthūr Labaniyya* (Acne vulgaris). *Ṭila-i Muhāsā*, a topical polyherbal Unani formulation containing *Beikh-i Sosan* (*Iris ensata), Post-i Siras (Albizia lebbeck),* and *Barg-i Neem (Azadirachta indica)* is used in acne vulgaris, but there is no documentary clinical evidence on its efficacy and safety (Sīnā, 2010; Arzānī, YNM; Kabīruddīn, 1977). Therefore, a randomized controlled clinical study was conducted to evaluate the efficacy and safety of *Ṭila-i Muhāsā* versus 5% Benzoyl Peroxide (BPO) in patients with acne vulgaris on modern scientific parameters.

METHODS AND MATERIALS

This prospective, single-blind (assessor blinded), randomized, active-controlled, parallel-group clinical study was conducted in patients with acne vulgaris at Central Research Institute of Unani Medicine (CRIUM), Hyderabad (now upgraded to NRIUMSD). *Ṭila-i Muhāsā* was selected for the study from *Al-Qarābādīn* (a Pharmacopoeia of Unani Medicine). It is a topical polyherbal Unani formulation, containing three herbs, *Iris ensata, Albizia lebbeck*, and *Azadirachta indica* (Table 1). All the ingredients in equal weight were powdered in a pulveriser and passed through a sieve of mesh number 80 to prepare a fine powder. Thus, the test drug *Ṭila-i Muhāsā* in powder form was prepared and supplied in air tight containers by the Institute's GMP-certified Pharmacy. The study protocol was approved by the Institutional Ethics Committee (38-18/2015-16/CRIUM/Hyd/IEC/03/M) and registered in Clinical Trial Registry of India (CTRI/2017/12/010974). Written informed consent was obtained from all the participants before participating in the study. Patients younger than 18 years signed the assent for the study and their parents or legal guardians signed the informed consent form. Patients reached the age of consent during the study were re-consented at the next study visit.

Table 1. Composition of *Ṭila-i Muhāsā*

S. No.	Plant Drug	Botanical Name	Part Used	Quantity
1.	*Beikh-i Sosan*	*Iris ensata* Thunb.	Root	100 g
2.	*Post-i Siras*	*Albizia lebbeck* Linn. Willd.	Stem Bark	100 g
3.	*Barg-i Neem*	*Azadirachta indica* A. Juss.	Leaves	100 g

Patients clinically diagnosed as acne vulgaris attending the outpatient department (OPD) of CRIUM, Hyderabad, between August, 2018 and April, 2019 were screened for the study. Of the 113 patients screened during this period, 73 acne patients aged between 14 and 40 years were enrolled into this study. Patients below 12 and above 40 years of age, patients with other variants of acne (acne rosacea/ acne fulminans/ acne necrotica), pregnant and lactating women, mentally retarded persons, patients on corticosteroid or anticonvulsant therapy or taking oral contraceptives, patients with renal, hepatic, or severe cardiac co-morbidities and patients unable to attend treatment schedule regularly were excluded from the study. Patients who had used any topical and systemic treatments in previous 4 weeks were also excluded.

Each acne patient was subjected to a detailed medical history taking and a complete dermatological examination. The patients who met inclusion criteria were randomly allocated to test group (n=37) and control group (n=36) through block randomization method with block size of 4, using sequentially numbered, sealed, opaque envelopes containing computer-generated block

randomization codes supplied by the Institute's Statistics Unit. Of the 73 patients randomized, 30 patients in each group completed 6 weeks of treatment. Seven patients in test group and six patients in control group were lost to follow-up.

Unani treatment group (test group): Patients were given a topical Unani formulation, *Ṭila-i Muhāsā* in fine powder form and advised to make its homogenous paste with lukewarm water, and apply on the acne-affected skin of the prewashed & dried face at night and then wash the face with lukewarm water in the morning.

Benzoyl peroxide group (active control group): Patients were given a conventional allopathic drug, 5% Benzoyl Peroxide (BPO) cream and advised to use the medication in the same manner as paste in test group.

The duration of treatment was 6 weeks and patients were followed up at 2, 4, and 6 weeks of treatment. Treatment compliance was evaluated by examining the quantity of drug left at each follow-up visit, and <75% treatment compliance was considered for withdrawal of patients from the study. Concomitant use of any topical or systemic anti-acne medications was not allowed during the study.

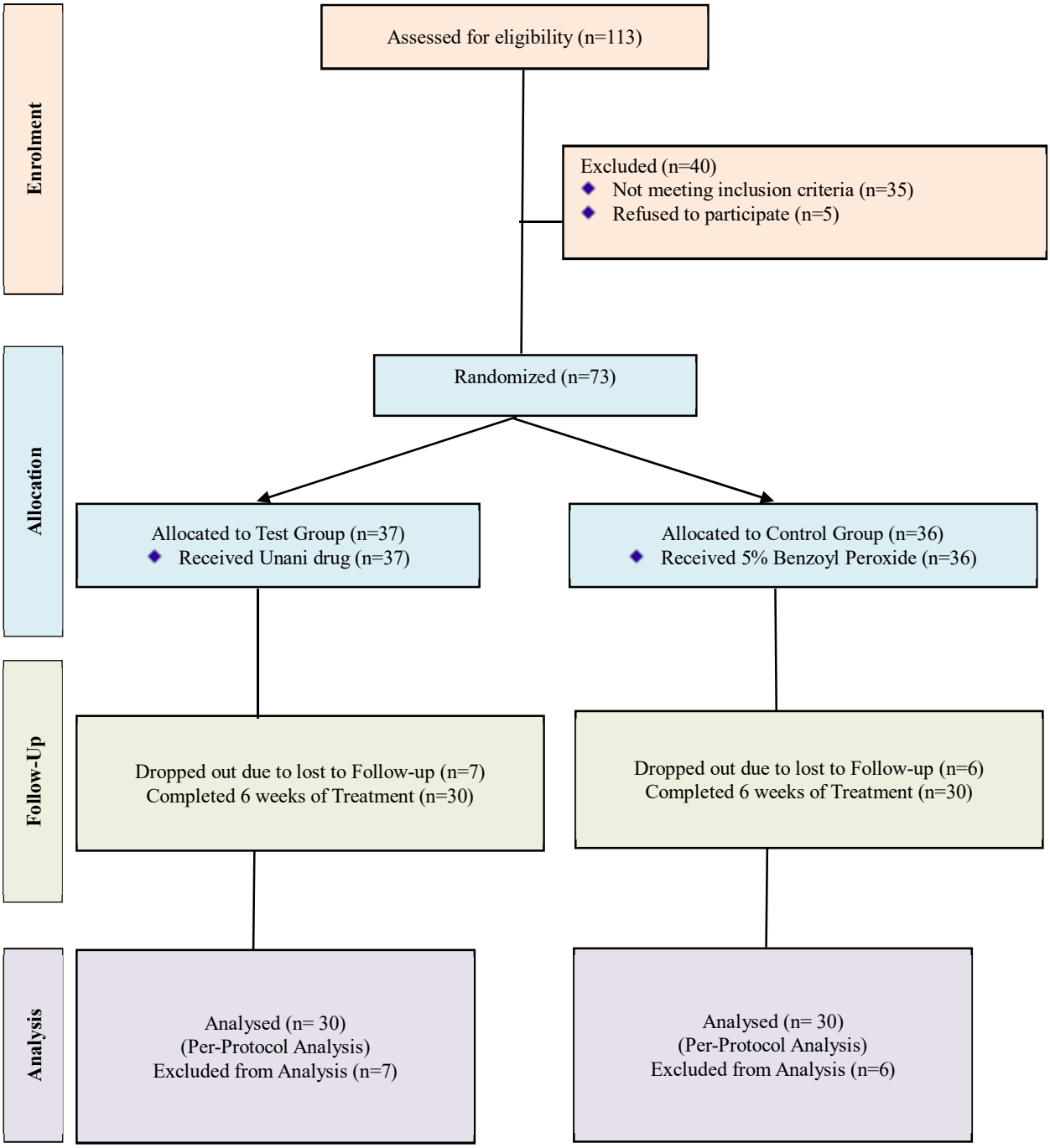

Fig. 1. CONSORT Flow Diagram of Study

Global Acne Grading System (GAGS) (Doshi *et al*, 1997; Adityan *et al*, 2009) and Patient Global Assessment (PGA) scores were used to assess clinical severity of acne and efficacy of treatment. PGA of acne severity was scored using 0-100mm horizontal Visual Analogue Scale (PGA-VAS), and patients were asked to place a vertical mark on the scale corresponding to their disease severity level at each clinical visit, and the distance in millimetres from zero to the patient's mark was measured and recorded. In addition, overall severity of acne was evaluated on a 5-point (0-8 grades) Cook's acne grading scale using photographic standards (Cook *et al*, 1979). Facial photographs of patients were taken by using digital zoom camera (Sony IMX398 Exmor RS Sensor, 16 MP f/1.7) with fixed distance and the same place at baseline and after 6 weeks of treatment. Assessment of safety was performed through adverse drug reactions, local dermal tolerability, vital signs at each follow-up visit, and routine laboratory investigations, including haemogram (Hb, TLC, DLC, ESR), urine examination (routine and microscopic), liver function tests (SGOT, SGPT, S. Alkaline Phosphatase, and S. Bilirubin) and kidney function tests (S. Creatinine and Blood Urea Nitrogen) at baseline and after treatment. Any local or systemic adverse effects were recorded at each clinical follow-up visit for both groups.

Statistical Analysis

Statistical analysis of data was performed on per-protocol (PP) basis, using the statistical software IBM SPSS Statistics 23.0 (Mac OS). Categorical variables were expressed as number (%), whereas continuous data were presented as mean ± standard deviation (SD). Chi-Square test was used to compare the categorical variables between the test and control groups. Continuous data were compared between the groups using independent samples t test. Paired t test was used to compare continuous data before and after treatment in each group, and P-value <0.05 was considered statistically significant. The primary outcome was a reduction in GAGS score from baseline to 6 week.

RESULTS

A total of 113 patients were screened, of them, 73 patients who met the eligibility criteria were enrolled in the study, and they were randomized with 37 patients receiving Unani treatment and 36 patients receiving 5% Benzoyl Peroxide (BPO). Of 73 patients randomized, 60 patients (30 in each group) completed 6 weeks of treatment; seven patients from Unani group and six patients from BPO group were dropped out of the study due to loss to follow-up. Data were analysed by per-protocol (PP) analysis, and 13 non-completers were excluded from the analysis (Fig. 1).

Clinico-demographic Profile

The mean age ± SD of patients in Unani treatment group was 22.47±6.08 years, 10 were male and 20 were female with male to female ratio 1:2. The mean age ± SD of patients in BPO Group was 21.13±5.12 years, 11 were male and 19 were female with male to female ratio 1:1.7. The mean disease chronicity ± SD in Unani treatment group was 5.4±4.2 months, and the mean disease chronicity ± SD in BPO Group was 3.97±2.66 months. There were no significant differences (P>0.05) in the baseline demographic and clinical characteristics of acne patients between Unani treatment group and BPO group (Table 2).

Table 2. Clinico-demographic Profile of Acne Patients in Unani and BPO Groups (PP Population)

Variables	Unani Group (n=30)	BPO Group (n=30)	*P*-Value
Age, Years (Mean±SD)	22.47±6.08	21.13±5.12	0.458
Age Distribution, years, n (%)			
14-20	19 (63.3)	14 (46.7)	
20-30	10 (33.3)	14 (46.7)	0.458
30-40	1 (3.4)	2 (6.6)	
Gender, n (%)			
Male	10 (33.3)	11 (36.7)	0.500
Female	20 (66.7)	19 (63.3)	
Age of Onset, Years (Mean±SD)	17.47±3.53	17.13±3.25	0.494
Disease Chronicity, months (Mean±SD)	5.4±4.2	3.97±2.66	0.739

PP = Per Protocol; SD = Standard Deviation

GAGS (Global Acne Grading System) Score

In Unani treatment group, the mean GAGS score was significantly reduced from 28.97±8.24 at baseline to 9.57±5.39 after 6 weeks of treatment (P<0.0001). In BPO group, the mean GAGS score was significantly reduced from 31.70±10.47 at baseline to 12.97±5.13 after 6 weeks of treatment (P<0.0001) (Table 3).

Table 3. GAGS Score in Unani Treatment Group vs BPO Group

Visit	GAGS Score			
	Unani Group (n=30)		BPO Group (n=30)	
	Mean±SD	*P*-value	Mean±SD	**P*-value
Baseline	28.97±8.24		31.70±10.47	
After 2 weeks	23.50±7.03	0.0076	25.93±9.65	0.0304
After 4 weeks	16.06±5.18	<0.0001	18.67±6.14	<0.0001
After 6 weeks	9.57±5.39	<0.0001	12.97±5.13	<0.0001

SD = Standard Deviation; *P = p-value of comparison from baseline visit (Paired t test)

Percentage Reduction in GAGS Score

The percentage reduction in the mean GAGS score after 6 weeks of treatment from baseline in the Unani treatment group was 66.97%, and in BPO group was 59.09% (Table 4).

Table 4. Percentage Reduction in Mean GAGS Score in Unani Group vs BPO Group

Visit	Percentage Reduction in GAGS Score (%)	
	Unani Group (n=30)	BPO Group (n=30)
Baseline	00.00	00.00
After 2 weeks	18.88	18.2
After 4 weeks	44.56	41.1
After 6 weeks	66.97	59.09

PGA Score on VAS

In Unani treatment group, the mean PGA score was significantly reduced from 72.83±9.79 at baseline to 31.0±13.48 after 6 weeks of treatment (P <0.0001). In BPO group, the mean PGA score was significantly reduced from 74.0±11.02 at baseline to 36.83±10.87 after 6 weeks of treatment (P <0.0001) (Table 5).

Table 5. PGA Score in Unani Treatment Group vs BPO Group

Visit	PGA Score			
	Unani Group (n=30)		BPO Group (n=30)	
	Mean±SD	*P*-value	Mean±SD	*P*-value
Baseline	72.83±9.79		74.0±11.02	
After 2 weeks	61.5±9.21	<0.0001	64.0±10.7	0.0007
After 4 weeks	44.83±8.79	<0.0001	50.67±11.12	<0.0001
After 6 weeks	31.0±13.48	<0.0001	36.83±10.87	<0.0001

Percentage Reduction in PGA Score

The percentage reduction in the mean PGA score after 6 weeks of treatment from baseline in the Unani treatment group was 57.44%, and in BPO group was 50.23% (Table 6).

Table 6. Percentage Reduction in Mean PGA Score in Unani Group vs BPO Group

Visit	**Percentage Reduction in Mean PGA Score(%)**	
	Unani Group (n=30)	**BPO Group (n=30)**
Baseline	00.00	74.0
After 2 weeks	15.56	13.51
After 4 weeks	38.45	31.53
After 6 weeks	57.44	50.23

Cook's Acne Severity Scale Score

The overall severity of acne measured by Cook's acne grading scale was significantly reduced after treatment in both groups. In Unani treatment group, acne severity grades on Cook's scale at baseline were 2, 4, and 6 in 1 (3.3%), 4 (13.3%), and 25 (83.4%) patients respectively, and after treatment, Cook's severity grades were 0, 2, and 4 in 8 (26.7%), 18 (60%) and 4 (13.3%) patients respectively. In BPO group, acne severity grades on Cook's scale at baseline were 2, 4, 6 and 8 in 1 (3.3%), 4 (13.4%), 24 (80%), and 1 (3.3%) patients respectively, and after treatment, Cook's severity grades were 0, 2, and 4 in 4 (13.3%), 24 (80%) and 2 (6.7%) patients respectively (Table 7).

Table 7. Cook's Acne Severity Score

Grade	**Unani Group (n=30), n (%)**		**BPO Group (n=30), n (%)**	
	Baseline	**After treatment**	**Baseline**	**After treatment**
0	---	8 (26.7)	---	4 (13.3)
2	1 (3.3)	18 (60.0)	1 (3.3)	24 (80.0)
4	4 (13.3)	4 (13.3)	4 (13.4)	2 (6.7)
6	25 (83.4)	---	24 (80.0)	---
8	---	---	1 (3.3)	---

Safety Evaluation

Clinical adverse effects, including dry skin, itching and burning at the site of application were reported in 3 (10%) patients in Unani treatment group and 9 (30%) patients in BPO group. Hyperpigmentation was observed in some patients, but it was considered to be post-inflammatory and was not associated with treatment. These adverse effects were minimal and self-limited, and no serious adverse events (SAEs) were reported in both treatment groups. Moreover, values of all haematological and biochemical safety parameters measured at baseline and after 6 weeks of treatment were within the normal range.

DISCUSSION

Acne vulgaris is a multifactorial disorder, in which the pilosebaceous units of the skin become plugged and distended, presenting as comedones, papules, pustules, or nodules (Zeind and Carvalho, 2018). Despite various scientific studies, complete pathogenesis of acne remains unclear, and a single, primary cause has not been identified. The development of acne involves the combination of four factors, including hyperkeratinization, excess sebum production, overgrowth of *P. acnes*, and inflammation (Bolognia *et al*, 2018; Williams *et al*, 2012). *Propionibacterium acnes* (now renamed *Cutibacterium acnes*), a gram-positive anaerobic bacterium is a member of the normal human skin microbiota essential for the maintenance of a healthy skin. It is predominant in pilosebaceous follicles, and it plays a critical role in the development of inflammatory lesions in acne (Ingham, 1999).

Anti-acne therapies exhibit one or more of the four mechanisms: normalizing follicular keratinization, decreasing sebum production, suppressing *P. acnes*, and reducing inflammation (Zeind and Carvalho, 2018). Oxidative stress-initiated inflammation and its maintenance within the pilosebaceous unit are important initial steps for the subsequent pathogenic processes of acne development. The favourable environment created by the oxidation of sebum for *P. acnes* to grow is also an important contributing factor for these steps. This mechanism suggests the oxidative stress as a therapeutic target in the treatment of acne vulgaris (Grange *et al*, 2009).

Long-term use of oral antibiotics is associated with the development of bacterial resistance and disruption of the gut microbiota. So, the use of antibiotics for acne is discouraged in clinical practice. Topical therapies are the most appropriate first-line treatment for comedonal acne. Benzoyl peroxide is an effective topical agent for the treatment of acne, which works through three mechanisms: antimicrobial, anti-inflammatory, and keratolytic effects (Zeind and Carvalho, 2018).

Ṭila-i Muhāsā, a topical polyherbal Unani formulation containing *Beikh-i Sosan (Iris ensata), Post-i Siras (Albizia lebbeck),* and *Barg-i Neem (Azadirachta indic*a) has been used as an effective topical treatment for acne vulgaris. This clinical study was conducted to evaluate the efficacy and safety of *Ṭila-i Muhāsā* in the treatment of acne vulgaris. In this study, *Ṭila-i Muhāsā* was compared with 5% Benzoyl Peroxide (BPO) in patients with acne vulgaris. *Ṭila-i Muhāsā* was effective in acne vulgaris, as it showed a statistically significant reduction in GAGS and PGA scores from baseline to 6 weeks of treatment (P<0.0001).

The GAGS score was significantly reduced after 6 weeks of treatment in BPO group also (P<0.0001), and no statistically significant difference was found between the efficacy of test and control drugs. Thus, both drugs were found to be equally

effective in the treatment of acne vulgaris. However, *Ṭīla-i Muhāsā* showed rapid onset of efficacy, as a significant reduction in GAGS score was evident as early as week 2 and GAGS score was continued to reduce till the end of treatment. Moreover, topical application of *Ṭīla-i Muhāsā* once daily for 6 weeks showed significant improvement in the overall severity of acne assessed by the Cook's acne severity grading scale.

In present study, none of the patients reported any serious adverse events (SAE) in both treatment groups. However, clinical adverse effects, including dry skin, itching and burning at the site of application were observed in both groups, but frequency of these adverse effects was higher (30%) in BPO group than in Unani treatment group (10%), and this difference was statistically significant. These adverse effects were minimal and self-limited. Hyperpigmentation was observed in some patients, but it was considered to be post-inflammatory and was not associated with treatment. Moreover, pathological and biochemical safety parameters were remained within normal limits after treatment. Thus, once-daily topical application of *Ṭīla-i Muhāsā* for up to 6 weeks did not exhibit significant adverse effects, and it was found to be safe and well tolerated during study.

The exact mechanism of action in the treatment of acne is not yet known. The antimicrobial, anti-keratinization, and anti-inflammatory and antioxidant effects have been implicated. In the present study, the analyzed results showing the efficacy of *Ṭīla-i Muhāsā* in acne vulgaris may theoretically be attributed to *Muḥalil-i-Waram* (anti-inflammatory), *Dāfi'-i-'Ufūnat* (antiseptic), *Musaffī-i-Dam* (blood purifier), *Muaḥmmir* (Rubefacient), *Jālī* (detergent), and *Mujaffif* (desiccative) properties of its ingredients as described in the classical texts of Unani Medicine. Its *Mujaffif* (Desiccative) property may help in reducing sebum production, and it may improve the post-inflammatory hyperpigmentation due to its *Jāli* (Detergent) property (Ali, 2010; Ibn Sīnā, 2010; Ghani, 2010; Anonymous, 2007; Hakīm, 2002; Anonymous, 1987).

Recent studies have indicated that inflammation and oxidative stress may play an early role in initiating the pathogenesis of acne. The activities of antioxidant defense enzymes, including superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in leukocytes are decreased in acne patients. Thus, the drugs with antioxidant effects may be valuable in acne treatment (Basak *et al*, 2001). In patients with acne, the cutaneous and systemic oxidative stress is increased as indicated by observation of oxidative stress biomarkers. Administration of local and systemic antioxidants appears to be effective against acne (Bowe *et al*, 2012).

It has been reported that *Iris ensata* (roots) possesses a broad spectrum antibacterial activity (Ganaie *et al*, 2018; Wagay and Jain, 2018) and a significant free radical scavenging activity. Its antioxidant activity may contribute the anti-inflammatory effects (Ganaie *et al*, 2018). The root extract of *Iris ensata* is used in cosmetic preparations for the prevention of skin roughness and ageing (Khare, 2007).

Albizia lebbeck (stem bark) has been reported to possess remarkable anti-inflammatory (Babu *et al*, 2009; Saha and Ahmed, 2009; Pramanik *et al*, 2005; Das *et al*, 2003), broad spectrum antibacterial (Ali *et al*, 2018; Abriham and Paulos, 2015; Tasnim *et al*, 2014; Salem *et al*, 2013; Chulet *et al*, 2010; Khare, 2007; Rashid *et al*, 2003; Ganguli and Bhatt, 1993), antioxidant & free radical scavenging (Ali *et al*, 2018; Abriham and Paulos, 2015; Tasnim *et al*, 2014; Zia-Ul-Haq *et al*, 2013; Suruse *et al*, 2013), immunomodulatory (Chaudhary *et al*, 2012), antiseptic, and anti-allergic activities (Khare, 2007).

Azadirachta indica (leaves) has been reported to possess antibacterial (Benisheikh *et al*, 2019; Ghonmode *et al*, 2013; Maragathavalli *et al*, 2012; Yerima *et al*, 2012; Aditi *et al*, 2011; Sarmiento *et al*, 2011; Mahfuzul Hoque *et al*, 2007; Khare, 2007), anti-inflammatory (Dinda *et al*, 2011; Schumacher *et al*, 2011; Mosaddek *et al*, 2008; Khare, 2007; Kaur *et al*, 2004; Jain and Basal, 2003; Chattopadhyay, 1998; Chattopadhyay *et al*, 1993), antioxidant (Patel *et al*, 2011; Ghimeray *et al*, 2009; Sithisarn *et al*, 2005), immunomodulatory (Durrani *et al*, 2008; Renu *et al*, 2003; Sadekar *et al*, 1998; Ray *et al*, 1996; Upadhyay *et al*, 1992; van der Nat *et al*, 1987), wound healing activities (Osunwoke *et al*, 2013; Barua *et al*, 2010), and skin renewal (epidermal turnover) effect (Wadher *et al*, 2009; Kamlesh *et al*, 2009). *Propionibacterium acnes*, an anaerobic pathogen, plays an important role in the pathogenesis of acne by inducing certain inflammatory mediators. *Azadirachta indica* possesses anti-inflammatory activity and it significantly suppresses the capacity of *P. acnes*-induced ROS and pro-inflammatory cytokines (IL-8 and TNF-α), the two important inflammatory mediators in acne pathogenesis (Jain and Basal, 2003). *Azadirachta indica* leaf extract is profoundly anti-microbial and capable of inhibiting the growth of *Propionibacterium acnes* (Abiya *et al*, 2018; Balakrishnan *et al*, 2011).

Thus, anti-acne effect of this combination of three ingredients in the form of *Ṭīla-i Muhāsā* shows that it may act by targeting the different steps in the pathogenesis of acne vulgaris, including inhibition of hyperkeratinization, reduction in sebum secretion, suppression of *P. acnes*, and reduction in inflammation by inhibiting the production of reactive oxygen species (ROS) and pro-inflammatory cytokines.

In summary, findings from the present study suggest that *Ṭīla-i Muhāsā* containing *Iris ensata, Albizia lebbeck* and *Azadirachta indica* may provide the safe and effective alternative treatment for acne vulgaris.

CONCLUSION

In this randomized controlled clinical trial, *Ṭīla-i Muhāsā* was successfully evaluated for its antiacne activity. There was no statistically significant difference in the efficacy of *Ṭīla-i Muhāsā* compared to 5% Benzoyl Peroxide (BPO) in the management of acne vulgaris; however *Ṭīla-i Muhāsā* reported less adverse effects than BPO. In conclusion, the results of the present study suggest that *Ṭīla-i Muhāsā*, a topical polyherbal Unani formulation containing *Beikh-i Sosan (Iris ensata), Post-i Siras (Albizia lebbeck),* and *Barg-i Neem (Azadirachta indica)* may be an effective, safe and well-tolerated topical medication in the treatment of acne vulgaris. These therapeutic results may probably be attributed to antioxidant, anti-inflammatory, immunomodulatory, and antimicrobial activities of the ingredients of *Ṭīla-i Muhāsā*. However, the limitation of this study were small sample size and short duration of therapy; to overcome these limitations, additional clinical studies with larger sample size and longer duration of therapy need to be conducted in the future, which could further reinforce the scientific evidence.

ACKNOWLEDGEMENTS

Authors express their sincere thanks to Dr. Tasleem Ahmed, Research Officer (Biochemistry), Dr. Syeda Hajra Fatima, Research Officer (Pathology), Technicians of NRIUMSD, and

all the patients who participated in this study for their cooperation in conducting the trial. Authors acknowledge the Central Council for Research in Unani Medicine (CCRUM), Ministry of AYUSH, Government of India, New Delhi, for providing financial support. The presenting author is also grateful to the Kaloji Narayana Rao University of Health Sciences (KNRUHS), Warangal, Telangana State for approval of this work as a part of his M.D. Dissertation.

CONFLICT OF INTEREST

The authors have no conflicting financial interests.

REFERENCES

Abiya SE, Odiyi BO, Falarunu LR, Abiya NU. Antimicrobial activity of three medicinal plants against acne-inducing bacteria *Propionibacterium acnes*. *Brazilian Journal of Biological Sciences*. 2018; 5(10):277-288.

Abriham H, Paulos B. *In vitro* Antioxidant and Antibacterial Activity of *Albizia lebbeck* (L) Benth Stem Bark. *Sci. Technol. Arts Res. J*. 2015; 4(2):204-206.

Aditi G, Bhandari BS, Rai N. Antimicrobial activity of medicinal plants *Azadirachta indica* A. Juss, *Allium cepa* L. and *Aloe vera* L. *Int. J Pharm Tech Res*. 2011; 3(2):1059-1065.

Adityan B, Kumari R, Thappa DM. Scoring systems in acne vulgaris. *Indian J Dermatol Venereol Leprol*. 2009; 75(3):323-326.

Ali MT, Haque ST, Kabir ML, Rana S, Haque ME. A comparative study of *in vitro* antimicrobial, antioxidant and cytotoxicactivity of *Albizia lebbeck* and *Acacia nilotica* stem bark. *Bulletin of Faculty of Pharmacy, Cairo University*. 2018; 56:34-38.

Ali SS. Unani Adviya Mufarrada, 4th Ed. (New Delhi, India: National Council for Promotion of Urdu Language, Ministry of HRD), pp. 57-58,275-276, 2010.

Anonymous. Standardization of Single Drugs of Unani Medicine, Vol-1,2,3,4. (New Delhi, India: CCRUM, Ministry of Health and Family), pp. 42-46,86-90,262-266,7-12,79-83,262-265,79-83,256-260, 1987.

Anonymous. The Unani Pharmacopoeia of India, Part-I, Vol.-2. (New Delhi, India: Dept. of AYUSH, M/o Health & FW), pp. 57,58,81,82, 2007.

Anonymous. The Unani Pharmacopoeia of India, Part-I, Vol.-4. (New Delhi, India: Dept. of AYUSH, M/o Health & FW), pp. 102,103, 2007.

Anonymous. Standard Unani Medical Terminology. (New Delhi, India: CCRUM, Dept. of AYUSH), pp. 303, 2012.

Anṭākī D. *Tadhkira Ūlī al-Albāb*. (New Delhi, India: CCRUM, Ministry of Health & Family Welfare), pp. 87, 2010.

Arzāni HMA. *Ṭibb-i Akbar*, Translated by Hussain HM. (Deoband, India: Faisal Publications), pp. 697,722.

Arzānī HMA. *Mīzān al-Ṭibb*, Translated by Ḥakīm Kabīr al-Dīn. (New Delhi, India: Idārā Kitāb al-Shifā), pp. 249, 2001.

Babu NP, Pandikumar P, Ignacimuthu S. Anti-inflammatory activity of *Albizia lebbeck* Benth., an ethnomedicinal plant, in acute and chronic animal models of inflammation. *Journal of Ethnopharmacology*. 2009; 125:356-360.

Baghdādī IH. *Kitāb al-Mukhtārāt fi'l Ṭibb*, Vol. 4. (New Delhi, India: CCRUM, Dept. of AYUSH, Ministry of Health & Family Welfare), pp. 188-189.

Balakrishnan KP, Narayanaswamy N, Subba P, Poornima EH. Antibacterial activity of certain medicinal plants against acne inducing bacteria. *Int J Pharma Bio Sci*. 2011; 2(3):476-481.

Barnes LE, Levender MM, Fleischer AB, Feldman SR. Quality of life measures for acne patients. *Dermatologic Clinics*. 2012; 30(2):293-300.

Barua CC, Talukdar A, Barua AG, Chakraborty A, Sarma RK, Bora RS. Evaluation of the wound healing activity of methanolic extract of *Azadirachta indica* (Neem) and *Tinospora cordifolia* (Guduchi) in rats. *Pharmacologyonline*. 2010; 1:70-77.

Basak PY, Gultekin F, Kilinc I. The role of the antioxidative defense system in papulopustular acne. *J Dermatol*. 2001; 28(3):123-127.

Benisheikh AAG, Muhammad FM, Kelluri H, Aliyu ZM, Mallam UB, Jibrin MW. Phytochemical Extraction and Antimicrobial Studies on Crude Leaf Extract of *Azadirachta indica* (Neem) in Semi-Arid Region of Borno State, Nigeria. *International Journal of Research & Review*. 2019; 6(12):516-522.

Bolognia JL, Schaffer JV, Cerroni L, Callen JP, Cowen EW, . Dermatology, Vol. 1, 4th Edn. (US: Elsevier Limited), pp. 588-603, 2018.

Bowe WP, Patel N, Logan AC. Acne vulgaris: the role of oxidative stress and the potential therapeutic value of local and systemic antioxidants. *J Drugs Dermatol*. 2012; 11(6):742-746

Chattopadhyay RR, Chattopadhyay RN, Maitra SK. Possible mechanism of anti-inflammatory activity of *Azadirachta indica* leaf extract. *Indian J. Pharmacol*. 1993; 25:99-100.

Chattopadhyay RR. Possible biochemical mode of anti-inflammatory action of *Azadirachta indica* A. Juss in rats. *Indian Journal of Experimental Biology*. 1998; 36(4):418-420.

Chaudhary M, Sharma AK, Kumar R, Chauhan B, Kaushik K, Agarwal V. Comparative Immunomodulator Activity of Leaves and Bark of *Albizia lebbeck* (Linn.) Benth. *Int. J. Res. Dev. Pharm. L. Sci.* 2012; 1(1):25-27.

Chulet R, Pradhan P, Sharma KS, Jhajharia KM. Phytochemical screening and antimicrobial activity of *Albizia lebbeck*, *J. Chem. Pharm. Res.*, 2010; 2(5): 476-484.

Cook CH, Centner RL, Michaels SE. An Acne Grading Method

Using Photographic Standards. *Archives of Dermatology*. 1979; 115(5):571-575.

Das AK, Ahmed F, Bachar SC, Kundu J, Dev S. Anti-inflammatory effect of *Albizzia lebbeck* (Benth.) Bark. *Online Journal of Biological Science*. 2003; 3:685-687.

Dinda A, Das D, Ghosh G, Kumar S. Analgesic and anti-inflammatory activity of hydro-alcoholic extract of *Azadirachta indica* Leaf. *Pharmacologyonline*. 2011; 3:477-484.

Doshi A, Zaheer A, Stiller MJ. A comparison of current acne grading systems and proposal of a novel system. *International Journal of Dermatology*. 1997; 36(6):416-418.

Durrani FR, Chand N, Jan M, Sultan A, Durrani Z, Akhtar S. Immunomodulatory and growth promoting effects of Neem leaves infusion in broiler chicks. *Sarhad J. Agric.* 2008; 24:655-659.

Ganaie AA, Mishra RP, Allaie AH. Antioxidant activity of some extracts of *Iris ensata*. *J Pharmacogn Phytochem*. 2018; 7(2):230-235.

Ganguli NB, Bhatt RM. Mode of Action of active principles from stem bark of *Albizia lebbeck*. *Indian J Experiment Biol*. 1993; 31:125-129.

Ghani N. Khazāin al Adviā. (New Delhi, India: Idāra Kitāb al-Shifā), pp. 242-3,370-1,482-5,595-6,616-9,720-1,800-1, 2010.

Ghimeray AK, Jin CW, Ghimire BK, Cho DH. Antioxidant activity and quantitative estimation of azadirachtin and nimbin in *Azadirachta indica* A. Juss grown in foothills of Nepal. *Afr. J. Biotechnol.* 2009; 8(13):3084-3091.

Ghonmode WN, Balsaraf OD, Tambe VH, Saujanya KP, Patil AK, Kakde DD. Comparison of the antibacterial efficiency of Neem leaf extracts, grape seed extracts and 3% sodium hypochlorite against *E. feacalis* - an *in vitro* study. *J Int Oral Health*. 2013; 5(6):61-66.

Goldsmith LA, Katz SI, Gilchrest BA, Paller AS, Leffell DJ, Klaus Wolff K. Fitzpatrick's Dermatology in General Medicine, 8th Edn. (US: McGraw-Hill Companies), pp. 897-917, 2012.

Goodman G. Acne and Acne Scarring - the case for active and early intervention. *Australian Family Physician.* 2006; 35(7):503-504.

Goodman GJ. Post-acne scarring: A short review of its pathophysiology. *Australas. J. Dermatol.* 2001; 42:84-90.

Grange PA, Chéreau C, Raingeaud J, Nicco C, Weill B, *et al*. Production of Superoxide Anions by Keratinocytes initiates *P. acnes*-induced Inflammation of the Skin. *PLoS Pathogens*. 2009; 5(7):1-14.

Griffiths C, Barker J, Bleiker T, Chalmers R, Creamer D. *Rook's Textbook of Dermatology,* 9th Edn. (US: John Wiley & Sons, Ltd.), pp. 90.1-50, 2016.

Hakīm MA. Bustān al Mufradāt Jadīd. (New Delhi, India: Idāra Kitāb al-Shifā), pp. 85,106,129,176,286,335-7, 2002.

Ibn Sīnā. Al Qānūn fi'l Ṭibb. (New Delhi, India: Idāra Kitāb al-Shifā), pp. 1420,1432, 2010.

Ingham E. The immunology of *Propionibacterium acnes* and acne. *Curr Opin Infect Dis*. 1999; 12(3):191-197.

Jain A, Basal E. Inhibition of *Propionibacterium acnes*-induced mediators of inflammation by Indian herbs. *Phytomedicine.* 2003; 10(1):34-38.

Kabīruddīn HM. *Bayāḍ-i Kabīr*, Vol. 2, 15th Edn. (Gujrat, India: Shaukat Book Depot), p. 126, 1977.

Kamlesh JW, Lakhotiya CL, Umekar MJ. Formulation and Evaluation of Cream of *Azadirachta indica* leaves extracts on Skin Renewal rate. *Int. J. ChemTech Res*. 2009; 1(1):88-95.

Kaur G, Alam MS, Athar M. Nimbidin suppresses functions of macrophages and neutrophils: relevance to its anti-inflammatory mechanisms. *Phytotherapy Research.* 2004; 18(5):419-424.

Khare CP. Indian Medicinal Plants - An Illustrated Dictionary, New York, USA: Springer Verlag, Berlin Heidelberg), pp. 30,75,76,336, 2007.

Mahfuzul Hoque MD, Bari ML, Inatsu Y, Juneja VK, Kawamoto S. Antibacterial activity of guava (*Psidium guajava* L.) and neem (*Azadirachta indica* A. Juss.) extracts against foodborne pathogens and spoilage bacteria. *Foodborne Pathog Dis.* 2007; 4(4):481-488.

Maragathavalli S, Brindha S, Kaviyarasi NS, Annadurai B, Gangwar SK. Antimicrobial activity in leaf extract of Neem (*Azadirachta indica* Linn.). *Int. J. Sci. Nat*., 2012; 3:110-113.

Mosaddek ASM, Rashid MMU. A comparative study of the anti-inflammatory effect of aqueous extract of Neem leaf and dexamethasone. *Bangladesh J. Pharmacol*. 2008; 3(1):44-47.

Osunwoke EA, Olotu EJ, Allison TA, Onyekwere JC. The wound healing effects of aqueous leave extracts of *Azadirachta indica* on wistar rats. *J. Nat. Sci. Res.* 2013; 3(6):181-186.

Papadakis MA, McPhee SJ, Rabow MW. Current Medical Diagnosis & Treatment. 58th Edn. (US: McGraw-Hill Education), pp. 134-136, 2019.

Patel P, Bhalodia Y, Gohil T, Malavia S, Devmurari V. In-vitro antioxidant activity of *Azadirachta indica* leaves. *J Advances Pharmacy Healthcare Res*. 2011; 1(3):22-7.

Patterson JW, Hosler GA. Weedon's Skin Pathology. 4th Edn. (USA: Churchill Livingstone, Elsevier Ltd.), pp. 459-461, 2016.

Pramanik K., Bhattacharya P, Chatterjee TK, Mandal SC. Anti-inflammatory activity of methanol extract of *Albizzia lebbeck* (Mimosaceae) bark. *Eur Bulletin Drug Res.* 2005; 13:71-75.

Ralston SH, Penman ID, Strachan MWJ, Hobson RP. Davidson's Principles and Practice of Medicine. 23rd Edn. (Edinburgh, Scotland: Elsevier Ltd.), pp. 1241-1243, 2018.

Rashid RB, Chowdhury R, Jabbar A, Hasan CM, Rashid MA. Constituents of *Albizia lebbeck* and antibacterial activity of an

isolated flavone derivatives. *Saudi Pharm. J.*, 2003; 11(1-2):52-56.

Ray A, Banerjee BD, Sen P. Modulation of humoral and cell-mediated immune responses by *Azadirachta indica* (Neem) in mice. *Indian J Exp Biol*. 1996; 34(7):698-701.

Rāzī ABMZ. *Kitāb al-Ḥāwi fi'l Tibb*. Vol. 23. (Aligarh, India: Saba Publishers), pp. 36-37, 1994.

Renu S, Rakha NK, Sandeep G, Mishra SK. Effect of Neem (*Azadirachta indica*) leaf extract administration on immune responses of broiler chickens. *J. Immunol. Immunopathol*. 2003; 5:47-50.

Sadekar RD, Kolte AY, Barmase BS, Desai VF. Immunopotentiating effects of *Azadirachta indica* (Neem) dry leaves powder in broilers, naturally infected with IBD virus, *Ind J Exp Biol.* 1998; 36(11):1151-1153.

Saha A, Ahmed M. The analgesic and anti-inflammatory activities of the extract of *Albizia lebbeck* in animal model. *Pak. J. Pharm. Sci*. 2009; 22(1):74-77.

Salem MZM, Aly H, Gohar Y, El-Sayed AW. Biological activity of extracts from *Morus alba* L., *Albizzia lebbeck* (L.) Benth. and *Casuarina glauca* Sieber against the Growth of some Pathogenic Bacteria. *Int J Agri Food Res*. 2013; 2(1):9-22.

Sarmiento WC, Maramba CC, Gonzales MLM. An in vitro study on the antibacterial effect of Neem (*Azadirachta indica*) leaf extracts on methicillin-sensitive and methicillin-resistant *Staphylococcus aureus*. *PIDSP Journal.* 2011; 12(1):40-45.

Schumacher M, Cerella C, Reuter S, Dicato M, Diederich M. Anti-inflammatory, pro-apoptotic, and anti-proliferative effects of a methanolic neem (*Azadirachta indica*) leaf extract are mediated via modulation of the nuclear factor-κB pathway. *Genes and Nutrition*. 2011; 6(2):149-160.

Sehgal VN. Textbook of Clinical Dermatology. 5th Edn. (New Delhi, India: Jaypee Brothers Medical Publishers), pp. 12-16, 2011.

Sīnā I. *Al-Qānūn fi'l Ṭibb*, Urdu Translation by Ḥakīm Ghulām Ḥasnayn Kintūrī. Vol. IV. (New Delhi, India: Idāra Kitāb al-Shifā), pp. 1420, 1432, 2010.

Sithisarn P, Supabphol R, Gritsanapan W. Antioxidant activity of siamese neem tree. *J Ethnopharmacol.* 2005; 99(1):109-112.

Suruse PB, Bodele SB, Duragkar NJ, Saundankar YG. *In-Vitro* Evaluation of Antioxidant Activity of *Albizia lebbeck* Bark. *Int. J. Biol. Sci. Ayur. Res*. 2013; 1(1):6-17.

Tasnim J, Saha A, Ahmed S, Sultana N, Muslim T, Rahman MA: Biological Studies of the Bark of *Albizia Lebbeck* (L.) Benth. *Int J. Pharm Sci Res*. 2014; 5(11):4969-4974.

Upadhyay SN, Dhawan S, Garg S, Talwar GP. Immunomodulatory effects of Neem (*Azadirachta indica*) oil. *Int. J. Immunopharmacol*. 1992; 14(7):1187-1193.

van der Nat JM, Klerx JP, van Dijk H, de Silva KT, Labadie RP. Immunomodulatory activity of an aqueous extract of *Azadirachta indica* stem bark. *J Ethnopharmacol*. 1987; 19(2):125-131.

Vos T, Allen C, Arora M, Barber RM, Bhutta ZA, . Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet*. 2016; 388(10053):1545-1602.

Wadher KJ, Lakhotiya CL, Umekar MJ. Skin renewal effect of different extracts of leaves of *Azadirachta indica*. *Int. J. PharmTech Res*. 2009; 1(4):1350-1353.

Wagay JI, Jain K. Phytochemical Analysis and Antimicrobial Activity of Iris kashmiriana and Iris ensata Extracts against Selected Microorganisms, *J Drug Delivery & Therapeutics*, 2018; 8(6):28-34.

Williams HC, Dellavalle RP, Garner S. Acne vulgaris. *Lancet. 2012;* 379(9813)*:*361-372*.*

Wolff K, Johnson RA, Saavedra AP. Fitzpatrick's Color Atlas and Synopsis of Clinical Dermatology. 7th Edn. (New York, USA: McGraw-Hill Education), pp. 2-7, 2013.

Yerima MB, Jodi SM, Oyinbo K, Maishanu HM, Farouq AA, Junaidu AU. Effect of Neem extracts (*Azadirachta indica*) on bacteria isolated from adult mouth. *J. Basic & Appl. Sci*. 2012; 20:64-67.

Zeind CS, Carvalho MG. Applied Therapeutics: the clinical use of drugs. 11th Edn. (Philadelphia, US: Wolters Kluwer Health), pp. 824-831, 2018.

Zia-Ul-Haq M, Ahmad S, Qayum M, Sezai E. Compositional studies and antioxidant potential of *Albizia lebbeck* (L.) Benth. pods and seeds, *Turk J. Biol.*, 2013; 37:25-32.