



A Review on Polyherbal Gel Used in Treatment of Acne

¹Pranay Bharat Pawar, ²Assistant Prof.-Mayuri Bhoir, ³Chetan Manohar Patil, ⁴Divya Vilas Patil, ⁵Mrunali Balkrushna Patil, ⁶Riya Ravindra Patil

¹C-43 sector no: 2, 301, Shantinagar, Miraroad (E), Post code-401107 INDIA.

²Ideal institute of pharmacy, Tal. -Wada, Dist.-Palghar, Post code-421303 INDIA.

³206, Neel Sagar Apt Shraddhanand Road, Vileparle East, Post Code-400057, INDIA.

⁴At. Post umroli, Tal. -Palghar, Dist.-Palghar, Post code-401404 INDIA.

⁵At. Post Parol Tal. Vasai, Dist.- Palghar, Post code- 401303 INDIA.

⁶Saphale Tal. -Palghar, Dist.-Palghar, Post code- 401102 INDIA.

ABSTRACT:

Acne vulgaris is a common skin condition that affects a large number of individuals worldwide. Despite the availability of several treatment options, many of them have undesirable side effects or are not effective for all individuals. Polyherbal gels have recently gained interest as a natural and effective treatment option for acne vulgaris. These gels are made up of a combination of herbal extracts that work synergistically to target the underlying causes of acne. This review article aims to summarize the current research on the efficacy and safety of polyherbal gels for acne vulgaris. The article discusses the various mechanisms of action of these gels, including anti-inflammatory, antimicrobial, and antioxidant properties, which can effectively control the factors responsible for acne formation. Additionally, the review examines the tolerability and safety of polyherbal gels, highlighting their potential as a promising new treatment option for individuals with acne. Overall, the evidence suggests that polyherbal gels have the potential to offer a safe and effective treatment for acne vulgaris, with fewer side effects than traditional treatments.

Keyword: Acne vulgaris, Herbal, poly herbal, gel, effective, side effects, traditional medicine, semi solid dosage form

Introduction:

Polyherbal formulations are herbal preparations that combine two or more therapeutically effective plant extracts. Due to their low toxicity, lack of negative side effects, and potential therapeutic benefits, these formulations, which have been utilized in traditional medicine for many years, are now receiving attention from the modern medical community. Polyherbal gels are topical preparations that combine a variety of plant extracts in a foundation of gel to make them easier to apply to the skin. They can be used to provide anti-comedogenic, anti-inflammatory and anti-microbial effects as per the ratio of the amount of herbal extract used in preparation of polyherbal gel. (Reference 1) They have the ability to provide increased bio availability and better absorption rate for herbal extract used. Idea of polyherbal remedies is derived from Ayurvedic knowledge, a traditional medical system with its roots in India for more than three thousand years. The origins of Ayurveda, its pharmacological principles, and the value of polyherbal formulations in Ayurvedic therapy have always been effective and reliable. According to Ayurveda, each plant has its own set of characteristics and applications, and the mixing of various herbs in particular ratios can produce synergistic effects that increase the medicinal action of the formulation. There are different ways that polyherbal formulations are made in Ayurvedic medicine, which include decoctions, powders, pastes, and oils. The use of multi-herbal treatments for a variety of illnesses, such as diabetes, cancer, heart disease, and skin conditions including acne vulgaris. (Reference 2) Acne vulgaris is one of the most commonly found skin diseases which could be caused by many bacterial strains ranging from P. acnes to streptococcus and staphylococcus. (Reference 3) they can be caused by certain number of factors ranging from hormonal to diet and lifestyle. (Ref 4)

Concept of Polyherbal Formulation Against Acne Vulgaris:

Polyherbal formulations are herbal products that contain a mixture of multiple plant extracts with therapeutic properties. These formulations have been used in traditional medicine for centuries and have gained renewed interest in modern medicine due to their low toxicity, fewer side effects, and potential therapeutic benefits. Polyherbal gels are topical formulations that contain a mixture of plant extracts in a gel base, which facilitates their application on the skin. Several studies have investigated the use of polyherbal gels for the treatment of acne vulgaris. In one study, a polyherbal gel containing extracts of *Azadirachta indica*, *Curcuma longa*, and *Ocimum sanctum* was formulated and evaluated for its efficacy in the treatment of acne vulgaris. The gel was applied twice daily on the affected areas for four weeks, and the results showed a significant reduction in the number of comedones, papules, and pustules. (Ref 1)

A polyherbal formulation comprising extracts of *Azadirachta indica*, *Centella asiatica*, and *Curcuma longa* was tested for effectiveness in treating acne vulgaris in a randomized controlled pilot study. According to the research, the polyherbal formulation reduced the number of acne lesions just as well as a conventional treatment that included benzoyl peroxide. (Ref 5)

Another study evaluated the efficacy of a polyherbal gel containing extracts of *Aloe vera*, *Azadirachta indica*, and *Curcuma longa* in the treatment of acne vulgaris. The gel was applied twice daily on the affected areas for six weeks, and the results showed a significant reduction in the number of acne lesions and improvement in the overall appearance of the skin. (Ref 6)

The potential advantages of polyherbal formulations over single-herb preparations, such as broader therapeutic activity, reduced side effects, and improved patient compliance. A study that evaluated the efficacy of a polyherbal formulation containing extracts of *Gymnema sylvestre*, *Momordica charantia*, and *Curcuma longa* in reducing blood glucose levels in patients with type 2 diabetes. The study found that the polyherbal formulation was effective in reducing fasting and postprandial blood glucose levels compared to placebo. The authors also mention a study that investigated the anticancer activity of a polyherbal formulation containing extracts of *Withania somnifera*, *Tinospora cordifolia*, and *Ocimum sanctum*. The study found that the polyherbal formulation exhibited potent cytotoxic effects against cancer cells in vitro and in vivo. (Ref 2)

The use of polyherbal formulations is a novel approach for the treatment of acne vulgaris. There are potential benefits of using polyherbal formulations as an alternative or complementary therapy for this condition. A study that investigated the efficacy of a polyherbal cream containing extracts of *Azadirachta indica*, *Curcuma longa*, and *Hemidesmus indicus* in reducing acne lesions. The study found that the polyherbal dose was effective in reducing the number and severity of acne lesions compared to placebo. Another study evaluated the efficacy of a polyherbal gel containing extracts of *Aloe vera*, *Azadirachta indica*, and *Curcuma longa* in reducing acne lesions. The study found that the polyherbal gel was effective in reducing the number and severity of acne lesions and improving skin texture compared to placebo. (Ref 7)

Acne:

Common skin conditions such as comedones, papules, pustules, nodules, and/or inflamed infectious growths like cysts are indicative of acne vulgaris. It is a multifactorial disorder caused by several factors, including increased sebum production, bacterial colonization of the pilosebaceous unit, and inflammation. Various topical agents are available for the treatment of acne vulgaris, including antibiotics, retinoids, benzoyl peroxide, and azelaic acid. However, the use of polyherbal formulations has gained popularity in recent years due to their potential benefits against acne.

Acne is a chronic inflammatory skin condition that affects the hair follicles and sebaceous glands. The most common types of acne include non-inflammatory acne (such as whiteheads and blackheads) and inflammatory acne (such as papules, pustules, and nodules). Factors that contribute to the development of acne include genetics, hormonal changes (such as during puberty or pregnancy), stress, diet, and certain medications. The authors also note that the presence of the bacterium *Propionibacterium acnes* can contribute to the development of acne. The authors explain that the pathogenesis of acne involves several interconnected factors, including increased sebum production, hyper keratinization (an abnormal buildup of skin cells), inflammation, and bacterial colonization. They also describe the role of various inflammatory mediators, such as cytokines, in the development of acne lesions. The condition can be treated by both topical and systemic therapies. Topical treatments may include benzoyl peroxide, retinoids, and antibiotics, while systemic therapies may include oral antibiotics, hormonal therapies (such as oral contraceptives), and isotretinoin (a potent retinoid). Acne is classified as inflammatory and non-inflammatory, where the Non-inflammatory type has higher chances of leaving a scar on skin surface, whereas inflammatory acne is generally more severe than non-inflammatory acne. Some examples of inflammatory type acne include Papules, Pustules, Nodules, Cysts. The non-inflammatory type of acne consists of blackheads and whiteheads. (Ref 3)

Microbial or bacterial colonization of the pilosebaceous unit is the largest factor. The pilosebaceous unit is the area of the skin that contains hair follicles and sebaceous glands. The bacterium *Propionibacterium acnes* (*P. acnes*) is one of the most common microbial strains associated with acne vulgaris. However, recent research has identified several other microbial strains that may also contribute to the development of acne, including:

1. **epidermidis Staphylococcus (S. epidermidis):** This bacterium is a normal resident of the skin but may contribute to the development of acne by producing enzymes that break down sebum and promote inflammation.
2. **Staphylococcus aureus (S. aureus):** This bacterium is another normal resident of the skin but can cause infection in individuals with weakened immune systems. In acne vulgaris, *S. aureus* may contribute to inflammation and exacerbate the disease.
3. **Malassezia species:** These fungi are also normal residents of the skin but may contribute to the development of acne by inducing inflammation and stimulating sebum production.

Targeting these microbial strains may be an effective approach for treating acne vulgaris. For example, topical and oral antibiotics may be used to target *P. acnes* and other bacteria, while antifungal agents may be used to target *Malassezia* species. (Ref 4)

Gram-negative folliculitis (GNF):

Another type that causes severe levels of acne vulgaris is Gram-negative folliculitis (GNF). Gram-negative bacteria are a type of bacteria that are often associated with infections and can play a role in the pathogenesis of acne vulgaris. The authors specifically discuss several strains of bacteria that have been found in association with acne, including *Propionibacterium acnes* (*P. acnes*), *Staphylococcus epidermidis*, and various Gram-negative bacteria such

as *Escherichia coli*, *Klebsiella* species, and *Pseudomonas aeruginosa*. While *P. acnes* is the most commonly isolated bacterium in acne, Gram-negative bacteria are also frequently found in acne lesions, particularly in cases of inflammatory acne. GNF is a rare complication of acne vulgaris that is associated with the overgrowth of Gram-negative bacteria in the hair follicles, leading to the formation of pustules and nodules. The examples of bacteria found in Gram-negative bacteria are *Escherichia coli*, *Klebsiella* species, and *Pseudomonas aeruginosa*, etc. Gram-negative bacteria can produce various inflammatory mediators, such as lipopolysaccharides and endotoxins, which can trigger an immune response and contribute to the formation of inflammatory acne lesions. They have a pathogenesis of production of various inflammatory mediators, such as lipopolysaccharides and endotoxins, which can trigger an immune response and contribute to the formation of inflammatory acne lesions. They can also produce enzymes that can break down sebum, which may contribute to the formation of comedones and further exacerbate the inflammatory response in acne lesions. (Ref 8)

Designing and development of polyherbal formulation:

Designing of polyherbal formulations by Ayurveda, which emphasizes the use of natural substances and the concept of synergy among various plant components to achieve therapeutic effects. Furthermore, the article highlights the need for standardization and quality control of polyherbal formulations to ensure their safety and efficacy. The authors suggest that modern scientific methods, such as chromatography and spectroscopy, can be used to identify and quantify the active constituents of herbal formulations and to ensure consistency and reproducibility. (Ref 9)

The process is split into two phases as given below:

Phase a:

In the first phase, a survey is conducted of herbal medicines used traditionally for the treatment of acne. The selected five herbs that were commonly used and had potential anti-acne activity were: *Salvia officinalis*, *Zataria multiflora*, *Hypericum perforatum*, *Achillea millefolium*, and *Viola odorata*. The selected herbs were then subjected to phytochemical screening and evaluation of their antimicrobial activity.

Phase b:

In the second phase, the formulation of topical herbal-based products takes place by using the five selected herbs. The formulations were prepared by using a gel base like carbomer or natural origin, with different concentrations of the herbal extracts. The chosen five herbs are as follows: Neem: antibacterial, antifungal, anti-inflammatory, and antiseptic properties, Tea tree oil: antibacterial, antifungal, and anti-inflammatory properties, Aloe vera: anti-inflammatory and wound-healing properties, Lavender: antibacterial and anti-inflammatory properties, Chamomile: anti-inflammatory and soothing properties. (Ref 10)

Herbs against acne vulgaris:

Various herbs and natural products commonly used in the treatment of acne vulgaris, including neem, aloe vera, turmeric, tea tree oil, and green tea. They are also compared for their efficacy for each herbal remedies with that of conventional therapies, such as topical retinoids, benzoyl peroxide, and antibiotics. The review article also discussed the limitations of conventional therapies, such as the development of antibiotic resistance and side effects like dryness and irritation of the skin. The authors suggested that herbal remedies may be a safer alternative to conventional therapies for the treatment of acne vulgaris. (Ref 11)

A study conducted by the authors by collection 22 different plant species from various regions of India and extracted their active compounds using organic solvents. The extracted compounds were then tested against a range of microorganisms, including bacteria and fungi by using agar well diffusion method. (Ref 12)

The results of the study showed that several of the plant species had significant antimicrobial activity. For example, extracts from the plants *Terminalia chebula*, *Emblca officinalis*, and *Terminalia bellerica* showed potent activity against a range of bacteria and fungi, including some antibiotic-resistant strains.

Some of the herbs screened in the study and their properties are:

<i>Azadirachta indica</i> (Neem)	antimicrobial activity against a variety of bacteria and fungi
<i>Allium sativum</i> (Garlic)	Garlic showed antimicrobial activity against both Gram-positive and Gram-negative bacteria.
<i>Curcuma longa</i> (Turmeric)	antimicrobial activity against several bacterial and fungal strains.
<i>Ocimum sanctum</i> (Holy basil)	antimicrobial activity against several bacterial and fungal strains
<i>Emblca officinalis</i> (Amla)	antimicrobial activity against several bacterial strains

Other Herbs Used Against Acne: (ref 13, 14, 15, 16)

Tea tree oil: anti-inflammatory, antibacterial, and antifungal

Aloe vera: Shows anti-inflammatory and antibacterial

Neem: anti-inflammatory, antibacterial, and antifungal

Turmeric: anti-inflammatory, antioxidant, and antibacterial

Licorice root: anti-inflammatory and antibacterial

Chamomile: anti-inflammatory and antioxidant

Green tea: anti-inflammatory and antioxidant

Witch hazel: shows astringent and anti-inflammatory

Some botanicals used in treatment of acne: (ref 17)

Calendula (*Calendula officinalis*): Calendula has anti-inflammatory and antimicrobial properties

Licorice (*Glycyrrhiza glabra*): Licorice contains compounds that have anti-inflammatory properties

Echinacea (*Echinacea purpurea*): Echinacea has antimicrobial properties and can help boost the immune system

Method Of prep: (ref 18, 19, 20, 21, 22)

There are several methods for preparing polyherbal gel, and the specific method used will depend on the intended use, the properties of the herbs, and the desired texture and consistency of the gel. Some common methods for preparing polyherbal gel include:

1. **Hot infusion method:** In this method, the herbs are steeped in hot water or oil to extract their active compounds. The resulting infusion is then mixed with a base material such as glycerin or aloe vera gel to create the gel.
2. **Cold infusion method:** This method is similar to the hot infusion method, but the herbs are steeped in cold water or oil instead. This method is often used for more delicate herbs that may be damaged by heat.
3. **Decoction method:** This involves simmering the herbs in water to extract their active compounds. The resulting liquid is then strained and mixed with a base material to create the gel.
4. **Emulsification method:** In this method, the herbal extracts are mixed with a base material and an emulsifying agent to create a stable emulsion. This method is often used for polyherbal gels that contain oils or other ingredients that are not water-soluble.
5. **Micronization method:** In this method, the herbs are ground into a fine powder, and the powder is then mixed with a base material to create the gel. This method is often used for herbs that are difficult to extract using traditional methods.
6. **Alcohol extraction method:** In this method, the herbs are soaked in alcohol to extract their active compounds. The resulting extract is then concentrated and mixed with a base material to create the gel.

Evaluation of Polyherbal gel:

The evaluation of polyherbal gel would depend on its quality and efficacy. The efficacy was determined by randomized, double-blind, vehicle-controlled study conducted to evaluate the efficacy and safety of a polyherbal formulation for the treatment of acne vulgaris as follows:

The polyherbal gel contained extracts of Aloe vera, Centella asiatica, and Camellia sinensis, and was compared to a vehicle gel (placebo) in the study.

A total of 60 patients with mild to moderate acne vulgaris were enrolled in the study, and they were randomized to receive either the polyherbal gel or the vehicle gel twice daily for four weeks. The primary efficacy endpoint was the reduction in the number of acne lesions (papules, pustules, and comedones) at the end of four weeks.

The results showed that the polyherbal gel was significantly more effective than the vehicle gel in reducing the number of acne lesions. At the end of four weeks, there was a 50.5% reduction in the number of acne lesions in the polyherbal gel group compared to an 18.7% reduction in the vehicle gel group. The polyherbal gel was also found to be safe and well-tolerated by the patients. (Ref 23)

Another study for efficacy was conducted on randomized 60 patients, with a polyherbal gel containing extracts of Aloe vera, Curcuma longa, and Azadirachta indica to a placebo gel. The study found that after 4 weeks of treatment, the polyherbal gel was significantly more effective than the placebo gel in reducing the number of acne lesions, with a reduction of 68.47% in the polyherbal gel group compared to 30.31% in the placebo group. (Ref 24)

Another study was conducted on randomized 60 patients, with mild to moderate acne vulgaris, comparing the efficacy of a polyherbal formulation containing extracts of Azadirachta indica, Curcuma longa, and Symplocos racemosa to a placebo formulation. The study found that after 6 weeks (about 1 and a half months) of treatment, the polyherbal formulation was significantly more effective than the placebo formulation in reducing the number of acne lesions, with a reduction of 60.3% in the polyherbal group compared to 32.5% in the placebo group. (Ref 25)

Another efficacy study with a formulation consisting of extracts of Azadirachta indica, Curcuma longa, Hemidesmus indicus, and Ocimum sanctum was compared to its placebo group. The results after 12 weeks (about 3 months) of treatment by the polyherbal formulation was significantly more effective

than the placebo formulation in reducing the number of acne lesions, with a reduction of 64.7% in the polyherbal group compared to 25.6% in the placebo group. (Ref 26)

A pilot study that evaluated the efficacy and safety of a polyherbal gel for the treatment of acne vulgaris containing polyherbal gel formulation with extracts of neem, turmeric, sandalwood, and aloe vera for 20 patients with mild to moderate acne vulgaris. The patients applied polyherbal gel twice daily to the affected areas for four weeks. The severity of acne was evaluated using the Global Acne Grading System (GAGS) and the Acne Severity Index (ASI) at baseline and at the end of the treatment period. The results showed a statistically significant reduction in the mean GAGS and ASI scores after four weeks of treatment with the polyherbal gel. (Ref 27)

The evaluation of quality was carried by Determining the following parameters:

A. Physical properties:

pH: this was determined by pH paper or pH meter. The standard value of pH for polyherbal gel is meant to be between 5 to 7. (Ref 28)

Appearance: the gel should appear uniformly, free from any Discoloration or any visible amount of residue.

Viscosity: the Brookfield viscometer is an ideal equipment for determining the viscosity of the gel. The standard value lies between 20,000 to 50,000 centipoises for poly herbal gel. (ref 29)

B. Chemical properties:

Solid matter: the percentage of solid matter in the poly herbal gel should be between 1% to 2% after drying a sample of gel at certain temperatures.

Drug content: The gel should contain a specified amount of active ingredients as per the label claim. (Ref 29)

C. Microbiological properties:

Microbial contamination: the gel should be free from microbial contamination, and the total aerobic microbial count should be less than 10^3 CFU/g. Total yeast or mold count should be less than 10^2 CFU/g. Salmonella and Escherichia strains of bacteria should be absent. (ref 30)

D. Stability:

The stability of poly herbal gel is carried out by an ideal device called Stability chambers. A typical stability study for a polyherbal gel may involve storing the gel at $25^\circ\text{C} \pm 2^\circ\text{C}$ and $60\% \pm 5\%$ relative humidity for a period of 3 months or more. During this time, the gel should be evaluated periodically for changes in physical appearance, pH, drug content, and microbial contamination. (Ref 29)

Conclusion:

For the development of innovative therapeutic treatments, plants are regarded as a significant source of potentially advantageous ingredients because the vast majority of them are safe and have little to no side effects. (s). As comparison to cream and ointment, topical administration of gels at pathological locations offers substantial advantages in a faster release of a medicine directly to the site of action. This study clearly indicated that all herbal ingredients have different chemical constituents and shows presence of antimicrobial activity.

Reference:

1. Kedia, S., Jain, S., & Jain, R. (2016). A review on herbal antidermatophytic formulations. *International Journal of Pharmaceutical Sciences and Research*, 7(6), 2340-2354.
2. Tiwari, V., Darmani, N. A., Ahmad, M., & Haque, S. (2017). Polyherbal formulation: concept of ayurveda. *Journal of traditional and complementary medicine*, 7(2), 234-244
3. "A Review of Epidemiology, Pathogenesis, and Treatment" by James Q. Del Rosso and Leon H. Kircik, published in *The Journal of Drugs in Dermatology* in 2013. PMID: 23377320.
4. "Microbial Strains in Acne Vulgaris: New Insights for Improved Therapy" by Christos C. Zouboulis, published in *Dermatology* in 2009. PMID: 19752542
5. Jain, A., Basal, E., & Inamdar, S. (2011). Clinical evaluation of polyherbal formulation in the management of acne vulgaris. *International Journal of Ayurveda Research*, 2(1), 22-26. doi: 10.4103/0974-7788.83176.
6. Singla S, Harjai K, Katare OP, Singh R. A polyherbal gel for the treatment of acne vulgaris: formulation and evaluation. *Drug Dev Ind Pharm*. 2012;38(5):574-581. doi:10.3109/03639045.2011.632109
7. Kaur, S., & Gupta, A. (2018). Polyherbal formulation: a novel approach in the treatment of acne vulgaris. *Journal of pharmaceutical sciences and research*, 10(5), 1125-1129.

8. Gram-Negative Folliculitis in Acne Vulgaris: A Review of Pathogenesis, Diagnosis, and Management" by Michael J. Alam and Jeffrey P. Callen, published in American Journal of Clinical Dermatology in 2003. PMID: 12762826.
9. Choudhary S, Bisht S, Bist SS. Polyherbal formulation: Concept of ayurveda. *Pharmacogn Rev.* 2014;8(16):73-80. doi:10.4103/0973-7847.134229
10. Moghimipour E, Afsordeh O, Hajimonfarednejad M, Taghizadeh M, Shakeri MT. Design and development of novel herbal-based formulations for treatment of acne. *Res Pharm Sci.* 2015;10(5):417-424.
11. Zaidi KU, Ali SA, Ali AS, Alam MS, Ahmad S. Acne vulgaris: Herbal remedies vs conventional therapy. *J Pharm Res.* 2012;5(2):932-935.
12. Ahmad I, Ahmad F, Pasha A. Screening of some Indian medicinal plants for their antimicrobial properties. *J Ethnopharmacol.* 1998;62(2):183-193. doi: 10.1016/S0378-8741(98)00055-9
13. Dudhe, R., Upadhaya, V., & Srivastava, S. (2020). Herbal Medicines for the Management of Acne Vulgaris: A Review of Recent Advances. *Phytotherapy Research*, 34(11), 2761-2785. PMID: 31990060.
14. Pillai, S. I., Sharma, P., & Mehta, M. (2020). Herbal remedies for acne vulgaris: a systematic review. *Journal of Dermatological Treatment*, 31(8), 832-841. PMID: 32003229.
15. Wagle, N., Pawar, S., & Pawar, P. (2021). Herbal Medicines for Acne Vulgaris: A Systematic Review. *International Journal of Pharmaceutical Sciences and Research*, 12(1), 240-254.
16. Javadi, H., & Rezaeizadeh, H. (2021). A review of herbal medicines for the treatment of acne vulgaris. *Current Pharmaceutical Design*, 27(13), 1425-1436. PMID: 33261580.
17. Abascal K, Yarnell E. Botanical treatments for acne. *Altern Complement Ther.* 2003;9(5):281-287. doi: 10.1089/107628003322166861
18. Rizvi, S. A., Shakir, S. A., & Zubair, M. A. (2012). Formulation and evaluation of herbal gel containing methanolic extract of *Ficus carica* for treatment of acne. *Journal of Pharmacy Research*, 5(5), 2698-2701.
19. Naik, J. B., & Dashputre, N. L. (2012). Development and evaluation of polyherbal gel for wound healing. *Indian Journal of Pharmaceutical Sciences*, 74(3), 220-225.
20. Shetty, S., & Udupa, S. (2013). Evaluation of herbal gel formulation of *Cucumis trigonus* Roxb. for anti-acne activity. *Journal of Young Pharmacists*, 5(2), 64-67.
21. Bhattacharjee, N., & Patel, R. (2018). Development of a herbal gel containing extracts of neem, tulsi and aloe vera for the treatment of acne vulgaris. *Journal of Applied Pharmaceutical Science*, 8(12), 133-137.
22. Jain, A., Kumar, D., & Jain, S. K. (2012). Preparation and evaluation of polyherbal gel containing *Azadirachta indica* and *Curcuma longa* for anti-acne activity. *Journal of Advanced Pharmaceutical Technology & Research*, 3(1), 48-53.
23. Halder S, Mehta AK, Mediratta PK, Sharma KK. Evaluation of efficacy and safety of a polyherbal formulation for acne vulgaris: a double-blind, randomized, vehicle-controlled study. *Indian J Dermatol Venereol Leprol.* 2017;83(2):155-160. doi:10.4103/0378-6323.200414
24. Gautam, N., Mantha, A. K., & Mittal, S. (2013). Evaluation of polyherbal gel for treatment of acne vulgaris. *Journal of pharmacology & pharmacotherapeutics*, 4(Suppl1), S51.
25. Naik, R. R., & Munshi, R. P. (2016). A randomized controlled study to evaluate the efficacy of polyherbal formulation in acne vulgaris. *International Journal of Green Pharmacy*, 10(4), 238-243.
26. Parekh, J., Chanda, S., & Ingle, P. (2011). Efficacy and safety of polyherbal formulation in acne vulgaris: a single-blind randomized controlled trial. *Ayu*, 32(3), 353-359.
27. Gupta M, Mahajan VK, Mehta KS, Chauhan PS (2004). Efficacy and safety of 3% polyherbal cream in mild to moderate acne vulgaris: A randomized, double-blind, placebo-controlled study. *Indian Journal of Dermatology, Venereology and Leprology*, 70(3), 163-165.
28. C. V. Patil et al., "Development and evaluation of herbal gel for acne," *International Journal of Drug Development and Research*, vol. 7, no. 3, pp. 227-233, 2015.
29. S. A. Patel et al., "Development and Evaluation of Polyherbal Gel for Treatment of Acne Vulgaris," *Journal of Pharmaceutical Sciences and Research*, vol. 4, no. 12, pp. 2402-2407, 2012. Viscosity
30. The United States Pharmacopeial Convention (2021). *United States Pharmacopeia (USP) General Chapter <61> Microbial Examination of Nonsterile Products: Microbial Enumeration Tests*. Ref no: USP 44-NF 39 microbial limit test