International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Optimizing Polyherbal gels for enhanced antibacterial efficacy in acne and skin disorders: A comprehensive formulation design and evaluation

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Abstract

Acne and bacterial skin disorders, prevalent and impactful, stem from factors like sebum production and androgen stimulation. They often cause psychological distress, emphasizing the need for effective yet safer treatments. Herbal remedies with minimal side effects have gained prominence for their antibacterial properties. Incorporating these properties into gel formulations has attracted global interest. This study aimed to develop polyherbal gels utilizing Rubia cordifolia extract, Aloe vera gel, and tea tree oil. Six formulations (PHG-1 to PHG-6) with varying concentrations were prepared. Evaluation encompassed physical parameters, skin irritation tests, stability studies, drug content estimation, in vitro drug release, and antibacterial efficacy against acne-derived bacteria, S. epidermis, and E. coli. The gels exhibited varying consistency, color and fragrance. All formulations showed no skin irritation in albino rats. Stability studies revealed no significant changes in physical parameters or drug content. In vitro studies unveiled diverse drug release profiles; PHG-3 and 5 demonstrated sustained releases over 150 minutes. Antibacterial assays indicated PHG - 4, 5, and 6 as significantly superior to the standard clindamycin gel. PHG-2 and 3 displayed similar activity, while PHG-1 showed lower efficacy. The formulation process, balancing Rubia, Aloe vera, and tea tree oil, showcased distinct properties in the gels. Aloe vera primarily contributed to structural aspects rather than antibacterial potency. PHG-5 emerged as a promising formulation due to notable antibacterial efficacy, sustained drug release, and favorable physical attributes. This comprehensive analysis underscores the intricate balance essential for optimal polyherbal gel performance, emphasizing PHG-5 as a potential candidate for further development.

Keywords: Antibacterial gels, Rubia, Aloe vera, Tea Tree oil, Acne

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1. Introduction

Acne and bacterial skin disorders affect a significant portion of the population at least once in their lifetime. The severity of these disorders hinges on factors such as sebum production and the stimulation of androgens [1]. Acne, particularly, is prevalent among nearly 80% of young individuals and, albeit less common, still occurs in adults and children [2]. Various physiological factors, including hyperproliferation of follicles and sebaceous glands, facilitate the growth and colonization of bacteria such as Staphylococcus, Propionibacterium, and E. coli. These gram-positive and gram-negative bacteria play crucial roles in the pathogenesis of acne and contribute to delayed wound healing. They prolong the inflammatory phase of wound healing and induce acne formation due to inflammation [3]. The attraction of neutrophils to the infection site, metabolization of triglycerides into fatty acids, and their accumulation further promote acne development and delay wound healing [4]. While acne and skin disorders mentioned above aren't life-threatening, they often lead to psychological conditions like low self-esteem, anxiety, and depression, significantly impacting the affected person's lifestyle and productivity [5]. Various topical drugs, including salicylic acid, nystatin, retinoids, and antibiotics like minocycline, erythromycin, and clindamycin, form part of chemotherapy for treating acne and bacterial skin disorders. Additionally, anti-inflammatory agents are recommended to alleviate pain and itching resulting from inflammation. However, these antibacterial chemotherapy drugs frequently lead to severe side effects and adverse drug reactions [6]. As a result, herbal

drugs and other external therapies are highly recommended for their antibacterial activity, gaining importance due to their minimal side effects [1][7][8]. Herbal drugs are extensively relied upon by the majority of the global population for their daily medical needs, particularly for managing skin diseases [9]. Topical application of herbal drugs at the infection site enhances drug release directly into the acne or bacterial site. Moreover, gel formulations offer advantages over other topical forms like creams or ointments [10]. This has drawn global researchers' attention to integrating antibacterial herbal components into gel formulations to treat infectious skin diseases [11].

Rubia cordifolia, a well-known Ayurvedic herb, is commonly used to treat bacterial skin infections, especially acne [12]. Tea tree oil exhibits significant effectiveness against both gram-negative and gram-positive bacteria, methicillin-resistant bacteria, and candida. Its topical cosmeceutical application for anti-acne purposes has shown fewer side effects compared to synthetic formulations and is also effective on wounds [13][14]. Additionally, Aloe vera gel possesses anti-inflammatory properties and efficiently combats topical bacterial infections [15]. Therefore, the current research aimed to incorporate the methanol extract of Rubia cordifolia into Aloe vera-based carbopol gels and other ingredients based on previous research [16][17]. Tea tree oil was also added to contribute to antibacterial activity alongside Rubia and Aloe vera. The prepared gels underwent evaluation for pH, viscosity, spreadability, stability, extrudability, in vitro drug release, and were examined for their antibacterial efficacy against bacteria extracted from the acne of human volunteers, S. epidermis, and E. coli, commonly found in skin wounds and infections.

2. Materials and Methods

2.1 Chemicals and Reagents

All chemicals and reagents utilized in the study were of analytical grade and meticulously sourced. Melaleuca alternifolia (Tea Tree) oil and anthraquinone analytical standard were obtained from Merck Ltd, India. Propylene glycol and triethanolamine were supplied by Fisher Scientific. Clindamycin gel (1% w/w, Wallace Pharmaceuticals Pvt. Ltd) was acquired from a local drugstore. Carbapol-940 and other necessary chemicals were procured from SD Fine Chem Ltd, India. Throughout the study, distilled and sterile water were consistently used. All remaining chemicals and solvents were of analytical grade and utilized as received.

2.2 Plant Collection and extraction

The dried stems of Rubia cordifolia and fresh Aloe vera leaves were obtained from a local supplier and underwent authentication by a certified botanist. A specimen was deposited in the department herbarium at the college. The Rubia stems were powdered and subjected to extraction using methanol in a soxhlet apparatus, followed by filtration. The resulting extract (27.43% w/w) was then desiccated using a rotary evaporator. For Aloe vera, gel extraction involved squeezing and scraping the fresh leaves. The yellow exudate was removed, and the translucent gel obtained was homogenized to achieve a free-flowing consistency. Both the Rubia extract and Aloe vera gel were stored in airtight containers at 4°C until further use.

2.3 Preparation of polyherbal gels

The materials specified in Table 1 were precisely weighed and utilized for the gel preparation process. Carbopol-940 was dispersed in 40ml of distilled water and left to soak overnight under mechanical stirring. In a 100ml flask, 50ml of distilled water was heated to 60°C, and potassium sorbate, along with propylene glycol and lauric acid, was dissolved with continuous stirring. Separately, the required quantity of Rubia extract was dissolved in 5ml of ethanol and ultrasonicated for 5 minutes to ensure dissolution and eliminate any immiscible lumps. This mixture was then blended into the above solution along with the overnight-soaked carbopol gel, ensuring even mixing through gentle stirring. After 20 minutes, the specified amount of Tea tree oil was introduced into the gel, and stirring continued for an additional 20 minutes. Parabens were incorporated to inhibit fungal growth and enhance gel stability. Triethanolamine was added to adjust the pH to neutral on the scale. Warm distilled water was used to adjust the volume to 100ml. The prepared gel was carefully stored in an airtight container for future use [18].

2.4 Evaluation of the polyherbal gels

2.4.1 Visual appearance:

The prepared polyherbal gels were inspected for their colour, greasiness, consistency and odour.

2.4.2 Determination of pH and Viscosity:

A digital pH meter was used to determine the pH and all the measurements were taken in ambient room temperature after calibrating the instrument. Viscosity of prepared polyherbal gels was determined using Brookfield viscometer at 50 rpm and 25°C [19]. Values were taken as triplicate readings.

2.4.3 Determination of Spreadability and Extrudability:

Spreadability and extrudability were performed using the methods described without alterations [20]. Standard formulae were used as per procedures prescribed.

2.4.4 Determination of Drug content:

1g of the gels was mixed in ethanol and serially diluted to make a solution of final concentration of 10mg/ml and subsequently Standard anthraquionone (0.5mg/ml) in ethanol was also prepared. They were analyzed using double beam UV spectrophotometer at 325nm and the absorbance values were taken as triplicates. Total anthraquinone content in the gels was determined and expressed as mg/g [21].

2.4.5 Stability studies:

The stability of the prepared gels was investigated using cycles of freezing and thawing. the prepared gels were stored at 4°C for 1week in refrigerator and then exposed to

40°C for another week in a hot air oven [16]. pH, Viscosity, Drug content were determined to identify and physical or chemical changes as a result of variable temperatures.

2.4.6 Determination of in-vitro Drug Release:

The Franz diffusion cell method was employed to assess the in vitro drug release from the prepared gels. A quantity of 25mg of gel was placed on the semipermeable membrane composed of cellulose acetate, with the diffusion medium being a phosphate buffer solution with a pH of 6.8. The temperature of the medium was maintained at 37°C while gently stirring it continuously. At regular time intervals of 15 minutes, 30 minutes, 60 minutes, 120 minutes, and 180 minutes, 1ml of the solution were collected from the medium. Each time a sample was withdrawn, an equal volume of fresh solution was added to maintain sink conditions. The collected solutions were subsequently analyzed for drug content using a UV spectrophotometer set at 325nm. The percentage of drug release was calculated based on these measurements [22].

2.4.7 Determination of antibacterial activity of polyherbal gels

a) Collection and culture of bacteria:

The strains of Staphylococcus epidermis and E. coli were obtained from the microbiology lab and subsequently incubated in nutrient Agar medium (Merck Millipore) for 48 hours under anaerobic conditions to facilitate their growth. For the human bacterial swab, a volunteer clinically diagnosed with acne vulgaris was chosen. The volunteer's face was cleansed with distilled water and dried using a sterile cotton wipe. A visible comedone from one of the pimples was gently ruptured using a sterile acne needle, and the exudate was collected using a sterile cotton swab. This swab was then immersed in 5ml of distilled water for 5min. The solution obtained was then evenly spread onto freshly prepared solid agar medium in petri dishes. All the petri dishes were incubated at 37°C for approximately 48 hours to allow for the proliferation of bacterial colonies [23].

b) Antibacterial activity of the gel

The antibacterial activity was assessed using the disc diffusion method against the three types of bacteria. Bacterial cultures were collected and the seed solution was adjusted with BHI broth to achieve a bacterial count of 108cells/ml. Subsequently, 10ml of this solution was evenly spread onto solid agar medium in petri plates. Circular filter paper discs, 1cm in diameter, were prepared and allowed to soak in the formulated gel preparations and Clindamycin gel 1% w/w (Wallace Pharm Pvt Ltd) for 5 minutes. Once thoroughly soaked, these discs were placed at the center of the agar plates and then incubated for 72 hours. Following incubation, the zone of inhibition was measured in millimeters, starting from the edge of the circular disc to the point where bacterial growth began. Values were recorded in triplicate for each individual experiment to ensure consistency and reliability of the results [1].

3. Results

Six formulations were created by adjusting carbopol, Rubia extract, and Aloe vera gel concentrations while maintaining a constant drug concentration of 4% w/w. These gels exhibited a clear, pale greenish-brown color with a jelly-like texture and varying consistency. The inclusion of tea tree oil imparted its distinct fragrance to the gels. The pH levels of the gels ranged between 6.7-7.0, effectively adjusted using triethanolamine. Viscosity analysis revealed a spectrum of values, detailed in Table 2. PHG-6, which contained the highest carbopol concentration, exhibited the highest viscosity at 6637 cps. Conversely, PHG-1, with the lowest carbopol concentration, was flowable and displayed the least viscosity, indicating the influence of carbopol concentration on gel consistency. While PHG-3 contained a decent carbopol concentration, its viscosity was comparatively lower than the second formulation, suggesting that tea tree oil concentration also impacts gel thickness. Spreadability increased as viscosity decreased, with PHG-1 being flowable and PHG-6 being thick and creamy. PHG-2, 4, and 5 demonstrated an ideal consistency with good spreadability. Additionally, extrudability appeared directly linked to spreadability, where PHG-3 and 5 displayed good extrudability with minimal effort, while PHG-1 flowed out even with a weight of less than 100g.

Stability studies performed on the polyherbal gels indicated no significant changes in physical parameters, pH, or viscosity, as depicted in Table 3. Moreover, there were no visible signs of syneresis observed during these stability studies. The drug content estimation at ambient temperature across all gels showed a total anthraquinone content ranging between 28-30mg/g of gel. Table 4 outlines the effect of stability studies on the variation of drug content in the prepared polyherbal gels, demonstrating no significant variation in overall drug content, suggesting both physical and chemical stability of the gels. In vitro drug release studies conducted over durations of 15 minutes, 30 minutes, 1 hour, 2 hours, and 3 hours using the Franz diffusion cell revealed noteworthy findings. PHG-1 exhibited approximately 90% drug release within 35 minutes, indicating immediate release. PHG-2 and PHG-4 demonstrated 90% release within 2 hours, while PHG-3 and PHG-5 exhibited 90% release in 150 minutes, an ideal duration for a formulation applied to the skin. The results of the in vitro diffusion studies have been tabulated in Table 5.

The antibacterial efficacy of the gels was investigated against bacteria isolated from human acne, including Staphylococcus epidermis and Escherichia coli, using both the cotton swab and disc diffusion methods. The zone of inhibition against each bacterium was recorded in Table 6.

Table 1: Design of polyherbal antibacterial gels

Ingredients	*PHG-1*	*PHG-2*	*PHG-3*	*PHG-4*	*PHG-5*	*PHG-6*
Carbopol-940 (g)	*0.5*	*1*	*1.5*	*2*	*2.5*	*3*
Propylene glycol (ml)	*5*	*5*	*5*	*5*	*5*	*5*
Lauric acid (mg)	*2*	*2*	*2*	*4*	*4*	*4*
Potassium Sorbate (ml)	*5*	*5*	*5*	*5*	*5*	*5*
Isopropyl myristate (ml)	*5*	*5*	*5*	*5*	*5*	*5*
Propyl paraben (mg) (0.2%w/v)	*0.1*	*0.1*	*0.1*	*0.1*	*0.1*	*0.1*
Methyl paraben (mg) (0.5%w/v)	*0.2*	*0.2*	*0.2*	*0.2*	*0.2*	*0.2*
Triethanolamine (ml)	*q.s.*	*q.s.*	*q.s.*	*q.s.*	*q.s.*	*q.s.*
Tea Tree oil (ml)	*0.5*	*1*	*1.5*	*0.5*	*1*	*1.5*
*Rubia Extract (g)**	*1*	*1.5*	*2*	*2*	*2.5*	*3*
Aloe Vera Gel (g)	*3*	*2.5*	*2*	*2*	*1.5*	*1*
*Distilled water (ml)***	*100*	*100*	*100*	*100*	*100*	*100*

*Extract solublized in enough volume of ethanol **Final Volume is made up to 100ml Using Distilled water

Table 2: Physical Parameters of the prepared Polyherbal gels

Formulation	pH	Viscosity (cps)	Spreadability (g-cm/sec)	Extrudability (g)
PHG-1	6.93±0.14	2326±38.8	65.88±4.42	72.19±4.29
PHG-2	6.64±0.25	3110±42.5	41.29±2.11	165.62±6.49
PHG-3	6.72±0.12	4434±84.1	31.44±1.92	243.28±10.32
PHG-4	6.78±0.17	3209±61.2	45.31±2.45	177.56±10.44
PHG-5	6.84±0.26	5122±88.3	39.05±1.03	390.55±18.42
PHG-6	6.65±0.35	6637±101.9	23.12±2.76	522.13±27.67

All values given as Mean±SEM (n=3)

Table 3: Stability studies of the prepared Polyherbal Gels

Formulation	pH*		Viscosity (cps)*		Syneresis	
	4°C	40°C	4˚C	40°C	4˚C	40˚C
PHG-1	6.91±0.11	6.88±0.08	2574±23.5	2218±88.5	NS	NS
PHG-2	6.68±0.18	6.66±0.1	3732±53.2	2938±52.4	NS	NS
PHG-3	6.62±0.09	6.67±0.21	4893±48.4	4321±67.2	NS	NS
PHG-4	6.71±0.13	6.72±0.12	3542±87.6	2935±91.6	NS	NS
PHG-5	6.88±0.21	6.83±0.18	5547±47.3	5045±104.4	NS	NS
PHG-6	6.72±0.22	6.69±0.15	6937±87.9	6253±103.2	NS	NS

All values given as Mean±SEM (n=3); NS-No-significant Change

Table 4: Drug content in the prepared Polyherbal Gels

Formulation	Drug Content (mg/g)		
	4˚C	Normal	40˚C
PHG-1	29.28±1.44	29.37±1.93	29.19±1.28
PHG-2	29.11±1.32	28.91±1.28	28.14±1.46
PHG-3	29.27±0.66	29.03±1.44	28.55±1.11
PHG-4	30.02±1.13	29.08±0.98	28.43±1.34
PHG-5	30.13±1.75	30.12±1.35	29.48±1.77
PHG-6	29.39±0.95	29.43±1.86	29.12±1.65

All values given as Mean±SEM (n=3); NS-No-significant Change

Table 5: Invitro Drug Diffusion Studies of prepared Polyherbal Gels

Formulation	Drug Release				
	15min	30min	60min	120min	180min
PHG-1	71.47±0.54	86.56±0.85	93.67±0.88	97.91±0.35	99.53±0.25
PHG-2	68.72±0.72	78.57±0.55	86.59±0.51	95.92±0.59	99.36±0.14
PHG-3	56.35±0.62	66.18±0.63	77.12±0.16	87.76±0.48	99.49±0.17
PHG-4	72.15±0.51	81.95±0.35	90.49±0.6	93.56±0.54	98.43±0.44
PHG-5	49.13±0.29	59.96±0.78	74.07±0.82	86.64±0.49	97.49±0.82
PHG-6	35.78±0.54	55.28±0.97	76.37±0.37	86.83±0.18	94.52±0.38

All values given as Mean±SEM (n=3); NS-No-significant Change

Table 6: Invitro Anti-bacterial activity of prepared Polyherbal Gels

Formulation	Zone of inhibition (mm)		
	Acne Swab	*S.epidermis*	*E.coli*
PHG-1	5.67±0.67*	6.83±0.44*	11.33±0.72*
PHG-2	11.33±0.88*	13.96±0.18	17.97±0.86
PHG-3	14.17±0.41	16.51±0.68	20.13±0.31
PHG-4	15.51±0.52	18.03±0.41*	22.13±0.21*
PHG-5	16.02±0.57	17.43±0.57*	23.42±0.47*
PHG-6	17.67±0.33*	18.03±0.76*	24.06±0.37*
Standard	14.67±0.33	15.56±0.41	19.03±0.78

All values were expressed as mean±SEM (n=3); *indicates significant at P<0.001 in comparison to the standard drug

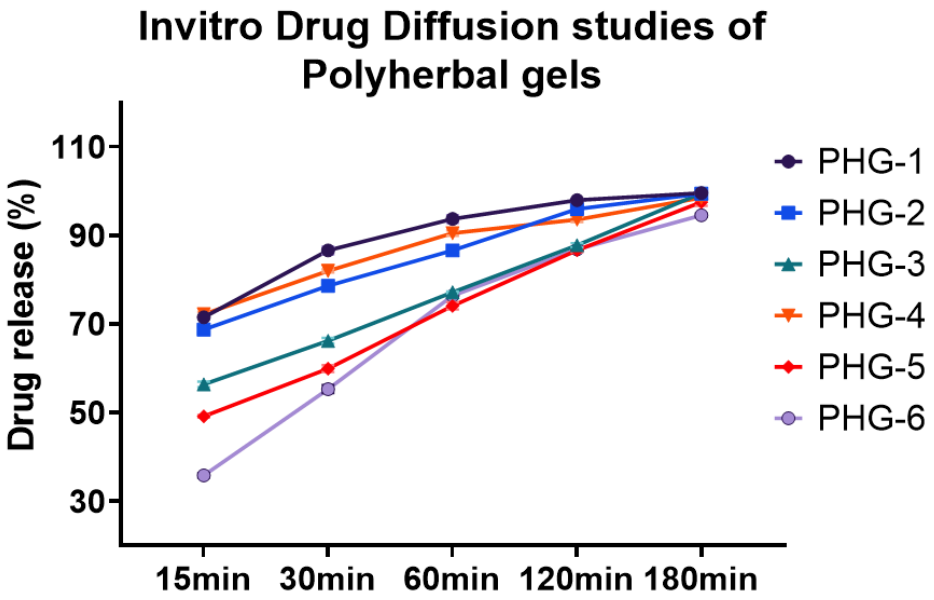

Figure 1: Invitro Drug Release study of Polyherbal Gels

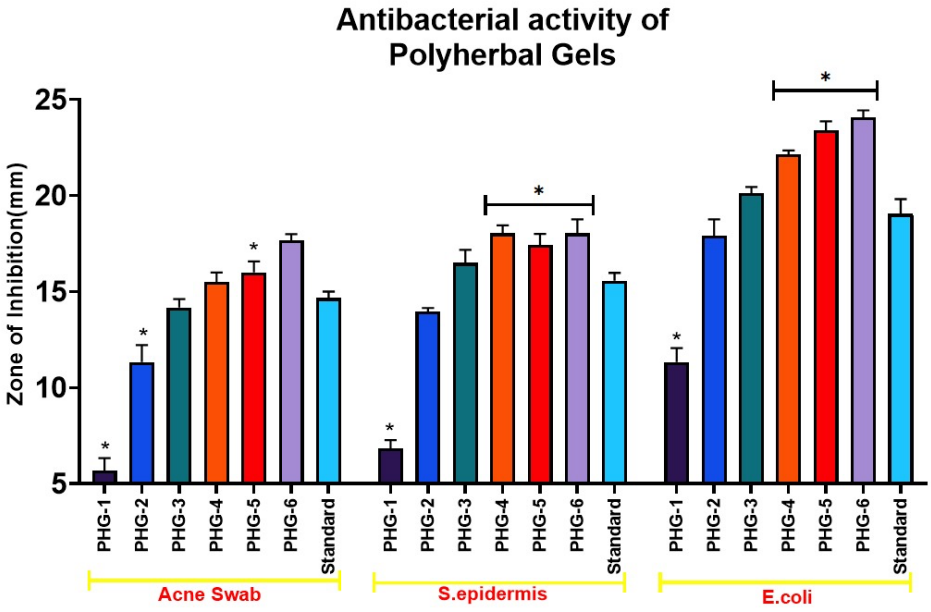

Figure 2: Antibacterial activity of Polyherbal Gels

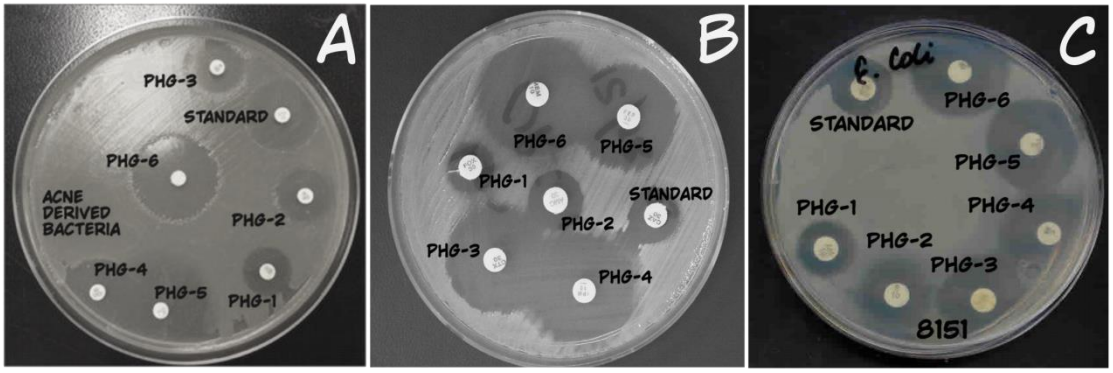

Figure 3: Antibacterial activity showing zone of inhibition

A. Acne derived bacteria, *B. S.epidermis, C. E.coli*

PHG-4, 5, and 6 displayed significantly superior inhibitory effects against the bacteria compared to the standard clindamycin gel. PHG-2 and PHG-3 exhibited similar or non-significantly different activity compared to the standard. However, PHG-1 demonstrated significantly lower activity than the Clindamycin gel. This variance in activity might be attributed to the differences in the contents of Aloe vera gel and Rubia extract. Higher concentrations of Rubia extract seemed to correlate with better activity, observed particularly in PHG-4, 5, and 6 in comparison to PHG-1, 2, and 3.

4. Discussion

The formulation of polyherbal gels often hinges on the delicate balance between various constituents to achieve desired properties and efficacy. Here, the development of polyherbal gels centered on the intrinsic antibacterial and antiacne potentials of Rubia extracts and Aloe vera gel. Carbopol, renowned for its ideal gelling properties, was chosen as the primary agent due to its propensity to yield clear, consistent gels with commendable spreadability [16]. In parallel, Aloe vera gel, recognized for its multifaceted beneficial properties, was included not primarily for its antibacterial action, as supported by Arbab et al. 2021 [24], but rather for its gel-forming characteristics and its role in

maintaining gel spreadability. Our findings resonate with the premise that while Aloe vera gel does possess antibacterial attributes, in this specific context, its contribution was focused on enhancing the gel's structural attributes rather than exerting antibacterial potency. The observed characteristics of the different formulations, particularly PHG-1, which displayed remarkable spreadability potentially attributed to its lower concentration of carbopol and higher content of Aloe vera gel. On the other hand, PHG-4 and 5 successfully struck a balance between Aloe vera gel and carbopol concentrations, thereby achieving the desired consistency, spreadability, and viscosity.Recent investigations, as cited in the study, have emphasized the trend of incorporating herbal extracts into gel formulations to harness their antibacterial properties [18][25][26]. Notably, Rubia cordifolia, recognized for its robust antibacterial activity [12][27], was a pivotal component in the prepared polyherbal gels, effectively demonstrating substantial activity against targeted bacteria from acne and other sources.

The in vitro diffusion studies provided insights into varying release profiles among the formulations. While PHG-3 and 5 exhibited an optimal release pattern conducive to sustained potency over a prolonged duration, others demonstrated immediate drug release. PHG-6 showcased the most favorable drug release profile, albeit at the expense of other crucial parameters compared to PHG-3 and 5. Consequently, the comprehensive analysis inclined towards PHG-5, lauding its notable antibacterial activity and favorable spreadability as the most promising formulation. This detailed examination underscores the combined activity between various components in polyherbal gel formulations and their consequent impact on efficacy, highlighting the critical balance necessary for optimal performance.

5. Conclusion

The development of polyherbal gels targeting acne and bacterial skin disorders highlights the intricate synergy of herbal extracts, gel-forming agents, and antibacterial components. This study underscored the nuanced role of Aloe vera gel primarily in enhancing gel structure rather than exerting antibacterial potency. Among the formulations, PHG-5 emerged as a frontrunner, boasting commendable antibacterial efficacy, sustained drug release, and favorable physical attributes. This illuminates the critical balance required for optimal performance in polyherbal gel formulations. Further research avenues could explore optimizing the formulations to enhance specific properties while maintaining efficacy. Long-term stability studies and clinical trials would substantiate the feasibility and effectiveness of these polyherbal gels in real-world scenarios, potentially revolutionizing the treatment landscape for acne and bacterial skin disorders.

6. Conflicts of Interest

The authors declare no conflicts of interest.

7. Funding

No Funding

8. Author Contribution

All authors are contributed equally.

References

[1] S. Sandhya, E. V. Sravanthi, K. Vinod. (2013). Evaluation of a dermatological herbal hydrogel integrated with Ipomea pestigridis for anti acne activity. Inflammation. 1:5.

[2] L. Zaenglein, A. L. Pathy, B. J. Schlosser, A. Alikhan, H. E. Baldwin, D. S. Berson, W. P. Bowe, E. M. Graber, J. C. Harper, S. Kang, J. E. Keri. (2016). Guidelines of care for the management of acne vulgaris. Journal of the American Academy of Dermatology. 74(5): 945-73.

[3] Jain, E. Bansal. (2003). Inhibition of Propionibacterium acnes-induced mediators of inflammation by Indian herbs. Phytomedicine. 10: 34–38.

[4] Y. Zu, H. Yu, L. Liang, Y. Fu, T. Efferth, X. Liu. (2010). Activities of ten essential oils towards Propionibacterium acnes and PC-3, A-549 and MCF-7 cancer cells. Molecules. 15:3200–10.

[5] P. R. Behera, S. Palepu, C. S. Sirka, R. Ranjan, S. Pradhan, A. K. Singh. (2022). Psychosocial Distress and Quality of Life Among Patients With a Chronic Skin Disorder at a Tertiary Care Hospital in Eastern India: A Hospital-Based Case-Control Study. Cureus. 14(10):e29830.

[6] M. Patel, W. P. Bowe, C. Heughebaert, A. R. Shalita. (2010). The development of antimicrobial resistance due to the antibiotic treatment of acne vulgaris: a review. J Drugs Dermatol. 9(6):655-64.

[7] T. Rajesh, A. K. Roy, V. R. Erumalla, D. Goli, S. J. Basha. (2014). Development and evaluation of antimicrobial ointment formulation containing extracts of Ocimum sanctum, Anthocephalus cadamba, Allium sativum and Origanum vulgare. World J Pharm Res. 3(5):398-422.

[8] H. Azimi, M. Fallah-Tafti, A. A. Khakshur, M. Abdollahi. (2012). A review of phytotherapy of acne vulgaris: Perspective of new pharmacological treatments. Fitoterapia. 83(8):1306-17.

[9] K. S. Priya, A. Gnanamani, N. O. Radhakrishnan, M. Babu. (2002). Healing potential of Datura alba on burn wounds in albino rats. J ethnopharmacol. 83(3):193-9.

[10] S. Avinash, D. V. Gowda, J. Suresh, A. S. Ram, A. Srivastava, R. M. Osmani. (2016). Formulation and evaluation of topical gel using Eupatorium glandulosum michx. for wound healing activity. Der Pharm Lett. 8(8):255-66.

[11] K. Kaur, A. K. Gupta, S. Ahmad, P. Alam. (2011). Pharmacognostic studies on bark of Murraya koenigii Spreng. Int J Res Pharm Biomed Sci. 2:4.

[12] N. Khan, R. Karodi, A. Siddiqui, S. Thube, R. Rub. (2011). Development of anti-acne gel formulation of anthraquinones rich fraction from Rubia cordifolia (Rubiaceae). Int J Applied Res Natl Products. 4: 28-36.

[13] M. Gharipour, M. A. Ramezani, M. Sadeghi, A. Khosravi, M. Masjedi, H. Khosravi-Boroujeni. (2013). Sex based levels of C-reactive protein and white blood cell count in subjects with metabolic syndrome: Isfahan Healthy Heart Program. J Res Med Sci. 18(6): 467–72.

[14] K. A. Hammer, C. F. Carson, T. V. Riley, J. B. Nielsen. (2006). A review of the toxicity of Melaleuca alternifolia (tea tree) oil. Food chem toxicol. 44(5): 616-25.

[15] J. K. Lalla, S. Y. Nandedkar, M. H. Paranjape, N. B. Talreja. (2001). Clinical trials of ayurvedic formulations in the treatment of acne vulgaris. J Ethnopharmacol. 78(1): 99–102.

[16] Rasheed, G. Avinash Kumar Reddy, S. Mohanalakshmi, C. K. Ashok Kumar. (2011). Formulation and comparative evaluation of poly herbal anti-acne face wash gels. Pharm biol. 49(8):771-4.

[17] M. Bankar, M. N. Dole. (2016). Formulation and evaluation of herbal antimicrobial gel containing Musa acuminata leaves extract. J Pharmacog Phytochem. 5(1): 1-3.

[18] D. S. Sandeep, P. Nayak, J. Jose, M. Rishal Relita, D. R. Sumana. (2017). Formulation and evaluation of antibacterial herbal gels of Murraya koenigii leaves extract. Res J Pharm Tech. 10(6): 1798-801.

[19] D. P. Pawar, P. B. Shamkuwar. (2013). Formulation and evaluation of herbal gel containing Lantana camara leaves extract. Asian J Pharm Clin Res. 6: 122–4.

[20] Vats, P. Sharma. (2012). Formulation and evaluation of topical antiacne formulation of coriander oil. Int J Pharm Pharm Sci Res. 2: 61–6.

[21] R. Khoomsab, K. Khoomsab. (2019). Extraction and Determination of Anthraquinone from Herbal Plant as Bird Repellent. Sci Tech Asia. 24(1): 14-20.

[22] G. Singhvi, M. Singh. (2011). Review: In-vitro drug release characterization models. Int J Pharm Stud Res. 2: 77–84.

[23] S. Mayank, R. Vikas. (2014). Formulation development and evaluation of novel poly-herbal anti-acne gel. Int J Pharm Tech Res. 6: 58–62.

[24] S. Arbab, H. Ullah, W. Weiwei, X. Wei, S. U. Ahmad, L. Wu, J. Zhang. (2021). Comparative study of antimicrobial action of aloe vera and antibiotics against different bacterial isolates from skin infection. Vet med sci. 7(5): 2061-7.

[25] M. A. Shahtalebi, G. R. Asghari, F. Rahmani, F. Shafiee, A. Jahanian-Najafabadi. (2018). Formulation of Herbal Gel of Antirrhinum majus Extract and Evaluation of its Anti-Propionibacterium acne Effects. Adv Biomed Res. 7: 53.

[26] S. D. Bhinge, M. A. Bhutkar, D. S. Randive, G. H. Wadkar, S. S. Todkar, P. M. Kakade, P. M. Kadam. (2017). Formulation development and evaluation of antimicrobial polyherbal gel. InAnnales Pharmaceutiques Françaises. 75(5): 349-358. Elsevier Masson.

[27] Gorle, S. Patil. (2018). Development and evaluation of Rubia cordifolia gel for treatment of acne. Res J Top Cosmet Sci. 9(2): 39-43.