Section: Research Paper

ANTIMICROBIAL FORMULATION DEVELOPMENT, AND EVALUATION OF BLUEBERRY AND BANANA EXTRACT

Ms. SURABHI TRIPATHI[1*] Dr. SATKAR PRASAD[2] DR. REENU YADAV[3]

1*Department of pharmacy, PhD Research Scholar ,Bhabha University Bhopal

2* Department of pharmacy, Principal ,Bhabha University Bhopal

3*Department of pharmacy ,Principal ,IES institute of Pharmacy,Bhopal

Abstract

The proposed work's objective was to create herbal skin formulations with medicinal value that would treat or prevent various microbes as well as other skin problems like aging without wrinkles. The Polyherbal Gel formulations were developed, and numerous metrics were used to standardize and evaluate them. In polyherbal formulation concentration of extracts is kept fixed as selected and the quantity of other ingredients varied to get an optimized formulation and evaluation of each formulation was performed to select the best formulation. The final formulations were selected based on the results of the evaluation.

Results from testing the formulations against several parameters were satisfactory i.e. consistency, pH in range, Release profile, and stability.

Keywords: Gel, *Blueberry* (fruit) and *Banana* (fruit), Carbopol, Cremophor, Streak plate method

Introduction:

People have historically utilized both synthetic and natural medications to treat this infections[1]. Given that they have fewer or no negative effects than synthetic pharmaceuticals, herbal treatments are becoming more and more popular today[2]. For a very long time, medicinal herbs including tulsi, aloe vera, neem, turmeric, and turmeric have been utilized to cure infectious infections. [3-8]

Hence, herbal topical preparations for the treatment of skin conditions gained popularity. Scientists discovered thousands of phytochemicals from plants that inhibit various types of microorganisms through various mechanisms and act as safe, broad-spectrum antibiotics in the treatment of resistant microbial strains as the development of bacterial resistance to antibiotics and other synthetic antimicrobials. [9]. When compared to systemic therapy, topical antimicrobial

agent administration at the site of infection offers more benefits[10–11]. First off, topical dosage makes it simple to reach the drug's needed concentration for antibacterial activity at the target site. Second, systemic levels of the active components are significantly reduced or nearly undetectable[19] following topical treatment. Thirdly, it can prevent the gut flora from being unnecessarily exposed to antimicrobial medicines, which could result in drug resistance or a decrease in the GIT's natural bacterial population. Thus, topical application of antimicrobial agents is regarded as a significant substitute for antibiotic administration through the systemic route for the treatment of skin diseases[17].

There are two types of skin infections: primary and secondary. Primary infections typically originate in healthy skin, have distinct morphologies and progressions, and are caused by solitary organisms. Staphylococcus aureus, Streptococcus pyogen, and coryneform bacteria are the most common causes. Common examples include impetigo, folliculitis, boils, and erythrasma. Skin symptoms of systemic infections are possible. Skin disease is the primary site of origin for secondary infections. Examples of secondary infections include intertrigo and toe web infections.

Herbal remedies are said to have fewer adverse effects than ones with synthetic ingredients. According to market data, the herbal cosmetic sector is a major driver of the upward trend in the herbal trade, which is driving up demand for herbals globally. Products made from herbs have been touted for their efficacy, inherent acceptance, and lack of the adverse effects frequently associated with synthetic products. [12-18]

The most significant topical and systemic formulations with active herbal therapeutics, such as blueberries and bananas for antibacterial purposes, will be highlighted.

Material and Methods

Fresh fruits of blueberry and banana were purchased from market suppliers from Chhatarpur in May 2019, and authentication of plant material was done by taxonomist Dr. Manjusa Saxena at the Department of Botany, Govt. Maharaja College, Chhatarpur (M.P.).

A suitable amount of polysorbate was dissolved in 5 ml of hot water to make each formulation. The quantity of various ingredients needed to prepare gel bases was determined through experimental design. The supplied quantity of carbopol 940 was then thoroughly mixed with 50 ml of deionized water for 20 minutes. This mixture was retained for soaking the next day. In another beaker, ethanol, and deionized water were combined with the necessary amounts of propylene glycol, isopropyl myristate, and cremophor. The second beaker received the necessary concentration of a Blueberry fractionated extract that was diluted in ethanol 90% in consideration of its MIC.

Development and evaluation of poly herbal gel.

The formulation for antioxidant activity, and anti-microbial activity, was developed. In all formulations firstly mono herbal formulation-I was prepared including the first plant's extract (*Blueberries*) as a therapeutic agent and the concentration of the extract varied from 1% to 5% to get optimum antimicrobial activity in the minimum possible concentration, and the number of other ingredients kept fixed. Secondly, mono herbal formulation II was prepared in the same manner by including a second plant extract (*Banana*). And anti-microbial activity of the formulation at various concentrations of extract (1%- 5%) was determined and optimum activity at the lowest concentration was selected. After the selection of the minimum concentration of the extracts to be included, the polyherbal formulation was made by including both of the extracts in a fixed selected concentration based on their antimicrobial activity. Evaluation and standardization were performed at every stage of the development of the formulation. In polyherbal formulation concentration of extracts was kept fixed as selected and the quantity of other ingredients varied to get an optimized formulation and evaluation of each formulation was performed to select the best formulation. The final formulations were selected based on the results of the evaluation.

Ingredient /Formulations	F1	F2	F3	F4	F5	F6	F7	F8
Carbapol (g)	1	0.25	0.5	0.75	0.4	0.6	1	0.5
Propylene glycol (ml)	5	5	5	5	5	5	5	5
Potassium sorbate (ml)	0.5	0.5	0.5	0.25	0.25	0.25	0.25	0.25
Isopropyl myristate (ml)	5	5	5	5	5	5	5	5
Blueberry extract fraction (g)	2	2	2	2	2	2	2	2
Banana extract active fraction (g)	3	3	3	3	3	3	3	3
Alcohol (ml)	25	25	25	25	25	25	5	25
Cremophor (g)	2	2	2	2	2	2	2	2
Water Q.S (ml)	100	100	100	100	100	100	100	100

pH

pH was tested by dissolving 1 gm product into 9 ml of water and shaking vigorously then the aqueous solution and pH is observed by pH meter.

Fragrance test

It was based on individual observation for its acceptability.5 people were asked for the acceptability of fragrance and their opinion was taken. And fragrance was evaluated based on the below-described criteria;

A) Fragrance was good, as good as the fragrance of reference herbal gel.

B) Fragrance was not so good but comparable to the reference herbal gel

C) Fragrance of the gel was poor than the reference herbal gel

Moisture content

The product (10 gm) weighed in a Porcelain dish and dried in the oven at 105^{o}C. It was cooled in a desiccator. The loss of weight is recorded as a percentage of moisture content and calculated by the given formula.

$$\% \text{ moisture} = \frac{\text{Original sample weight} - \text{dry sample weight}}{\text{Original sample weight}} \times 100$$

Water soluble extractive

Accurately weighed air-dried powdered (2 g) material was taken in a glass stoppered flask and macerated with 100 ml of water .then it was shaken frequently for 6 hrs in a shaker and then allowed to stand for 18 hrs. After that 10 ml of filtrate was evaporated to dryness in a tared flat bottomed and transferred to the petri dish and dried at 105 c and cool in a desiccator. The percentage of water-soluble extractives was calculated regarding air-dried drugs.

- **Viscosity**

The viscosity of formulated gels was determined using Brookfield Viscometer at 500 rpm and 25^{0}C.

- **Spreadability**

The spreadability of gel formulations was determined by measuring the

Spreading diameter of 1g of gel between two horizontal plates

***In-vitro* release**

The cellophane membrane was presoaked in distilled water for 24 h (1 g)spread over the cellophane membrane and was mounted on Franz-type diffusion cells with a receptor compartment volume of 33.2 ml and an effective diffusion area of 3.14 cm2. The receptor fluid

was selected as phosphate buffer (pH 7.4) containing 25% (v/v) ethanol to maintain sink conditions. During the experiments, the receptor phase was kept at 37C and continuously stirred at 600 rpm. At certain time intervals, 1 ml samples were withdrawn from the receiver compartment and replaced with an equal volume of fresh receptor fluid.

Data Analysis via Drug Release Kinetics Study

Results of the in-vitro release profile obtained for all the formulations were plotted

in kinetic models as follows,

1. Cumulative of drug released versus time (zero-order kinetic model).

2. Log cumulative percent drug remaining to be absorbed versus time (First order model)

3. Cumulative amount of drug release versus square root of time (Higuchi model)

4. Log cumulative drug released versus log time (Korsmeyer-Peppas model)

Result and Discussion

Evaluation of physical characteristics of herbal gel

The pH of herbal gel

the pH of the gel formulated was between 6.7 to 7.0 and the pH of the reference herbal gel was found to be 7.0.

Evaluation for Fragrance

The herbal gel has so many features, it exhibits a good and pleasant, and mood-elevating fragrance as compared to the reference herbal gel.

Moisture Content / Volatile Content

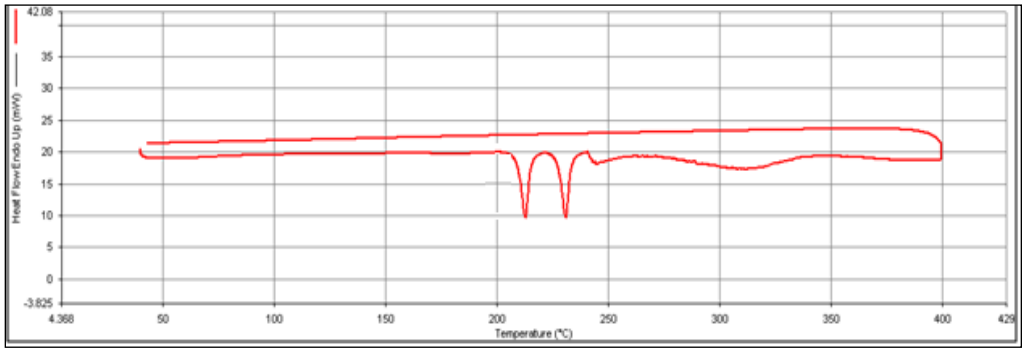
Formulation Code	F1	F2	F3	F4	F5	**Reference grade**
% moisture content	25	28	25	26	29	25

Drug- Excipient Interaction studies (By DSC)

Methanolic extract of *Blueberries*. Possess a 245^0C melting point and the Methanolic extract of *Banana* possess a 225^0C melting point. The whole formulation (drug and excipient) was kept in

accelerated conditions of temperature (400C) and RH (75%) for 15 days and subjected to DSC analysis.

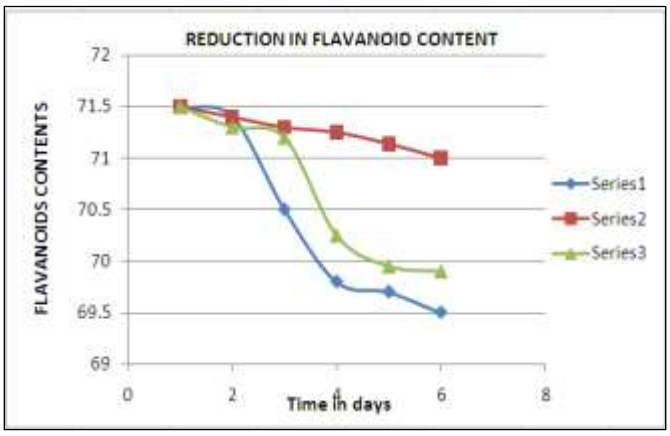
The characteristic peak of the Methanolic extract of *Blueberry* and Methanolic extract of *Banana* not deviated from its position of 248^{0}C and 225^{0}C that shows no interaction between the extract and other excipients. The DSC graphs are as follows.



(Drug- Excipient Interaction)

Storage stability of the optimized formulation

Results obtained from the accelerated stability study of the formulation can be concluded that the flavonoid contents reduced with time on long storage. Initially, on 0 days the concentration of flavonoid was 71.50 mg/gm, on the 54th day it was observed 69.80 mg/gm. and on the 90$^{th\ day,}$ it was observed at 69.50 mg/gm from the results it can be concluded that the tannin contents reduced with time by the effect of the temperature and moisture.

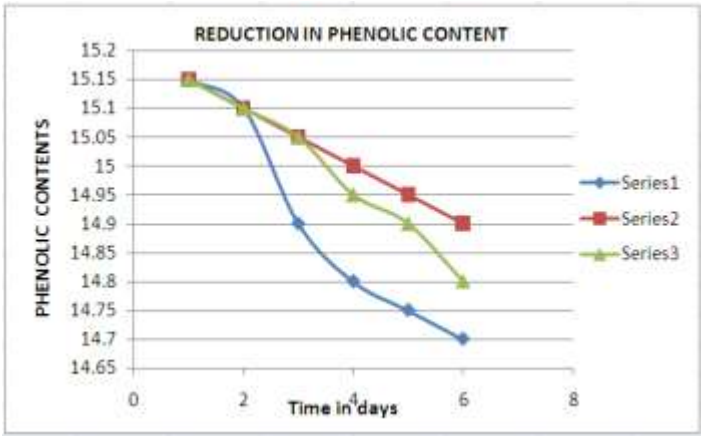


(Reduction in Flavonoids contents)

Estimation of Total Phenolic Content in Formulation F3

Results obtained from the accelerated stability study of the formulation can be concluded that the Phenolic contents reduced with time on long storage. Initially, on 0 days the concentration of

Phenolic was 15.15 mg/gm, on the 54th day it was observed at 14.80 mg/gm. and on the 90$^{th\ day,}$ it was observed 14.70 mg/gm from the results it can be concluded that the phenolic contents reduced with time by the effect of the temperature and moisture.



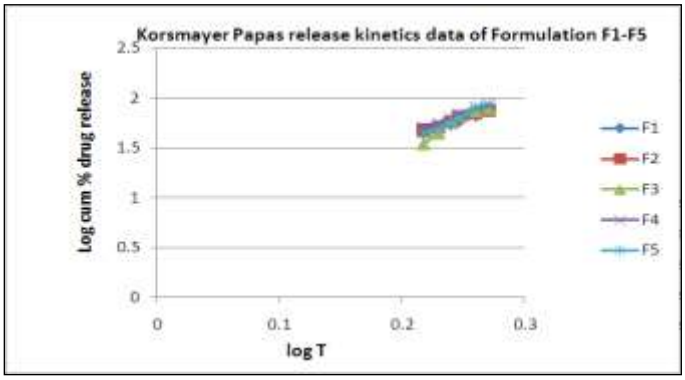
(Reduction in Phenolic contents)

Result of Assay of Formulation (Total Flavonoids and Phenolic compound)

In comparison to all formulations, Formulation F3 shows maximum Flavonoids and phenolic content of 72.98 mg/gm and 18.56 mg/gm respectively. Based on the results of various evaluation parameters, F3 was selected as the best formulation.

***In vitro* drug release study of Polyherbal Formulation**

In all the cases, the R values of the korsmayer papas model were close to 1. The diffusion coefficients (n) values ranged from 0.6655 to 0.9164 (Shown in Figure No.). Since the R values of Korsmayer papas were close to 1, the drug release follows matrix diffusion kinetics. Hence it was concluded that diffusion was the mechanism of the drug release from the herbal gel. Further, observed diffusion coefficient values are indicative of the fact that the drug release from the formulation follows a non-Fickian transport mechanism.

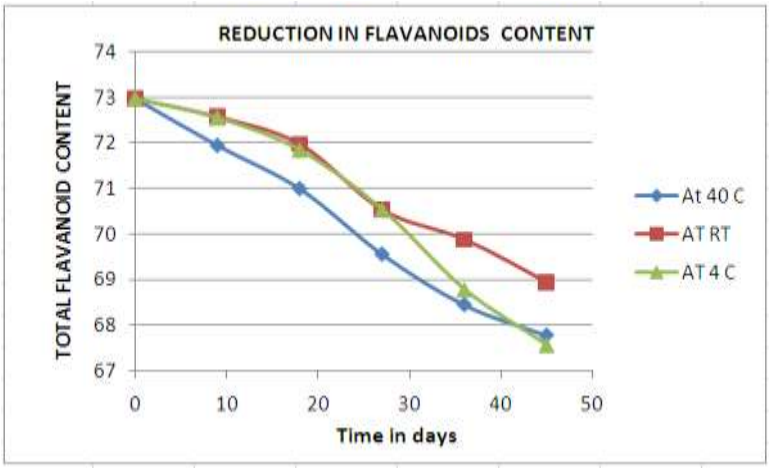


Korsmayer Papas release kinetics data of Formulation F1-F5

Estimation of Total flavonoid content in Formulation F3

Results obtained from the accelerated stability study of the formulation can be concluded that the flavonoid contents reduced with time on long storage. Initially, on 0 days the concentration of flavonoid was 72.98 mg/gm, on the 54th day it was observed 69.56 mg/gm. and on the 90$^{th\ day,}$ it was observed 67.78 mg/gm from the results it can be concluded that the flavonoid contents reduced with time by the effect of the temperature and moisture.

Results obtained from the accelerated stability study of the formulation can be concluded that the Phenolic contents reduced with time on long storage. Initially, on 0 days the concentration of Phenolic was 18.56 mg/gm, on the 54th day it was observed at 16.50 mg/gm. and on the 90$^{th\ day,}$ it was observed 16.00 mg/gm from the results it can be concluded that the phenolic contents reduced with time by the effect of the temperature and moisture.



Estimation of Total Phenolic Content in Formulation F3

Conclusion

In my present work two plants *Blueberry* (fruit) and *Banana* (fruit) were extracted and screened for antimicrobial activity by disc diffusion method against some oral pathogens and their methanolic extract was found to have good and moderate antimicrobial activity. And then these extracts were subjected to their bioactivity-directed fractionation. After fractionation antimicrobial activity of each fraction was screened and the acetone fraction (fraction- III) of the methanolic extract *Blueberry* and the ethyl acetate fraction of the methanolic extract *Banana* were found to be active against skin pathogens. These active fractions were again subjected to isolation of the compound, which may be responsible for the antimicrobial activity of these plants against some oral pathogens. Isolation of active compounds was carried out using column

chromatography and their characterization was done by spectroscopic methods. These spectral data confirmed the identity of the isolated compounds.

Our Polyherbal Gel formulations were developed, and numerous metrics were used to standardize and evaluate them. Results from testing the formulations against several parameters were satisfactory again these formulations were subjected to evaluation of their antimicrobial activity. An accelerated stability study; Temperature and humidity-dependent degradation for the Stability of compounds was also performed. It can be concluded from the results that these formulations will be able to protect/ cure various skin problems without any side effects.

The plant extract in the experiment has shown antibacterial action against Gram-negative, Gram-positive, and fungal strains, which suggests that it may be a source for the development of medications with a wide range of effects. The results of the study also support the traditional application of the plants and suggest that the plant extracts possess compounds with antimicrobial properties that can be used as potential antimicrobial agents. Further development and evaluations of these herbal dental formulations including the isolated compounds on a commercial scale and their clinical and toxicological studies are the future challenges.

References

1. Richard E (2005). Google Books -- Biotechnology of fruit and nut crops By Richard E. Litz. ISBN 9780851996622.
2. Naumann, W. D. (1993). "Overview of the Vaccinium Industry in Western Europe". In K. A. Clayton-Greene (ed.). Fifth International Symposium on Vaccinium Culture. Wageningen, the Netherlands: International Society for Horticultural Science. pp. 53–58. ISBN 978-90-6605-475-2. OCLC 29663461.
3. A. Rasul* and N. Akhtar Formulation and in vivo evaluation for anti-aging effects of an emulsion containing basil extract using non- invasive biophysical techniques Daru. 2011; 19(5): 344–350.
4. Kalt W, Ryan DA, Duy JC, Prior RL, Ehlenfeldt MK, Vander Kloet SP (October 2001). "Interspecific variation in anthocyanin, phenolics, and antioxidant capacity among genotypes of highbush and lowbush blueberries
5. Gagnon A (2006). "Wild Blueberry Production Guide in a Context of Sustainable Development: Survey of the Wild Blueberry Industry in Québec" (PDF). Ministère de agriculture, des Pêcheries et de alimentation du Québec. Retrieved 4 February 2015
6. "Banana from 'Fruits of Warm Climates' by Julia Morton". Hort.purdue.edu. Archived from the original on April 15, 2009. Retrieved April 16, 2009.
7. Claudine & INIBAP, eds. (2000). Bananas (PDF) (English ed.). Montpellier: International Network for the Improvement of Banana and Plantains/International Plant Genetic Resources Institute. ISBN 978-2-910810-37-5. Archived from the original (PDF) on April 11, 2013. Retrieved January 31, 2013.

8. National Research Council (US) Panel on Neem. Neem: A Tree For Solving Global Problems. Washington (DC): National Academies Press (US), 1992.
9. Rao J, Ehrlich M, Goldman MP. Facial skin rejuvenation with a novel topical compound containing transforming growth factor β1 and vitamin C. Cosmet Dermatol. 2004;17:705–13.
10. Baumann L. Skin ageing and its treatment. J Pathol. 2007;211(2):241–51.
11. Norramon Thanyapanich,[1] Ampa Jimtaisong,[1,2,*] and Saroat Rawdkuen[2,3] Functional Properties of Banana Starch (*Musa* spp.) and Its Utilization in Cosmetics 2021 Jun; 26(12): 3637.
12. Henni Rosaini, Auzal Halim, Ica Elpitha Sandi, Indra Makmur, Ridho Asra, Wahyu Margi Sidoretno formulation and antioxidant activity of nano gel ethanol extract of kepok banana peel (musa x paradisiaca l.) International Research Journal of Pharmacy , Volume 12, pp 21-25
13. Danijela ŠeremetORCID,Ksenija Durgo,Anamaria Komljenović,Mihaela Antolić,Ana Mandura Jarić,Ana Huđek Turković,Draženka Komes * andBožidar Šantek ORCID Red Beetroot and Banana Peels as Value-Added Ingredients: Assessment of Biological Activity and Preparation of Functional Edible Films 2022, *14*(21), 4724
14. Ira Adiyati Rum, Husnul W Suherman, Idar Formulation and evaluation of peel-off gel mask from whole milk yogurt and seaweed (Eucheuma cottonii) as antioxidants sources June 29, 2021 | Published: July 22, 2021
15. Xiao Shen [a], Xiaohong Sun [a 1], Qingchao Xie [a], Haiquan Liu [a], Yong Zhao [a] Antimicrobial effect of blueberry (*Vaccinium corymbosum* L.) extracts against the growth of *Listeria monocytogenes* and *Salmonella* Enteritidis January 2014, Pages 159-165
16. Hazim O Khalifa et al "Antimicrobial Effects of Blueberry, Raspberry, and Strawberry Aqueous Extracts and their Effects on Virulence Gene Expression in Vibrio cholera" Phytother Res 2015 Nov;29(11):1791-7
17. Xiao Shen a , Xiaohong Sun a,1 , Qingchao Xie a , Haiquan Liu a , Yong Zhao a , Yingjie Pan a , Cheng-An Hwang b , Vivian C.H. Wu; Antimicrobial effect of blueberry (Vaccinium corymbosum L.) extracts against the growth of Listeria monocytogenes and Salmonella Enteritidis Food Control 35 (2014) 159e165
18. Haonan liu, han wu, ying wang, fan wang, xiaoli liu & jianzhong zhou "enhancement on antioxidant and antibacterial activities of brightwell blueberry by extraction and purification" *applied biological chemistry* volume 64,article number: 78 (2021)
19. Shen, Xiao; Sun, Xiaohong; Xie, Qingchao; Liu, Haiquan; et al. Antimicrobial effect of blueberry (Vaccinium corymbosum L.) extracts against the growth of Listeria monocytogenes and Salmonella Enteritidis [2014]
20. Xiaohong Sun Antimicrobial effect of blueberry (Vaccinium corymbosum L.) extracts against the growth of Listeria monocytogenes and Salmonella Enteritidis January 2014 Food Control 35(1):159-165

21. Sureeporn Suriyaprom 1,2,3,Pascale Mosoni 3,Sabine Leroy 3,Thida Kaewkod 1,Mickaël Desvaux 3,*ORCID andYingmanee Tragoolpua 1,4,*Antioxidants of Fruit Extracts as Antimicrobial Agents against Pathogenic Bacteria *Antioxidants* 2022, *11*(3), 602
22. Tuğba İduğ In Vitro Antimicrobial and Antioxidant Activity of Some Berry Species Acta Pharm. Sci. Vol 56 No: 3. 2018
23. Song Xiaoyong[1], Cheng Luming Phenolic Constituents, Antimicrobial and Antioxidant Properties of Blueberry Leaves *Journal of Food and Nutrition Research*, 2014 2 (12), pp 973-979.
24. Chen Liu Study on Antibacterial Activity of Anthocyanins from Blueberry Wine Pomace May 2015.
25. Benedict Jose Chellathurai 1, ,Ramyadevi Anburose 1,Mohammad H. Alyami,Mohan Sellappan 1,Mohammad F. Bayan,Balakumar Chandrasekaran,Kumarappan Chidambaram andMohamed Rahamathulla Development of a Polyherbal Topical Gel for the Treatment of Acne *Gels* 2023, *9*(2), 163;