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Formulation and Evaluation of Topical Polyherbal Antiacne Gels Containing *Garcinia mangostana and Aloe vera*

Gowda Bhaskar*, Shariff Arshia, Priyadarshini SRB

Department of Pharmaceutics, Dayananda Sagar College of Pharmacy, Kumarawsamy Layout, Bangalore-560078, India.
e:mail: bhasker_007@yahoo.com

***Corresponding Author** Email:Bhaskar-007@yahoo.com*

ABSTRACT

The objective of the study was to develop a topical poly herbal gel for the treatment of mild acne vulgaris. Aqueous extracts of *Garcinia mangostana* and *Aloe vera* were formulated in an aqueous based carbopol-934(1%w/w) gel system. Preformulation studies on solubility, partition co-efficient, MIC, MBC were determined along with compatibility studies using a validated HPLC method. Six formulations of the gel were prepared by varying the proportions of polymers and evaluated for their physicochemical properties like pH, spreadability, viscosity and microbial assay. Based on these tests, formulation F-6 containing 1% carbopol-934 was selected as best formulation and carried over to *in-vitro* drug diffusion studies wherein it showed Cumulative Drug Release of 81.03% at the end of 8 hours with a flux of 0.0879 mg/cm2/hr.The microbial assay of all the formulations demonstrated better inhibitory activity against *Propionibacterium acne* and *Staphylococcus epidermidis* compared to the marketed clindamycin phosphate gel in equivalent amounts of application.
Conclusion: It was concluded from the study that aqueous extract of *Garcinia mangostana* and *Aloe vera* can be formulated in an aqueous based gel system for topical therapy of mild acne vulgaris.

KEYWORDS: *Aloe vera*, Antiacne, *Garcinia mangostana*, Gel, Topical.

INTRODUCTION

Ideally, topical therapy is the first-line treatment in mild acne, whereas for moderate and severe acne, systemic therapy is required in addition to topical therapy. Topical therapy has associated side effects and the undesirable physicochemical characteristics of certain important agents like tretinoin and benzoyl peroxide affect their utility and patient compliance (1). The latest treatment regimen followed is the one-step acne solutions(2), but they too have disadvantages in that, they are 99% oil based creams and contain either (or both) benzoyl peroxide or (and) salicylic acid. Oil based products are counterproductive because they both fight and contribute to acne by clogging pores. Benzoyl peroxide (3) and salicylic acid (4) are generally more irritating than acne itself. So the authors felt a need to develop a formulation that is water based and devoid of harmful chemicals.

Garcinia mangostana is a proven herbal extract possessing anti bacterial, anti-inflammatory, antioxidant and antiallergenic properties (5). An ideal polyherbal product would be an antiacne gel with emollient properties. Although acne is not cured by *Aloe vera* but the symptoms of redness, flaky skin, and swelling is known to be rapidly decreased with its consistent use (6). *Aloe Vera* has mild astringent properties that prevents future acne outbreaks and helps reduce redness and swelling. Therefore it was thought off to formulate a polyherbal antiacne gel

comprising of *Garcinia Mangostana* and *Aloe vera* as a form of Complimentary and Alternative therapy (CAM) for the treatment of Acne.

MATERIALS AND METHODS

Plant Extracts

Aqueous extracts of *Garcinia mangostana* and *Aloe vera* were gifted by Green Chem, Bangalore

Microorganisms and Media

The test organisms used in this study were *Propionibacterium acnes* (MTCC 1951) and *Staphylococcus epidermidis* (MTCC 931). These bacteria were obtained from the Microbial Type Culture Collection and Gene Bank, Chandigarh, India. All media used were purchased from Himedia. Carbopol 934 and β cyclodextrin were gift samples from Sun Pharma Advanced Research Centre, Baroda. All the reagents and chemicals used were of analytical grade.

Solubility Study by Heating and Equilibration Method

The API was studied for its thermostability at 121°C for 20mins for 4 cycles using β-cyclodextrin as a solubilizer prior to conduct of solubility study by heating and equilibration method (7). Three disposable crimped top glass vials were taken and 1 gm of drug in 15 ml of distilled water was added and sealed. The glass vials were then heated in an autoclave (121°C for 20 mins). After cooling to ambient temperature, the vials were opened, small amount of solid drug was added to the vial to promote drug precipitation (i.e. 1gm, 1.25gm, 1.5gm). They were resealed and then all three vials were kept in an ambient temperature (22°C-23°C) under constant agitation for 3 to 7 days. Then the suspension was filtered through a 0.45µm membrane filter and the solution analyzed by HPLC method.

Partition Co-Efficient Study

The Partition co-efficient study(8) was carried out for both Aqueous extracts of *Garcinia mangostana* and *Aloe vera* using n-octanol as the oil phase and distilled water as the aqueous phase.

Determination of Minimum Inhibitory and Bactericidal Concentrations

The minimal inhibitory concentration (MIC) values were determined by broth dilution assay (9–11). The cultures were prepared at 24 h and 48 h broth cultures of *Staphylococcus epidermidis* and *Propionibacterium acnes*, respectively. The MIC was defined as the lowest concentration of the compound to inhibit the growth of microorganisms. Six sterile test tubes with 9ml sterile nutrient broth were taken. 1ml of different concentration of drug solution was added and 0.1ml inoculum was also added to the test tube aseptically and media blank with the nutrient broth and the drug solution was also prepared. A positive control, containing media with 0.1ml inoculum was maintained to indicate the growth promotion capacity of the media. Test samples of Staphylococcus epidermidis were incubated at 37°C for 24hours and those of Propionibacterium acnes were incubated under anaerobic condition in an anaerobic jar (Hi- Media) with gas pack for 48h.

MBC was determined by subculturing the samples on to sterile nutrient agar plates, from the three test tubes which had shown no growth during determination of MIC. The plates were incubated following the procedure as described in MIC determination. The minimum bactericidal concentration values were interpreted as the highest dilution (lowest concentration) of the sample, which showed no growth on the agar plates.

Compatibility Study by HPLC Method

The compatibility studies provide the framework for the drugs combination with the excipients in the fabrication of the dosage form. The physical mixtures of the extract of *Garcinia mangostana* and the various excepients used in the formulation of gels were mixed thoroughly and analyzed using HPLC for the integrity of the actives of the extract.

Formulation of Poly Herbal Gels

Gels were formulated using polymers in different ratios (Table 1) such as carbopol-934, Hydroxy Propyl Methyl Cellulose (HPMC) and excipients like trimethanolamine, methyl paraben and propyl paraben. Accurately weighed quantity of Carbopol-934 and HPMC were dispersed in water with the aid of heat, cooled and then triethanolamine was added drop by drop with constant stirring till pH was neutralized and gel was formed. Then measured quantity of aqueous extract of *Garcinia mangostana* and 1% *Aloe vera* extract were added to Carbopol gel to contain aqueous extract of *Garcinia mangostana* equivalent to 1MIC in 500mg gel and mixed by constant and continuous stirring.

Table I Thermostability Studies

Drug	Heating Cycle	Percentage of Drug (%)
Aqueous Extract of *Garcinia mangostana*	STD, Drug	100
+	1st Cycle	99.60
β-Cyclodextrin	2rd Cycle	99.69
	eed Cycle99.34	
	4th Cycle	99.36

Drug Content Estimation

Accurately weighed 2gm of gel was dissolved in 10ml of methanol and kept in water bath for 5 to 10mins at 80°C to 90°C. It was cooled, filtered and made up to 10ml with methanol. This sample is examined by HPLC.
Drug concentration was calculated by using following formula

$$\frac{\text{Standard weight}}{\text{Sample weight}} \times \frac{\text{Sample Area}}{\text{Standard Area}} \% \text{ of Standard Assay} = \% \text{ of Sample assay}$$

Microbial Assay

Three different concentrations of herbal gel equivalent to 1MIC(500mg of gel), 2MIC and 3MIC from each formula and 500mg of marketed clindamycin phosphate gel [Clincitop gel, universal twin labs]were weighed and diluted with 2ml of sterile water in sterile test tubes. Sterile solid nutrient agar plates were prepared by pour plate method and cup was bored in the center using sterile cork borer of diameter 3cm. The drug solution was carefully transferred into the cup and incubated at 37°C for 24hrs. Zones of inhibition were measured and compared with that of standard marketed product.

Determination of Viscosity

Viscosity of the formulated polyherbal gels was determined using a Brookfield Viscometer (model- MA02346) using Spindle type 93/T-C & 94/T-D.

Determination of Spreadability

Spreadability(12) denotes the extent of area to which the gel readily spreads on application to skin or the affected part. The bioavailability efficiency of a gel formulation also depends on its spreading value. The Spreadability was expressed in terms of time in seconds taken by two slides to slip off from the gel, placed in between the slides, under certain load. Lesser the time taken for separation of the two slides, better the Spreadability. Two sets of glass slides of standard dimensions were taken. The herbal gel formulation was placed over one of the slides. The other slide was placed on the top of the gel, such that the gel was sandwiched between the two slides in an area occupied by a distance of 6.0 cm along the slide. 100gm weight was placed upon the upper slides so that the gel between the two slides was pressed uniformly to form a thin layer. The weight was removed and the excess of gel adhering to the slides was scrapped off. The two slides in position were fixed to a stand without slightest disturbance and in such a way that only the upper slide to slip off freely by the force of weight tied to it. A 20 gm weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 6.0 cm and separated away from the lower slide under the influence of the weight was noted. The experiment was repeated by three times both formulated gel and marketed gel and the mean time taken for calculation.

Spreadability was calculated by using the following formula:

$$S = m \times \frac{l}{t}$$

Where,
S – Spreadability
m – Weight tied to the upper slide (20gm)
l - Length of the glass (6 cm)
t - Time taken in seconds

Drug Diffusion Study

Diffusion study(13)was carried by using Franz diffusion cell of diffusional area 10.52cm^2 and receptor compartment volume of 25ml. Phosphate buffer solution (pH 7.2) was used in the receptor compartment and maintained at 37°C at 100rpm. A sigma dialysis membrane (Mol.Wt 12000) was used as the diffusion membrane. The aliquots were analyzed by HPLC and replaced with same volume of fresh phosphate buffer pH 7.2 to maintain constant volume in the receptor compartment.

RESULTS:

The formulation development work was preceded by Preformulation studies in order to characterize and estimate the suitability for its inclusion into an aqueous gel system. The solubility studies conducted by the heating and equilibration method were ideally preceded by the aqueous extract of *Garcinia mangostana* being subjected to thermal stability studies for 4 cycles of 20 mins each at 121°C. Once found to be thermally stable (Table I), it was further carried over to the equilibration method by which it demonstrated a solubility of 44.28 ml/gm, thereby confirming it to be sparingly soluble in water. A partition co-efficient determination study for topical products is primarily carried out to ascertain the degree of partitioning of the drug from the vehicle into the stratum corneum. The log P values of −0.1669 and −0.4926 were obtained for aqueous extract of *Garcinia mangostana* and *Aloe vera* respectively. These values imply that aqueous extract of Garcinia mangostana has ideal hydrophilic-lipophyllic balance for permeation into the epidermis which will prevent future break-outs whereas Aloe vera gel is predominantly hydrophilic which satisfies our objectives for its inclusion as an emollient into the anti-acne gel.

Table II: Microbial Studies on Aqueous Extracts of *Garcinia mangostana*

Microorganisms	MIC µg/ml	MBC µg/ml	Viable cell count (CFU's)
Staphylococcus epidermidis	220	260	104×10^{-6}
Propionibacterium acne	220 200	260 200	584×10^{-5}

Table III: Evaluation Parameters of Gels

Sl.No	Formulation	Assay (%)	pH	Viscosity (Cps)	Spreadability (gm-cm/sec)
1	F-1	99.0	7.48	261550*	5.192
2	F-2	99.2	7.36	267500*#	1.884
3	F-3	96.8	7.02	267600*	2.735
4	F-4	98.1	7.65	267700*	4.045
5	F-5	99.2	7.18	535480#	4.591
6	F-6	99.4	7.23	534333#	3.313

*measurements made using spindle 93/T-C,
#measurements made using Spindle 94/T-D

The antimicrobial property of the aqueous extract of *Garcinia mangostana* was reconfirmed as depicted in Table II. During the compatibility study between actives and excipients, the herbal extracts were found to be compatible as seen in the HPLC chromatograms of the compatibility studies (Fig I–IV). Retention times for Aqueous extract of *Garcinia mangostana* (14.41 mins), *Aloe vera* gel (16.038 mins) and Carbopol 934 (9.565 mins) were accorded for identification. The compatible ingredients were formulated in different ratios of polymers and evaluated in comparison with a marketed antiacne gel containing Clindamycin Phosphate. F-6 was selected as optimum formulation based on its comparatively better physico-chemical properties for topical application like Assay, Spreadability, pH and Viscosity (Table III).

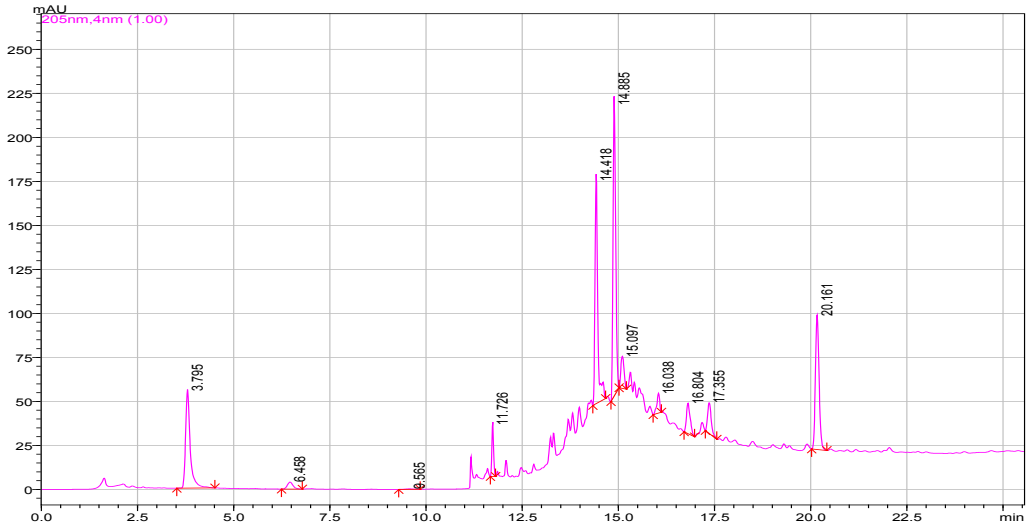

Figure I: Compatibility between Aqueous Extract of *Garcinia mangostana, Aloe vera* Gel Extract and Carbopol-934

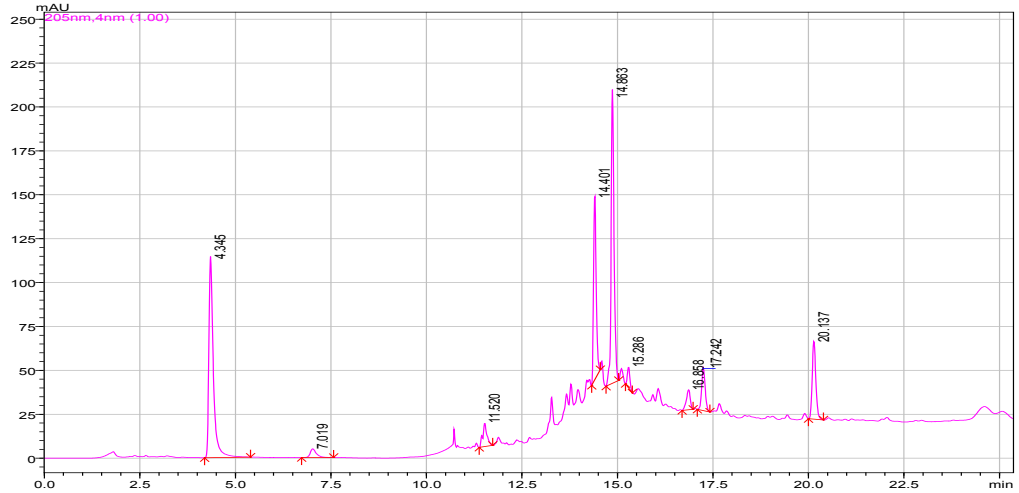

Figure II: Compatibility between Aqueous Extract of *Garcinia mangostana, Aloe vera* Gel and HPMC

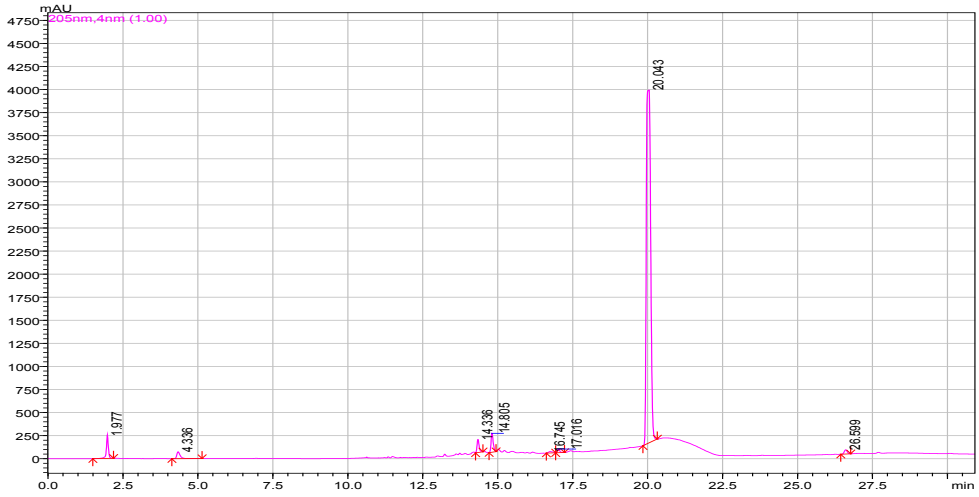

Figure III: Compatibility between Aqueous Extract of *Garcinia mangostana; Aloe vera* Gel and Methyl Paraben

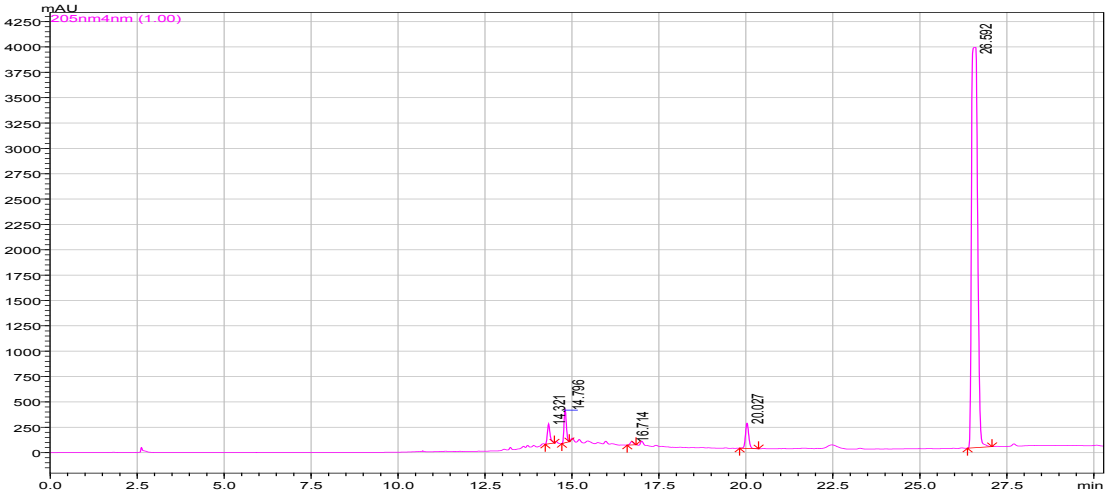

Figure IV: Compatibility between Methanolic Extract of *Garcinia mangostana, loe vera* Gel Extract and Propyl Paraben

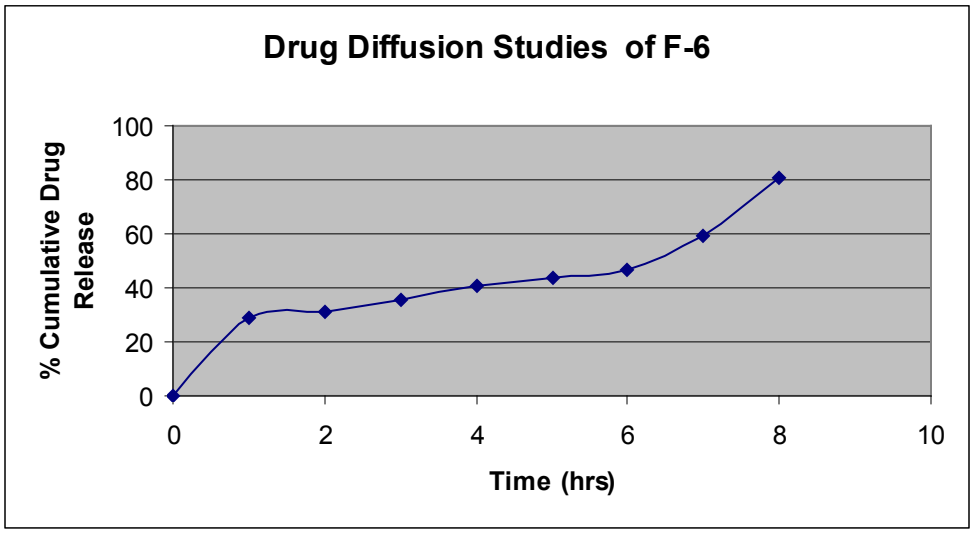

Figure 5: The Drug Diffusion Profile of F-6

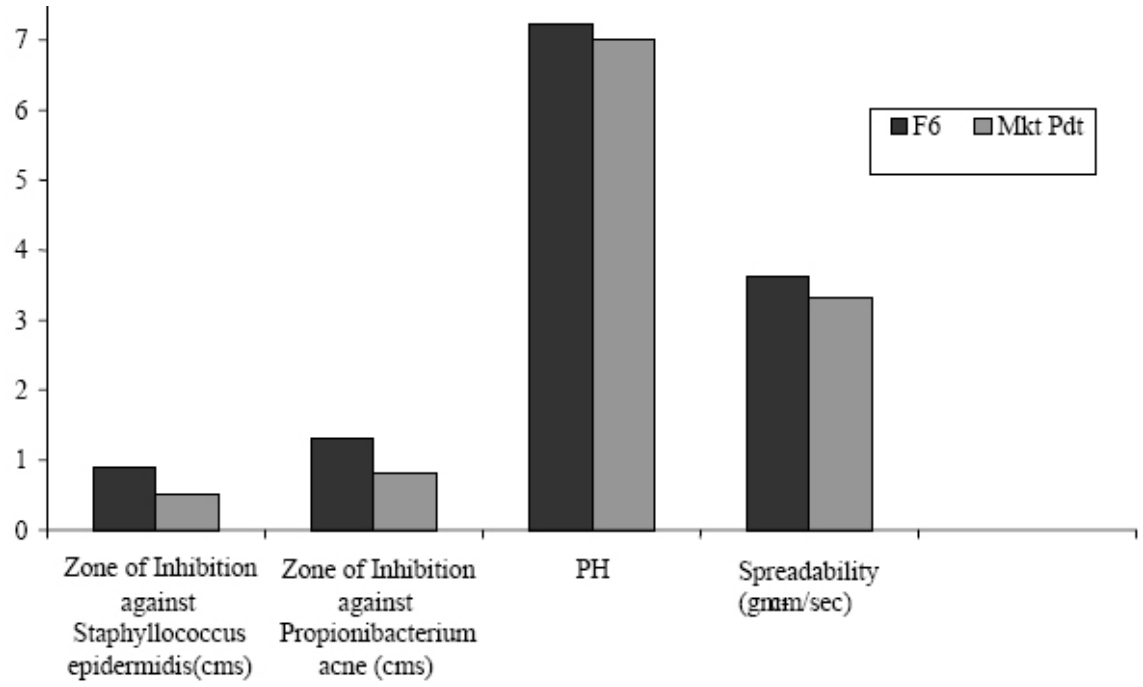

Figure VI: Comparative Evaluation of Gels with Marketed Antiacne Product

Table IV: Microbial Studies on the Gels

Sl. No	Amount of gel	F-1 (cm)	F-2 (cm)	F-3 (cm)	F-4 (cm)	F-5 (cm)	F-6 (cm)
Zone of Inhibition of Poly Herbal Gels against *Staphylococcus epidermidis:*							
1	500mg (1 MIC)	0.9	0.9	0.9	0.9	0.9	0.9
2	1gm (2 MIC)	1.0	0.9	1.0	0.9	1.0	1.0
3	1.5gm (3 MIC)	1.1	1.0	1.1	1.0	1.1	1.1
Zone of Inhibition of Poly Herbal Gels against *Propionibacterium acne*							
1	500mg (1 MIC)	1.2	1.2	1.9	1.2	1.2	1.3
2	1gm (2 MIC)	1.4	1.4	1.4	1.5	1.4	1.5
3	1.5gm (3 MIC)	1.5	1.6	1.7	1.6	1.6	1.7

The design of a gel for topical delivery is aimed at minimizing the flux of the drug through the stratum corneum and maximizing its retention in the epidermis. Drug diffusion study of the polyherbal gel was essential to confirm that the extract would partition from the vehicle and permeate through the semi permeable membrane which symbolizes the stratum corneum. The study was carried out for a period of 8 hours as shown in Fig V in which the gel demonstrated a maximum % CDR of 81.033% and a flux of 0.0879mg/cm^2/hr for the aqueous extract of *Garcinia mangostana*. Since the herbal gel has to finally compete with the marketed anti acne gel, it was deemed fit to conduct the microbial assay on the formulated gels in equivalent quantities (500mg ≡ 1MIC, 1gm ≡ 2MIC and 1.5gm ≡ 3MIC) which showed better zone of inhibition when compared to the marketed clindamycin phosphate gel (500mg of 1% gel) as shown in Table IV.

DISCUSSION

Currently the problem associated with the antiacne therapy is that the topical products available are either cream based (mostly oily) or associated with adverse effects contributed to their chemical nature. *Garcinia mangostana* is proven (14–15) to be active against methicillin- resistant *Staphylococcus aureus, Staphylococcus epidermidis* and *Propionibacterium acne* which are responsible for the outbreak of Acne. The results of the preformulation and antimicrobial studies obtained for the aqueous extract of *Garcina mangostana* reflect the potential for its delivery as a topical agent for treatment of acne vulgaris which forms the basis of our study. The studies (6) on *Aloe vera* extract have proved to significantly benefit in the control and treatment of acne when used in addition with other antiacne agents. So it was appropriately formulated along with Garcinia *mangostana* extract into a topical herbal aqueous based anti-acne gel. The topical approach is effective because the medication

is applied directly to the lesions and the herbs are less likely to cause side effects. The flux of 0.0879mg/cm^2/hr obtained for the aqueous extract of *Garcinia mangostana* also demonstrates the potential of the herb for topical delivery. Since the actives are expected to just permeate the skin into the epidermis which is the target for action along with limited absorption, the Log P values(−0.1669) reflect that the mangostins possess ideal hydrophilic-lipophillic balance for permeation into epidermis and solubility values of 44.28ml/gm reflect its low solubility that limits its absorption. The formulated herbal gel also has the added advantage over the currently used antibiotic treatment in the fact that the bacteria which often develop tolerance and resistance to the antibiotics over time may not be seen here.

CONCLUSION:

Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. So, a Herbal anti-acne solution which is non-toxic, safe, effective and improves patient compliance by the utilization of herbal extracts would be highly acceptable. In conclusion, the aqueous extracts of *Garcinia mangostana* was formulated along with *Aloe vera* gel into a 1%w/w carbopol-934 gel base in order to deliver it in the form of a non –oily(aqueous) topical therapy for treatment of mild Acne.

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