Research Article

Anti-inflammatory, Anti-oxidant and Anti-microbial Properties of Polyherbal Formulation in Acne Treatment

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ABSTRACT

Nowadays, individual herbs are insufficient to achieve a desired therapeutic effect. When it is optimized as multiple herbs composition in a particular ratio it will give a therapeutic effect in a better way with reduced toxicity. In order to develop such an intervention, the present study was intended to develop a polyherbal cream from extracts of *Santalum album, Rubia cordifolia, Ocimum sanctum, Emblica officinalis, Glycyrrhiza glabra, Persea americana, Simmondsia chinensis, Vitis vinifera*. The present study emphasizes on screening of polyherbalism as anti-inflammatory, antioxidant and anti-microbial in Acne treatment. The polyherbal cream showed significant activity against *P. acnes* and *S. auerus* with diameter of 10 mm and 15 mm inhibition zone respectively. The polyherbal cream exhibited moderate antioxidant activity with IC50 value of 8.9 mg/ml. Topical anti-inflammatory activity was assessed by carrageenan induced paw oedema compared with Diclofenac. The percentages of edema inhibition were 79.9 % ($p < 0.01$) after five hours. The outcome of the study suggested that polyherbal cream could be possible to use as the natural anti-acne formulations.

Keywords: Polyherbal cream, Anti-acne, Anti-inflammatory, Anti-microbial, antioxidant.

INTRODUCTION

Herbal treatments applied topically have gained considerable attention due to their widespread use and ill-defined benefit/risk ratio[1]. Topical application of cream and ointment at pathological sites offer great advantages in a faster release of a drug directly to site of action[2]. The concept of polyherbalism has mentioned in "Sarangdhar Samhita". This stated that products with combined extracts of plants are considered more effective rather than individual ones. The active phytoconstituents of individual plants have been recognized butare generally present in small quantities, which is not enough to produce the desired therapeutic action for curing acne. Medicinal plants with antimicrobial, antioxidant and anti-inflammatory properties used in the treatment of acne. Polyherbalism results in cheaper medication by reducing the duration of therapy or individual cost for anti-acne medications[3].

Acne vulgaris (acne) is one of the most commonly encountered skin diseases and usually affects nearly everybody during their lifetime[4]. Pathophysiology of acne is attributed to different notable factors such as androgen-mediated stimulation of sebaceous gland activity, follicular hyper keratinization, hormonal imbalance, inflammation and external bacterial infection. Propionibacterium acnes and Staphylococcus epidermidis are the major bacteria found on skin causes acne[5,6]. A number of topical and systematic therapies are available for acne; various antibiotics, comedolytic agents, and anti-inflammatory drugs are available as a topical therapy, whereas modern systematic cure includes antibiotics, hormones, zinc and laser treatment[7]. However, an excessive use of these drugs over a long time can lead to the rising resistance of bacteria. These drugs have limitations with respect to toxicity and side effects also such as skin drying, headache, nausea etc. To overcome these limitations, there is an imperative need for the development of effective, safe and low-cost anti-acne drugs. Exploration of herbal resources may provide valuable leads that can be further developed as anti-acne drugs [8].

Santalum album seed exhibited significant antioxidant and antimicrobial activity due to rich and diverse presence of saturated fatty acid[9]. The plant bioactives of *Rubia cordifolia* exhibited antioxidant and anti-microbial activities and has been found to have efficacy, traditionally in treatment of acne[10]. *Ocimum sanctum* contains fixed oil and linolenic acid having the ability to block cycloxygenase and lipoxygenase pathways of arachidonic acid metabolism. Therefore, they show anti-inflammatory activities[11]. *Emblica Officinalis* contains two hydrolysable tannins Emblicanin A and B which have antioxidant properties along with anti-microbial activities[12]. *Glycyrrhiza glabra L.* showed existence of numerous useful metabolites such as: flavonoids, saponins, alkaloids and so on. Because of these

constituents they exhibited effective antioxidant and anti-bacterial activities. β-glycyhrritinic acid has anti-inflammatory properties in different animal models[13]. *Persea Americana* which constitute a drug known as piascledine, has inhibited the release and activity of metalloproteinases and pro-inflammatory cytokines which play a major role in the development of osteoarthritis[14]. *Simmondsia Chinensis* extracts shown to possess antimicrobial and antifungal activities against several pathogens. The researchers isolated 10 flavonoids and four lignans from *Simmondsia Chinensis* extracts. They reported that flavonoidaglycosides showed stronger antioxidant and lipoxygenase inhibitory effects than their glycoside counterparts[15]. *Vitis vinifera* has its own significance in traditional medicine system. It has been used for decades for the treatment of various ailments like antioxidant, Antibacterial effects[16].

The selected herbs for this study aimed different pharmacological targets involved in the acne treatment like suppression of the production of inflammatory cytokines and inflammatory transduction cascades, reduction of oxidative factors, enhancement of anti-oxidative enzymes. The development of polyherbal formulation for the treatment of acne having antimicrobial, antioxidant and anti-inflammatory properties is the need of present times.

MATERIALS AND METHODS

Chemicals

Analytical grade chemicals were used for the study. The media and broth used for microbial culture were procured from Hi-Media Pvt. Limited, Bombay, India. The chemi-cals used for the experimental work included Carbopol 940(Merck Ltd), propylene glycol-400 (SD Fine Chemical Ltd), ethanol (Merck Ltd), methylparaben (Supreme Chemicals), propylparaben (Supreme Chemicals), triethanolamine (SD Fine Chemical Ltd), EDTA (S. D Fine lab India).

Plant Material

The dried extract of *Santalum album seed, Rubia cordifolia, Ocimum sanctum, Emblica Officinalis, Glycyrrhiza glabra, Persea Americana, Simmondsia Chinensis, Vitis vinifera* was purchased from local market, India.

Anti-inflammatory activities of polyherbal cream

Carrageenan induced paw edema in rats

The anti-inflammatory activities of the PR/HC/1718/013 Cream under study were evaluated by using the carrageenan-induced edema model. The in50 mg, 100mg, 200mg polyherbal cream and standard diclofenac cream was applied to the plantar surface of the left hind paw of negative control, standard and test drug treated group respectively by gently rubbing 50 times with the index finger. Three hours after the dose, 0.1 ml of 1% carrageenan solution in normal saline was injected via sub-plantar route in the left hind paw of each animal. The right hind paw however received 0.1 ml of saline. Paw edema was measured every 60 min up to 4 h after the injection of carrageenan. A digital Vernier calliper (Digi calliper) was used to measure the difference in footpad thickness between the right and left foot. Mean values of treated groups and control group were compared and analyzed using statistical methods [17].

Antioxidant activities of polyherbal cream

DPPH Radical Scavenging Activity

Antioxidant activities of extracts were measured spectrophotometrically by using 1, 1- diphenyl 2-picryl hydrazyl (DPPH). 0.1 mM DPPH solution was prepared in methanol. 1 ml of DPPH stock solution was mixed with 1 ml of PR/HC/1718/013 Cream solution of different concentrations (50, 100, 200 µg/ml). The mixer of 1 ml methanol and 1 ml DPPH stock solution was used as a control. Ascorbic acid was used as the standard reference compound with the same concentration. The reactions were carried out in triplicate and allowed to stand at room temperature for 30 min. Reduction in the absorbance was measured by UV–Vis spectrophotometer at 517 nm. The inhibition percentage was calculated using the following formula[18].

% Reduction = (Abs DPPH – Abs Dil.)/Abs DPPH x 100

Whereby:

Abs DPPH = Average absorption of the DPPH solution

Abs Dil. = Average absorption of the three absorption values of each dilution

Antimicrobial activity of polyherbal cream

Procedure for Propionibacterium acne activity

The dried surface of a blood agar plate was inoculated by spreading culture suspension of *Propionibacterium acne* (100µl) on agar surface. The wells were bored into the surface of the inoculated agar plate and the known concentration of sample (100µl) was added to the wells. Plates were kept in the freeze for pre-diffusion for 30 minutes and then placed under anaerobic conditions, in an incubator set to 37°C for 48 hours. All the samples were dissolved in methanol: water (50: 50) to obtain different concentrations. The cups were bored in agar medium spread with the test organism, using a sterile cork borer with 8 mm inner diameter. These cups were filled with 100 µl sample solutions and the plates were incubated at 37^0C for 48 hours under anaerobic conditions. The assessment of the antimicrobial activity was based on the measurement of the diameter of the zone of inhibition. Simultaneously zone of inhibition of diluent was also considered in the calculation [19].

Procedure for Staphylococcus aureus activity

Inoculation of Test Plates: The dried surface of a Mueller-Hinton agar plate was inoculated by spreading culture suspension (100 µl) on agar surface. The wells were bored

into the surface of the inoculated agar plate and the known concentration of sample (100 µl) was added to the wells in triplicates. Plates were kept in the freeze for pre-diffusion for 30 minutes and then placed in an incubator set to 37°C for 24 hours. Samples were dissolved in methanol: water (50:50) to obtain different concentrations. Muller Hinton agar as used for antibacterial test. The cups were bored in agar medium spread with the test organism, using a sterile cork borer with 8 mm inner diameter. These cups were filled with 100 µl sample solutions and the plates were incubated at 37^{0}C. The assessment of the antimicrobial activity was based on the measurement of the diameter of the zone of inhibition. Simultaneously zone of inhibition of diluent was also considered in the calculation[20].

RESULT AND DISCUSSION

Anti-inflammatory activities of polyherbal cream

Carrageenan induced paw edema in rats

Carrageenan-induced inflammation is useful in detecting orally active anti-inflammatory agents; therefore, it has significant predictive value for anti-inflammatory agents acting through mediators of acute inflammation. The development of edema induced by carrageenan injection causes an acute and local inflammatory response. In past studies, anti-inflammatory effects by plethysmometric measurement of formalin-induced paw edema on herbal cream formulation. The result indicates that the extracts inhibited the paw edema size and shows inhibition of the inflammation.

The herbal cream formulation has significantly inhibited edema at higher dose. The positive control group was compared with standard i.e. Diclofenac Tablet, Marketed product 1, 2, 3 and cream formulation of PR/HC/1718/013. As per statistical analysis, the herbal test cream has been shown highly significant effect after 3 Hr of drug administration and the standard cream has shown highly significant effect after 2 Hr of drug administration shown in Table 1 and Figure 1.

Table 1: Effect of Herbal cream on carrageenan-induced paw edema in rats

	% inhibition					
	30 min	**1 HR**	**2 HR**	**3 HR**	**4HR**	**5HR**
Diclofenac Tablet	8.56±1.56	5.23±0.07	36.59±1.31	56.91±0.06	72.69±0.43	85.63±1.23***
PR/HC/1718/013	1.23±0.29	4.60±1.21	19.63±0.35	34.09±0.09	59.76±1.04	79.90±0.09**
Marketed Product 1	0.96±2.98	1.90±1.13	12.07±2.01	29.80±1.63	41.06±0.08	52.80±0.82
Marketed Product 2	0.73±1.67	1.53±0.83	13.42±1.10	25.30±1.09	42.08±0.78	53.07±1.01
Marketed Product 3	1.78±0.05	2.27±0.81	16.78±0.85	31.03±0.56	46.75±0.89	59.98±0.94*

Results are expressed as mean ± S.E.M (n=6), one-way ANNOVA followed by Tukey multiple comparison test. *P<0.05, **P<0.01, ***P<0.001, When compared with control groups.

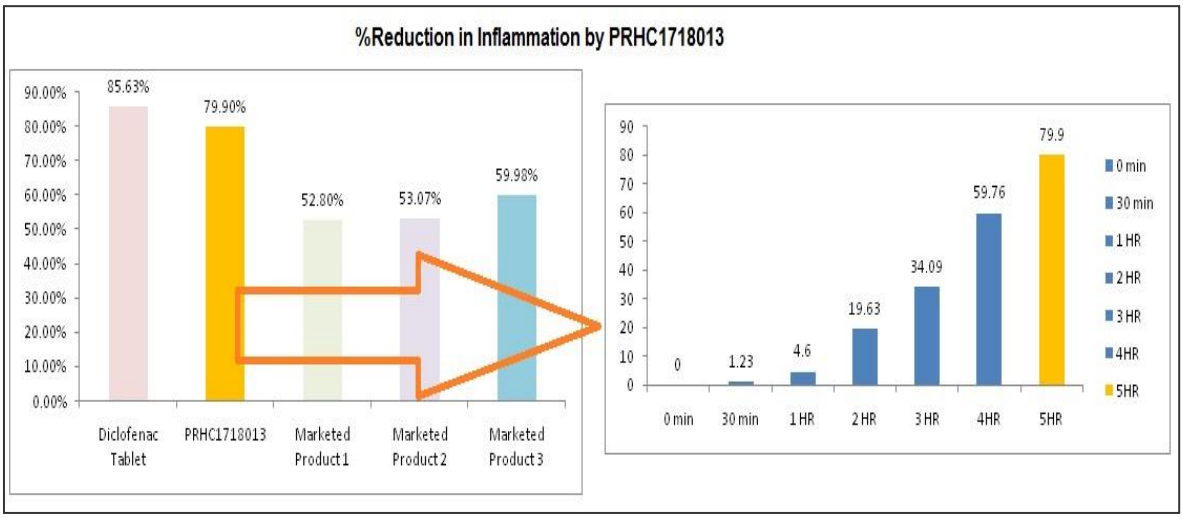

Figure 1: %Reduction in Inflammation by PR/HC/1718/013 cream

Antioxidant assay

DPPH-Free radical scavenging activity

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. In the DPPH assay, the antioxidants are able to reduce the stable radical DPPH to non-radical form, DPPH-H. The purple colored alcoholic solution of DPPH radical changes to yellow in the presence of hydrogen-donating antioxidant which could be measured at 517nm, the activity is expressed as effective concentration EC50, which is the concentration of the sample leading to 50% reduction of the initial DPPH concentration. Effectiveness of antioxidant properties is inversely correlated with

EC50 values. If the EC50 value of a cream less than 10mg/ml, that's mean the extract is an effective antioxidant. In this study, the EC50 value of cream was 8.9 mg/ml less than 10mg/ml this indicates that the samples have effective antioxidant activity (Table 2).

Table 2: Antioxidant properties of PR/HC/1718/013 cream

Sr. No.	Sample Name	DPPH free radical scavenging capacities (mg/ml)
1	PR/HC/1718/013	8.9
2	Marketed Product 1	4.6
3	Marketed Product 2	7.5
4	Marketed Product 3	1.8

Note: Data were expressed as mean ± (n = 3)

Antimicrobial activity of polyherbal cream

To determine antibacterial effects of PR/HC/1718/013 Cream against skin bacteria *Propionibacterium acne* and *Staphylococcus aureus* were chosen for the test. As listed in Table 3, PR/HC/1718/013 showed antimicrobial activities against the tested both strains. Tetracycline, Doxycycline and clindamycin, were active against both strains (Figure 2). PR/HC/1718/013 showed anti-acne activity by inhibiting *Propionibacterium acne* and *Staphylococcus aureus* micro-organisms.

Table 3: Antimicrobial activity of polyherbal cream

		Propionibacterium acne activity		*Staphylococcus aureus* activity	
SN	Name of compound	MIC (µg/mL)	Zone of Inhibition in mm	MIC (µg/mL)	Zone of Inhibition in mm
1	PR/HC/1718/013	75 (µg/mL)	10	140 (µg/mL)	15
2	Tetracycline	2 (µg/mL)	15	7.5 (µg/mL)	16
3	Doxycycline	3 (µg/mL)	14	7.5 (µg/mL)	14
4	Clindamycin	10 (µg/mL)	15	5 (µg/mL)	20

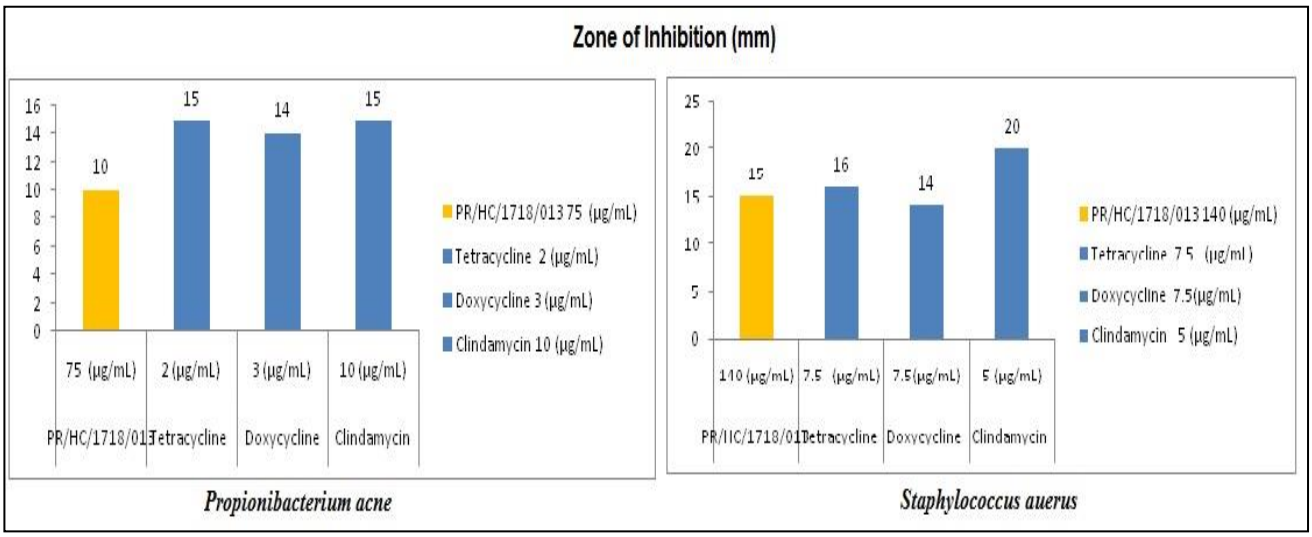

Figure 2: Zone of Inhibition in mm by *Propionibacterium acne* and *Staphylococcus aureus*

CONCLUSION

The results of this study indicate that PR/HC/1718/013 Cream show antibacterial activity with less MIC against acne causing bacteria. Additionally, PR/HC/1718/013 Cream also showed good antioxidant activity along with anti-inflammatory activity. The antioxidant, anti-inflammatory and antimicrobial activities of polyherbal composition might be among the contributing factors that showed remarkable improvement in collagen synthesis and reduced microbial load that could be involved in the healing process of acne. As these are the natural Cream, so they have no or less side effects. These can also be an alternative and better option for resistant acne causing bacteria. The topical anti-acne formulations can be developed using these plant extracts individually or in combination.

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