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Original Article

EVALUATION OFANTI-OXIDANT AND ANTI-ACNE ACTIVITIES (*IN-VITRO*) OF THE FORMULATED HERBAL GELS

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ABSTRACT

Objective: The objective of the present study was to evaluate the anti-oxidant and anti-acne activities (*in-vitro*) of the formulated herbal gels.

Methods: Herbal extracts and volatile oils were prepared and procured. Preliminary screenings for the anti-oxidant and anti-acne activities (in-vitro) were carried out to select the suitable candidates for the preparation of anti-acne herbal gels. Gels were further evaluated for the activities.

Results: The herbal gel (F2) containing the herbal extracts (*Azadirachtaindica*, *Ocmium sanctum*, *Curcuma longa)* each (1%) and volatile oils (*Melaleuca alternifoliae, Salviaesclareae a*nd *Citrus sinensis*) each (0.05%) showed maximum anti-oxidant activity (IC_{50} value 0.407 mg) amongst all four gels. Significant Anti-acne activity against *P. acne* and *S. epidermidis* was showed by F2 when compared with the marketed synthetic gel (Clindac gel).

Conclusion: The study proves that the herbal actives used in the formulation have promising anti-oxidant and anti-acne activity.

Keywords: Herbal extracts, Volatile oils, Gelling agents, Anti-oxidant activity and anti-acne activity.

INTRODUCTION

Acne is found be the universal skin disorder. The main target for the treatment of acne is to-

- Attack the *P. acne* and *S. epidermidis*
- Inhibit synthesis of free radical and release of histamine which causes inflammation
- Decrease sebum production
- Increase wound healing by cellular rejuvenation

The conventional therapies used for acne have following problems such as dryness of skin, dermatitis, darkening of skin, recurrence after withdrawal and the excessive use of antibiotics for longer period develops resistance against *P. acne* and *S. epidermidis*, and results in adverse effects like gram-negative folliculitis [1, 2].

The herbal gel is formulated by using natural gelling agents, herbal extracts and volatile oils. This gel provides multi-therapeutic effect with negligible adverse effect and has similar rheological properties of gel. Hence they are preferred over synthetic ones [3]. The potential candidates showing anti-acne activity are *Azadirachtaindica*, *Ocmium sanctum*, *Curcuma longa, Melaleuca alternifoliae, Salviaesclareae* and *Citrus sinensis*. Hence these plants were selected along with *Aloe vera* mucilage powder 200 X (gelling agent) and carbopol 940 (viscosity modifier) for the formulation [4].

Table 1: List of herbs used with their specification

Herbs	Part of herb	Extraction method	Solvent	Phyto-constituents
Neem	Leaves	Solvent extraction	Methanol	Quercetin
Tulsi	Leaves	Maceration	Methanol	Eugenol
Turmeric	Rhizome	Solvent extraction	Acetone	Curcumin

Table 2: List of volatile oils used with their specification

Volatile oils	Part of herb	Phyto-constituents
Tea tree	Leaves	Terpinen-4-ol
Clary sage	Flowering stem	Linayl acetate, linalool, α-Terpineol
Orange	Fresh peel	D-limonine, linalool

MATERIALS AND METHODS

Neem leaf powder, turmeric rhizome powder, tulsi leaf powder, euxyl 9010, tea tree oil, orange oil, clary sage oil, *Aloe vera* mucilage 200X powder was procured as gift sample. Ascorbic acid, carbopol 940, DPPH (2, 2-Diphenyl-1 picrylhydrazy) were obtained from Merck Pvt. Ltd, Mumbai. Anti-acne activity studies were conducted at Micro Bio Laboratory. All reagents used were of analytical grade.

Preparation of herbal extracts

The methanolic tulsi extract was prepared by maceration method. The methanolic neem and turmeric acetone extract were prepared by soxhlet extraction method.

Preparation of herbal gel

Step 1: Preparation of gel base

Required quantity of *Aloe vera* powder (200 X) was weighed and dissolved in water (100 gm) with continuous stirring using mechanical stirrer at 1000rpm (Remi motor RQT-127 HP1/8) at the temperature between 60-80 ^{o}C for 1hour in the reactor vessel (SS316). Gel forming polymers was added in various proportions to the solution (table 3). The pH of the solution was adjusted to 6.0-6.4 by addition of triethanolamine. Euxyl P. E. 9010 was used as preservative. All the gel bases were allowed to equilibrate for 12 hours at room temperature.

Table 3: Composition of herbal anti-acne gel

S. No.	Formulation codes Ingredients (gm)	F1	F2	F3	F4
Step1:Gelbase					
1.	*Aloevera*20 0Xpowder	0.4	0.4	0.5	0.5
2.	Carbopol-94 0	0.5	0.4	0.4	0.5
3.	Triethanolamine	0.1	0.1	0.1	0.1
4.	EuxylP. E.9010	0.6	0.6	0.6	0.6
5.	Distilledwaterupto	10 0	10 0	10 0	10 0
Step2:Formulationofherbalgel					
1.	Neemextract	1	1	1	1
2.	Turmericextract	1	1	1	1
3.	Tulsiextract	1	1	1	1
4.	Teatreeoil	0.05	0.05	0.05	0.05
5.	Orangeoil	0.05	0.05	0.05	0.05
6.	Clarysageoil	0.05	0.05	0.05	0.05
7.	Gelbase	10 0	10 0	10 0	10 0

Step 2: Preparation of the formulation

The herbal extracts (1% each) and volatile oils (0.05% each) were added with continuous stirring using mechanical stirrer at 1000rpm (Remi motor RQT-127 HP1/8)to different gel bases till the uniform dispersion of the ingredients was achieved.

All these batches were allowed to equilibrate for 24 hours at room temperature. The prepared gel was filled and stored ina wide mouth polypropylene container.

Biological evaluation

1) Anti-oxidant activity (*in vitro*DPPH Method)

❖ **Preparations**

a) 0.3 mM DPPH solution

0.012 gm of DPPH was weighed and dissolved in 90 ml of methanol and volume was made up to 100 ml.

b) Standard preparation

10 mg of ascorbic acid was weighed and dissolved in 100 ml of distilled water in an amber colored volumetric flask. The solution was vortexed on a cyclomixer and used as standard.

c) Sample preparation

The stock solution of 1 gm of sample in 10 ml of methanol was prepared. The solution was sonicated for 10 minsand vortexed. The clear methanolic solution obtained was used for assay.

❖ **Procedure**

i) Different concentrations of test samples were prepared in methanol. 2.5 ml of each test sample was mixed with 1 ml of 0.3 mM DPPH solution. These samples were kept in dark for incubationat room temperature for 30 mins.

ii) Absorbance was measured at 516 nm using UV-visible spectrophotometer (*Jasco V-630*).

iii) Blank was prepared by combining 1 ml of methanol with 2.5 ml of test sample. Control sample was prepared by adding 1 ml of 0.3 mM DPPH solution to 2.5 ml of methanol. Ascorbic acid was used as reference standard. All readings were taken in triplicates.

iv) The results were expressed as % Inhibition of DPPH radical induced by both the tests and standard samples. IC_{50} values were calculated and compared with that of the standard.

v) The inhibition of DPPH radicals by the samples was calculated according to the following equation:

DPPH-scavenging activity (%): $[1-(A_1-A_2)/A_0] \times 100$,
Where,
A_0=absorbance of control.
A_1=absorbance of the sample.
A_2= absorbance of blank

One month trial version of Graph Pad Prim 6.5 software was used to plot graphs and for the calculation of IC_{50} values by point to point curve method [5].

2) Anti-acne activity (*in vitro*)-was carried out by Paper disc diffusion method.

a) Micro-organism and media used

1. *Propionibacterium acne* (MTCC1951) andbrain heart infusion media (BHI)

2. *Staphylococcus epidermidis* (MTCC9041) andsoybean casein digest media (SCD)

b) Sterile Disc: The Whatmann paper No.1 (diameter = 5.42 mm)

c) Samples: Herbal gel formulation and Clindac A gel (Standard)

d) Procedure:

The petri plates were washed thoroughly and sterilized in hot air oven at 160 °C for 1 ½ hours. 20 ml of sterile molten antibiotic assay medium was seeded by micro-organism; it was poured aseptically in sterile plate and allowed to solidify at room temperature. After the solidification of soft agar, sterile disc impregnated with the gel sample were placed over the solidified agar plate.

The plates were left for 10 min's at room temperature to allow the diffusion of gel and incubated at 37 °C for 24 hours for *S. epidermidis* and 48 hrs for *P. acne* under anaerobic conditions. The plates were then observed for the zone of inhibition (mm). The zone of inhibition was calculated by measuring the diameter of the zone including the well diameter. Readings were taken in triplicates (in three different directions) and the average values were calculated

Measurement of zone of inhibition

The zone of inhibition for each sample was observed, measured and expressed in mm. From this the activity index (A. I.) and Percent Inhibition (P. I.) were calculated for all oils and extracts using the following formula.

A. I = Mean zone of inhibition of each formulation

Zone of inhibition obtained for standard

P. I. = Activity index x 100 [2, 6]

RESULTS

1) Anti-oxidant activity (*in vitro* DPPH Method)

a. Anti-oxidant activity of ascorbic acid

b. Anti-oxidant activity of herbal anti-acne gel.

From (fig. 1) the IC_{50} value of ascorbic acid was found to be39.563 µg/0.039563 mg.

Table 4: Anti-oxidant activity of ascorbic acid

Concentration (mcg/ml)	% Inhibition of DPPH radical	Standard deviation
0	0	0
10	11.234	0.58845
20	23.833	0.422383
30	36.83	0.29964
40	50.16	0.541516
50	71.55	0.277978
60	81.92	0.305054
70	87.38	1.036101
80	94.69	0.925993
90	95.49	0.981949

Table 5: Anti-oxidant activity of herbal gel formulations

Concentration mg/ml)	%Inhibition of F1 gel±SD	% Inhibition of F2 gel±SD	% Inhibition of F3 gel±SD	% Inhibition of F4 gel±SD
0	0	0	0	0
0.5	60.54±1.24	61.47±2.02	61.08±1.24	61.27±1.24
1	75.33±1.19	77.48±1.02	75.87±1.19	75.87±1.19
1.5	80.61±0.87	81.68±0.94	80.61±0.76	80.61±0.76
2	82.28±0.94	82.82±0.94	82.28±0.94	82.28±0.94
2.5	83.52±0.92	84.05±0.92	83.46±0.90	83.25±0.077

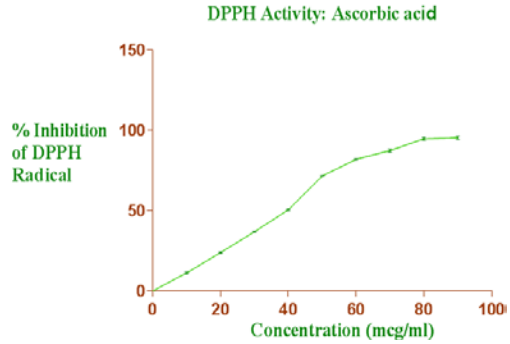

Fig. 1: Plot of % inhibition of DPPH v/s concentration of ascorbic acid

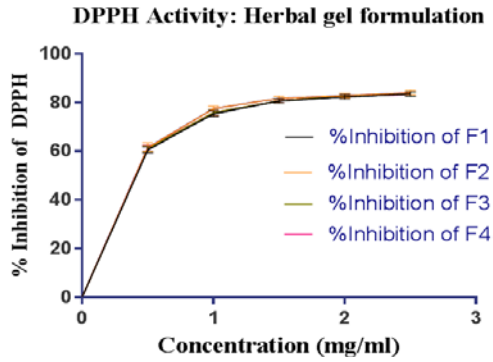

Fig. 2: Plot of % inhibition of DPPH v/s concentration of herbal gel formulations

From (fig. 2)

The IC_{50} value of F1 was found to be 0.413 mg/ml

The IC_{50} value of F2 was found to be 0.407 mg/ml

The IC_{50} value of F3 was found to be 0.409 mg/ml

The IC_{50} value of F4 was found to be 0.408 mg/ml

2) Anti-acne activity (*in vitro*)

The anti-acne activity of the herbal gel formulations showed significant and promising activity against *P. acne* and *S. epidermidis* in comparison with the marketed synthetic gel (Clindac gel).

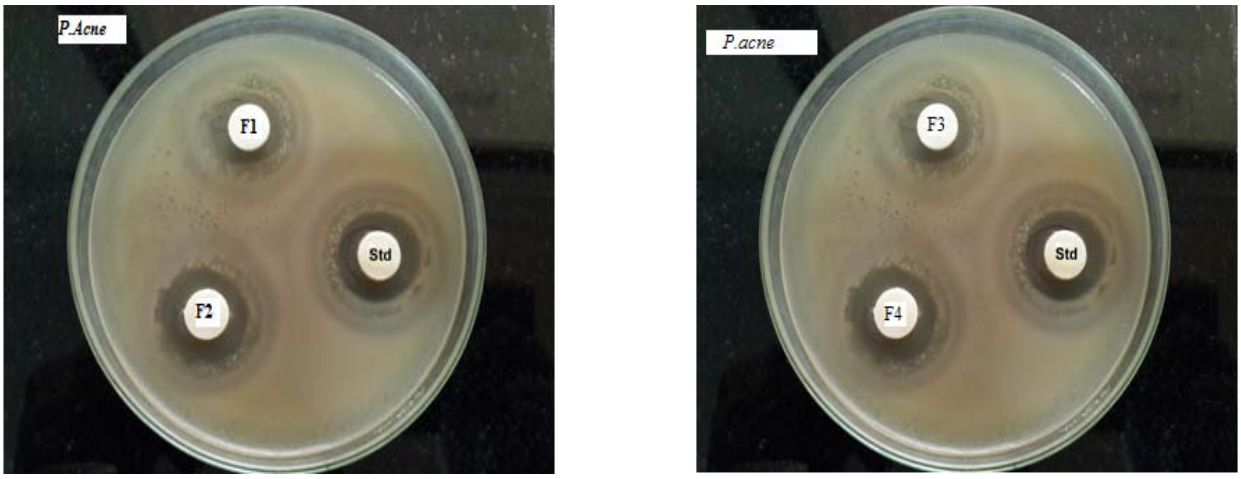

Fig. 3: Anti-acne activity against *P. acne*

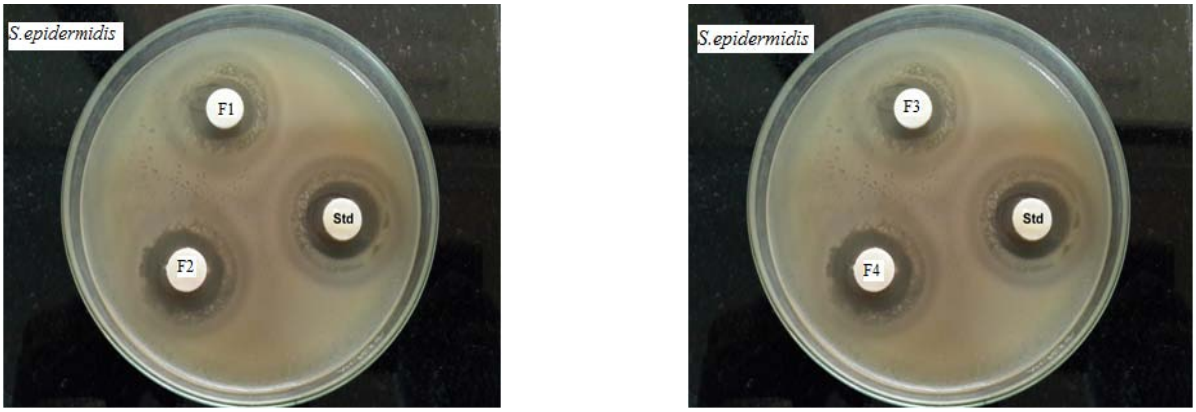

Fig. 4: Anti-acne activity against *S. epidermidis*

Table 6: Anti-acne activity of optimized formulations (*in vitro)*

S. No.	Formulation codes	Zone of inhibition (mm)±SD (n=3)	
		P.acne	***S.epidermidis***
1	F1	19.2±0.05	19.3±0.05
2	F2	19.7±0.05	19.8±0.05
3	F3	19.5±0.05	19.7±0.05
4	F4	19.6±0.05	19.5±0.05
5	Marketed formulation	20±0.05	20±0.05

Table 7: Percentage inhibition of optimized formulations

S. No.	Formulation codes	***P.acne***		***S.epidermidis***	
		A.I.	**P.I. (%)**	**A.I.**	**P.I. (%)**
1	F1	0.96	96	0.965	96.5
2	F2	0.985	98.5	0.99	99
3	F3	0.97	97	0.985	98.5
4	F4	0.98	98	0.97	97
5	Marketed formulation	1	100	1	100

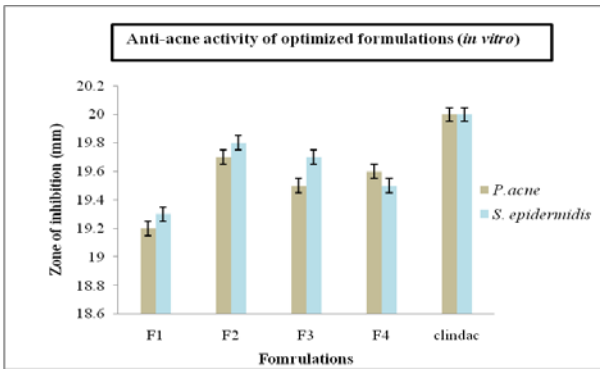

Fig. 5: Anti-acne activity profile of optimized formulations (*in vitro*).

DISCUSSION

The F2 gel containing *Aloe vera* mucilage powder (0.4%) and carbomer (0.4%) shows higher release of herbal actives. It showed maximum anti-oxidant (IC_{50} value 0.407 mg) amongst the four gels. Significant anti-acne activity against *P. acne* and *S. epidermidis* was showed by F2 when compared with the marketed synthetic gel (Clindac gel).

CONCLUSION

The studies conclude that all four herbal gels showed anti-oxidant and anti-acne activity. In addition to the promising biological activity it was also observed that the varying concentrations of gelling agent played an important role in the release of the activephyto-constituents.

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CONFLICT OF INTERESTS

Declared None

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