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Research Article

### FORMULATION DEVELOPMENT AND EVALUATION OF NOVEL HERBAL GEL OF PORTULACA QUADRIFIDA FOR THE TREATMENT OF ACNE

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**Abstract:**

*Portulaca quadrifida* is widely distributed in Africa and tropical Asia in India they are consumed as a cooked vegetable. review of records and scientific literature indicates that portulaca has many medicinal uses like in treatment of ulcers, eczema and dermatitis<sup>1</sup>.The 10%ethanolic extract of the aerial parts (dried leaves and stems) showed significant anti bacterial anti acne and anti inflammatory activity Commonly used benzoyl peroxide and clindamycin show many side effects herbal gel was formulated using extract of *Portulaca Quadrifida* for the treatment of acne .

**keywords:** *portulaca qadrifida* ,Anti acne, Anti inflammatory

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**INTRODUCTION:**

*Portulaca quadrifida* belonging to family portulacaceae is annual or short lived perennial herb used traditionally for treatment of ulcers, eczema and dermatitis [1-5]. The plant is usually eaten as vegetable. *Acne vulgaris* is extremely common skin disorder. *Propionibacterium acnes*, plays an important role in the pathogenesis of acne. It is implicated in the development of inflammatory acne. The 10% ethanolic extract of the aerial parts (dried leaves and stems) showed significant anti bacterial anti acne and anti inflammatory activity

**MAERIALS**

Carbapol 934, HPMV K15, glycerine, propyl paraben and triethanolamine required for formulation of gel were procured from SS Pharma, Hanmakonda, Warangal. Winstar rats weighing 250-300 g.

**Methodology:****Collection and Authentication of Plant:**

The aerial parts of plant material of *P. quadrifida* were collected from the Mulkanoor, Karimnagar, Telangana. The plant material was identified taxonomically and authenticated by Dr. Mustafa Department of Botany, Kakatiya University.

**Processing of Plant Parts:**

The aerial parts of *Portulaca quadrifida* are dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve and stored in airtight container for future use.

**Extraction:**

Dried aerial parts of plants are weighed into a round-bottomed flask fitted with condenser and a heated mantle. The whole content was refluxed with 10%

v/v aqueous ethanol (90 ml distilled water 10 ml ethanol) for 4 hrs. The resulting slurry was filtered through Whatman filter paper and the residue was again refluxed with fresh solvent as above.

The two volumes of the combined filtrate were reduced using a rota vapour under reduced pressure. The concentrated extract was transferred to tared dishes and dried on a water bath and finally in a vacuum oven at 40°C. The solid extract yellowish brown hygroscopic solid extract was scraped before complete drying and then dried to constant weight

**Acute Toxicity Testing:**

In many pharmacological screening, acute toxicity on mice will be performed before going to other activities. In acute toxicity test, a single dose of the drug is used in each animal on one occasion only for determining gross behaviour and LD<sub>50</sub>. LD<sub>50</sub> is the dose which kills 50% of animals in 24 hours

**Rabbit Blood Agar:**

Blood agar generally refers to an enriched base medium to which defibrinated mammalian blood has been added [6-9]. "Blood agar" is usually prepared from Tryptic Soy Agar or Columbia Agar base with 5% Sheep blood. Rabbit or horse blood may be used for growth of NAD-requiring organisms, such as *Haemophilus* species, but the hemolytic patterns may be inconsistent with those on sheep blood shown fig 1 & 2. (Human blood is discouraged because of the increased possibility of exposure to human blood-borne pathogens such as HIV or hepatitis.)



Fig 1: Preparation of Blood Agar Plate



Fig 2: Incubation of *P. Acnes*

**Formulation Development and Evaluation of Gel:**

Eight different formulations were prepared using different concentration of carbopol 934 P and HPMC separately [10,11]. Accurately weighed carbopol/HPMC was taken in a beaker and dispersed in 50 ml distilled water with constant stirring using a mechanical stirrer for 30 min at 1200 rpm. After all

the carbopol/HPMC was dispersed ,propylene glycol was added with constant stirring .To the above extract presevatives were added and mixed well.10 ml of triethanolamine was added to bring the pH close to neutral which results to a consistent gel shown in Table-1.

**Table 1: Compositions of P. Quadrifida Extract Gel Formulations**

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Carbopol 934 (g)	0.5	1	1.5	2.5	-	-	-	-
HPMC gm	-	-	-	-	0.5	1	1.5	2.5
Extract	2%	4%	6%	8%	2%	4%	6%	8%
Propylene glycol (mL)	5	5	5	5	5	5	5	5
Propyl Paraben (0.2%) (mL)	2	2	2	2	2	2	2	2
Triethanolamine (mL)	q.s.	q.s	q.s	q. s	q.s	q.s	q.s	q.s
Distilled water (mL)	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

**Fig 3: Preprated Gel Formulation**

### Evaluation of the Formulated Gels

#### Measurement of pH :

The pH of various gel formulations was determined by using digital pH meter. One gram of gel was dissolved in 100 ml distilled water and stored for two hours. The measurement of pH of each formulation was triplicate and average values were calculated.

#### Viscosity Study:

The measurement of viscosity of the prepared gel was done with a Brookfield Viscometer. The gels were rotated at 0.3, 0.6 and 1.5 rotations per minute. At each speed, the corresponding dial reading was noted. The viscosity of the gel was obtained by multiplication of the dial reading with factor given in the Brookfield Viscometer catalogues.

#### Spreadability:

One of the criteria for a gel to meet the ideal quantities is that it should possess good spreadability. It is the term expressed to denote the extent of area to which gel readily spreads on application to skin or affected part. The therapeutic efficacy of a formulation also depends upon its spreading value. Spreadability is expressed in terms of time in seconds taken by two slides to slip off from gel and placed in between the slides under the direction of certain load. Lesser the time taken for separation of two slides, better the spreadability. It is calculated by using the formula:

$$S = M \cdot L / T$$

Where,

- M = Wt. tied to upper slide
- L = Length of glass slides
- T = Time taken to separate the slides

#### Homogeneity:

All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their appearance and presence of any aggregates.

#### Stability Study:

The stability study was performed as per ICH guidelines. The formulated gel were filled in the collapsible tubes and stored at different temperatures

and humidity conditions, viz. 25°C ± 2°C / 60% ± 5% RH, 30°C ± 2°C / 65% ± 5% RH, 40°C ± 2°C / 75% ± 5% RH for a period of three months and studied for appearance, pH, viscosity and spreadability.

#### Skin Irritation Study:

Guinea pigs (400-500 g) of either sex were used for testing of skin irritation. The animals were maintained on standard animal feed and had free access to water. The animals were kept under standard conditions. Hair was shaved from back of guinea pigs and area of 4 cm<sup>2</sup> was marked on both the sides, one side served as control while the other side was test. Gel was applied (500 mg / guinea pig) twice a day for 7 days and the site was observed for any sensitivity and the reaction if any, was graded as 0, 1, 2, 3 for no reaction, slight patchy erythema, slight but confluent or moderate but patchy erythema and severe erythema with or without edema, respectively [11-14].

#### Evaluation of Anti acne activity:

##### Test Microorganisms and Growth Media:

*P. acnes* (MTCC 1951) were purchased from the M.T.C.C., Institute of Microbial Technology, Chandigarh (India). Blood agar base nutrient media was used as a growth media

##### Anti-Acne Testing Using Disc Diffusion Method:

All of the prepared formulations were checked for their antimicrobial activity against *Propionibacterium acnes*, one of the bacteria responsible for causing acne, using the disc diffusion method this method relies on the inhibition of bacterial growth measured under standard conditions. Filter paper discs impregnated with a specific concentration of a particular antibiotic are placed on the medium. The organism grows on the agar plate while the antibiotic "works" to inhibit the growth. If the organism is susceptible to a specific antibiotic, there will be no growth around the disc containing the antibiotic. Thus, a "zone of inhibition" can be observed and measured to determine the susceptibility to an antibiotic for that particular organism. This study was performed 4 times for each formulation [15-17].



**Fig 4: Zone Of Inhibition**

#### **Evaluation of Anti-Inflammatory Activity:**

##### **Animals:**

Albino Wistar rats of either sex, weighing 150–200 g were taken. They were housed in standard environmental conditions and fed with standard rodent diet with water and libitum. All animal are made in to three groups (Control, Test and Standard) of six animals in each group were used for experiment.

##### **Fresh Egg White Induced Paw Edema:**

The Swiss albino rats weighing between 150-200g were divided into three groups. Each consists of 4 animals, one group served as negative control. The second group served as positive control (received Diclofenac gel) The third group served as test (F4). Edema was induced by administration of 0.05 ml of undiluted fresh egg white in the sub- plantar region<sup>3</sup>. The gel (F4) and Diclofenac gel were applied topically at the site of inflammation. The paw volume was measured at 0hr- 3hr after the injection of fresh undiluted egg white using Plethysmograph [18].

**Statistical analysis:** Statistical analysis were performed by one way analysis of variance (ANOVA) followed by student's test.<sup>(4)</sup>At 95% confidence interval, P values < 0.001 were considered significant

##### **Carrageenan-Induced Rat Paw Edema:**

Animals were fasted for 24 hrs. Before the experiment with free access to water. Approximately 50 µl of a 1% suspension of carrageenan in saline was prepared 1 h before each experiment and was injected into the plantar side of right hind paw of rat. 0.2 g of herbal gel was applied to the plantar surface of the hind paw by gently rubbing 50 times with the index finger. Rats of the control groups received the plain gel base and 0.2 g 1% diclofenac gel applied in the same way was used as a standard. Drugs or placebo were applied 1 h before the carrageenan

injection. Paw volume was measured immediately after carrageenan injection and at 1, 2, 3 and 4 hrs intervals after the administration of the noxious agent by using a plethysmometer [19].

#### **RESULTS AND DISCUSSIONS:**

##### **Acute Toxicity Studies:**

The acute oral toxicity study was carried out as per the guidelines set by organisation for Economic Co-operation and Development (OECD) revised draft guidelines 423, received from Committee for the purpose of Control and supervision of Experimental animals (CPCSEA), Ministry of social justice and Empowerment, Govt. Of India.

The aqueous extract of leaf of *P.quadrifida* was evaluated for acute oral toxicity studies. The leaves were studied at doses of 2000mg/kg and 5000mg/kg observed that none of the doses of the extract produced any signs of toxicity or mortality. Hence, the extract was considered to be safe up to the dose levels of 5000mg/kg bodyweight. Therefore pharmacological studies were carried out at doses of 500mg/kg, by oral administration.

##### **Physical Evaluation of Topical Gel:**

Herbal gel prepared using 2, 4, 6 and 8 % *P.quadrifida* extract with two different polymers namely carbopol 934P and HPMC separately eight batches were prepared and found that all the formulations are greenish in colour translucent in appearance and homogeneous gels prepared using carbopol 934P had pH value between 6.4 to 7.0 where as HPMC based gels revealed a pH value in the range of 6.8 to 7.2. The gels which have pH range of 5.5 to 7.5 are most ideal, as they are near to pH of skin and cause practically no irritation. The viscosity (cps) of the prepared gel with carbopol 934P was 32170 to 64250 cps while HPMC based gels was found to be 55380 to 81548cps shown in table 2.

The spread ability (gm.cm/sec) of the carbopol 934P based gels were 22012 to 48.47gm.cm/sec while HPMC based gels viscosity was 16.44 to 30.30 gm.cm/sec.however it is observed that there is marked increase in viscosity range with HPMC verses carbopol 934 P.

Taking all the parameters into consideration the F4 batch is considered as optimised one and is chosen for stability study as per ICH guidelines .Stability studies for gels reveled good physical stabiliti, colour and consistency for the optimised formulations along with drug content shown in table3 &4.

**Table 2: Physical Evaluation of Topical Gel of P. Quadrifida Leaf Extract**

Formulation	Color	Appearance	Spreadability (g.cm/sec)	Viscosity (dyn.s/cm <sup>2</sup> )	pH	Skin irritation
F1	Greenish	Homogeneous	37.5.	32170	7.0	Nil
F2	Greenish	Homogeneous	25.46	48240	6.8	Nil
F3	Greenish	Homogeneous	22.12	53180	6.4	Nil
F4	Greenish	Homogeneous	48.47	64250	7.0	Nil
F5	Greenish	Homogeneous	30.0	55380	6.8	Nil
F6	Greenish	Homogeneous	24.07	65640	7.0	Nil
F7	Greenish	Homogeneous	17.79	78720	7.2	Nil
F8	Greenish	Homogeneous	16.44	81540	7.0	Nil

**Table 3: Intermediate Stability Studies**

30 °c ± 2°C/65% ± 5% RH	Colour	Appearance	Spreadability (gm.cm/sec)	pH
Initial	Greenish	Homogenous	48.47	7.0
1 month	Greenish	Homogenous	47.91	6.97
2 month	Greenish	Homogenous	46.41	6.94
3 month	Greenish	Homogenous	46.43	6.93

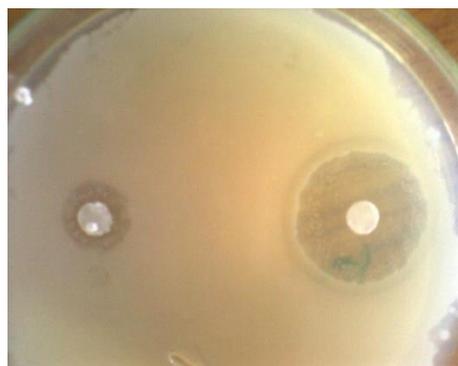
**Table 4: Accelerated Stability**

40 °c ± 2°C/75% ± 5% RH	Colour	Appearance	Spreadability (gm.cm/sec)	pH
Initial	Greenish	Homogenous	48.47	7.0
1 month	Greenish	Homogenous	47.86	6.90
2 month	Greenish	Homogenous	46.79	6.87
3 month	Greenish	Homogenous	46.58	6.93

**Evaluation of Anti-Acne Activity:**

The results of this investigation showed that all developed formulations had inhibitory effect on the *P.acnes*. Formulation 4 has higher activity than that of other developed formulations. The activity of the

developed formulation 4 has been comparable to that of marketed preparation. However, the activity of the standard was more than that of all developed formulations; the diameter of zones of inhibitions was shown in figures 5 & 6.

**Fig 5: Anti acne activity of F4****Fig 6: Anti acne activity of F4**

The zones of inhibitions for all the formulations are greater than 10.4 mm. This suggests that the other active ingredients of the formulations containing secondary metabolites like triterpenoids, flavonoid, tannins and saponins may have contributory antibacterial activity. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. The mechanism of action of terpenes is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds. *P. acnes*, an anaerobic pathogen, is implicated in the development of inflammatory acne. The formulations having antibacterial agents inhibiting the *P.acnes*, may also reduce the development of inflammatory acne

#### Evaluation of Anti-Inflammatory Activity:

The results of anti-inflammatory activity by fresh egg white induced paw edema method was shown in table 5. *P.quadrifida* produced a potent anti-inflammatory activity against the paw edema in Swiss albino rats when compared with reference standards ( $p < 0.001$ ). The potency was found to be inversely proportional to the time (table-6) taken for reduction in the paw volume. The inflammatory

response is a physiological characteristic of vascular tissue. Increased permeability seen in the inflammatory reaction leads to exudation of fluid rich in plasma proteins, coagulation factors and injured tissues with subsequent edema at the site. Exudation which is a consequence of vascular permeability is considered as major features of acute inflammation. Histamine and other mediators of inflammation increase vascular permeability at various times after injury. Chemically induced vascular permeability can cause an immediate reaction and its inhibition suggests that the topical administration of test formulation may effectively suppress the exudative phase of acute inflammation induced by undiluted fresh egg white. The results also show the effect of formulation on edematous response to egg white induced paw edema, provoking an inhibitory effect equal to that of standard Diclofenac gel.

#### Anti- Inflammatory Activity:

The results of anti-inflammatory activity by Carrageenan induced paw edema method was shown in table 6. The data presented in this study demonstrate that the herbal gel formulated possess significant topical anti-inflammatory properties,

**Table 5: Anti- Inflammatory Activity by Fresh Egg White Induced Paw Edema Method**

Drug	0 min	30 min	60 min	120 min	180 min
Control drug (Normal saline)	0	0.34±1.4	0.42±1.03	0.49±2.15	0.58±2.15
Standard drug (Diclofenac gel)	0	0.36±0.85	0.39±0.75	0.35±0.70	0.32±1.4
Formulated gel f4	0	0.37±0.85	0.34±0.9	0.30±1.03	0.28±1.25

**Table 6: Effect of Topical Herbal Gel on Carrageenan-Induced Paw Edema**

Mean	0 min	30 min	60min	90min	120min	150min	180min	210min	240min
Contr oll	0.20 ± 0.003	0.43 ± 0.022	0.41 ± 0.011	0.54 ± 0.012	0.65 ± 0.001	0.80 ± 0.01	0.74 ± 0.014	0.79 ± 0.001	0.77 ± 0.002
Stand ard	0.19 ± 0.016	0.21 ± 0.014	0.26 ± 0.013	0.31± 0.013	0.37± 0.011	0.42± 0.012	0.39 ±0.016	0.36 ±	0.014 0.35± 0.022
F4	0.17 ± 0.007	0.23 ± 0.016	0.29 ± 0.015	0.32 ± 0.017	0.35± 0.011	0.45± 0.012	0.41± 0.012	0.40 ± 0.015	0.37± 0.014

**CONCLUSION:**

Herbal formulations have growing demand in the world market. It is a very good attempt to establish the herbal gel containing hydro-alcoholic extract of *Portulaca quadrifida*.

This study revealed that the developed single herbal formulation F4 was comparatively better than other formulation. And developed formulation useful in overall treatment of acne with its anti acne anti-inflammatory properties.

In our study topical herbal gel was formulated using different polymers like carbopol 934 and HPMC and evaluated for physical and pharmacological parameters

From result of this study, that formulated gel was found to reduce the inflammation in carrageenan induced paw edema. The effect was comparable with the diclofenac sodium.

The formulation developed also proved for anti acne property. F4 showed 15.mm of zone of inhibition on *Propionibacterium acnes* (MTCC1951).

On the basis of the result obtained in the present investigation, it is possible to conclude that the gel of the extracts of above plants has significant in complete treatment of Acne.

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