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Development and evaluation of anti-acne gel containing *Luffa acutangula* Seeds extract

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Abstract
Acne is an inflammatory skin disease that occurs due to blockages in polysebase and inflammation that are caused by bacteria. *Propionibacterium acnes* are common pus-forming microbes responsible for the development of acne. Medicinal plants play an important role in the development of potent therapeutic agents. Plant based drugs provide outstanding contribution to modern therapeutics as a source of many valuable secondary metabolites which serves as plant defense mechanisms against predator such as microorganism, insects and herbivores which have been proved to be potentially active compounds. There is a tremendous increase in search of antimicrobial plant extracts due to the fact that the resistance offered against antibiotic by the microorganism, in short the effective life span of any antibiotic is limited. The herbal gel preparation was formulated based upon traditional knowledge and emphasis was to formulate a stable and functionally effective. The advantage of this gel is reduced adverse effects unlike synthetic drugs. The preliminary phytochemical screening of hydroalcoholic extract of *Luffa acutangula* seeds showed the presence of Carbohydrates, Amino acids, Proteins and tannins. The formulations were tested for the anti-acne activity by well diffusion method against *Propionibacterium acnes*. Results showed that the gels were non-irritant, stable and possess anti-acne activity. The efficacy when tested with a standard was almost same to that of marketed gel. This suggests that seeds of *Luffa acutangula*, have potential against acne causing bacteria and hence they can be used in topical anti-acne preparations and may address the antibiotic resistance of the bacteria.

Keywords: *Luffa acutangula*, Anti-acne gel, *Propionibacterium acnes*

1. Introduction
Acne is a skin disease with the highest prevalence among other skin disorders. Almost everyone has experienced acne prone skin, especially in an adolescent. Although it is considered not as a dangerous disease, but in fact, almost all acne sufferers feel disturbed appearance that often leads to lower levels of confidence and interfere with the daily activities [1-3]. *Propionibacterium acne* plays a role in the development of inflammatory acne by activating complements and can metabolize sebaceous triglycerides into fatty acids, which neutrophils were attracted [2]. In addition, *S. epidermidis* within sebaceous unit responsible in superficial infection [3]. When bacteria colonize into the comedons, and then the inflammatory factors are released by those bacteria. This made the comedons transformed into pustules and pimples. The inflamed acne becomes rupture and forms nodulus, also probably forms scars after healing [4-9]. The pathogenicity mechanism of acne was the production of sebums, follicular hyper keratinization, bacterial colonization, and inflammation. Acne vulgaris is characterized by various clinical conditions such as scaly red skin (seborrhea), erythematous papules and pustules, comedones, nodules, deep pustules, and sometimes piples [10-13].
Gel are semisolid system consisting of dispersion made up of either small inorganic particle or large organic molecule enclosing and interpenetrated by liquid. Gels are usually clear transparent semisolid containing the solubilized active substances, in which a liquid phase is constrained within a three-dimensional polymeric matrix (consisting of natural or synthetic gums) in which a high degree of physical (or sometimes chemical) cross- linking has been introduced. Some of these systems are as clear as water in appearance, visually an esthetically pleasing as in gelatin deserts and other are turbid. The clarity range from clear to a whitish translucent. The polymers are used between 0.5-15% and in most of the cases they are usually at the concentration between 0.5-2% [14-16].
Luffa acutangula (Family: Cucurbitaceae) is commonly known as Ridge gourd. It is a widely growing vegetative climber. The fruits are bas ball club shaped. Various pharmacological activities include hepatoprotective activity, antidiabetic activity, antioxidant activity, fungistatic poperty, CNS depressant activity.

Used in ayurveda to prevent premature greying of hair, seed possess enzymes that rest or melanin in hairs root. Apply directly to scalp can stimulate hair follicles, releave ichiness and dry skin, and potentially promote healthy hair growth. The roots of *Luffa acutangula* added to milk or water is helpful in the removal of kidney stones. In cosmetics, seeds of *Luffa acutangula* used as detoxifying agent [17-19].

2. Material

2.1 Selection and collection of plant material

Plant material (seeds) were collected and then rinsed with distilled water. Then the seeds were collected and shade dried for about 1 weeks. Dried seeds were grinded using electronic grinder. Powdered seed extract was observed for their color, odor, taste and texture.

2.2 Chemical reagents

All the chemicals used in this study were obtained from Himedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals and solvent used in this study were of analytical grade. The pathogenic microbes used in the current study are obtained from Microbial Culture collection, National Centre Forcell Science, Pune, Maharashtra, India.

3. Experimental methods

3.1 Extraction of seeds of *Luffa acutangula*

Dried powdered of seeds of *Luffa acutangula* has been extracted with hydroalcoholic solvent using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40 °C and stored in an air tight container free from any contamination until it was used [20].

3.2 Phytochemical Investigation

The prepared plant extract evaluated for preliminary phytochemical screening of Amino acids, Carbohydrates, Fixed oils and Fats, Proteins using standard procedure [21].

3.3 UV Visible Spectroscopy

3.3.1 Standard stock solution preparation:

The seed extract (10mg) was weighed accurately and dissolved in 100 ml of distilled water to prepare a stock solution of 100 ppm and scanned over range of 200-800 nm using Shimadzu 1800 UV spectrometer for determining the wavelength (λ_{max}) at which maximum absorbance occurs in distilled water.

3.3.2 Standard calibration curve

From the above prepared stock solution, 0.5-4.5 ml samples are withdrawn and diluted up to 10 ml to obtain 50-450 µg/mL of solutions. Then standard calibration curve was plotted against absorbance verses different concentrations at 265 nm.

3.4 Formulating anti-acne gel

Firstly carbopol 934 soaked in 40 ml water for overnight. Then measured quantity of methyl paraben, polyethylene glycol and hydroalcoholic seed extract of *Luffa acutangula* and dissolved in about 35 ml of water in beaker and were stirred at high speed using mechanical stirrer. Then carbopol 934 solution was added slowly to the beaker containing above liquid while stirring and make up the volume upto 100 ml. Neutralized the solution by slowly adding triethanolamine solution with constant stirring until the gel is formed [22-29].

Table 1: Formulation of anti-acne gel

Sr. No.	Chemicals	F1	F2	F3
1.	Seed Extract	1g	1g	1g
2.	Carbopol 934	0.25g	0.5g	1g
4	Polyethylene glycol	0.2ml	0.2ml	0.2ml
5	Methyl Paraben	0.08mg	0.08mg	0.08mg
6	Triethanolamine	0.5ml	0.5ml	0.5ml
8	Water	100ml	100ml	100ml

3.5 Evaluation of Prepared Herbal gel

To evaluate the prepared formulations, following test were carried out.

3.5.1 Physical appearance/visual inspection

The formulations prepared were evaluated in terms of their colour, odour, and consistency.

3.5.2 Determination of pH

Mix 1gm of gel with 10ml of water and determine the pH using digital pH meter at room temperature.

3.5.3 Rheological or Viscosity evaluations

All the samples were allowed to equilibrate for 24 hours at room temperature prior to performing rheological measurements. The viscosity of the gel was determined by using Brookfield viscometer. Gel is taken in a beaker and spindle is dipped in it for about 5min. and then reading was taken at 100 rpm.

3.5.4 Spread ability

An important criterion for semisolids is that it possesses good spread ability. “Spread ability is a term expressed to denote the extent of area to which the cream readily spreads on application to the skin”. The therapeutic efficacy of a formulation also depends on its spreading value. A special apparatus has been designed to study the spread ability of the formulations. Spread ability is Expressed in terms of “time in seconds” taken by two slides to slip off from the formulation, placed between, under the application of a certain load. Lesser the time taken for the separation of the two, better the spread ability. Two glass slides of standard dimensions were selected. The formulation whose spread ability had to be determined was placed over one of the slides. The other slide was placed on top of the formulations was sandwiched between the two slides across the length of along the slide. Weight was placed up on the upper slide so that the formulation between the two slides was pressed uniformly to form a thin layer. The weight was removed and the excess of formulation adhering to the slides was scrapped off. One of the slides was fixed on which the formulation was placed. The second movable slide was placed over it, with one end tied to a string to which load could be applied by the help of a simple pulley and a pan. Weight was put on the pan and the time taken for the upper slide to travel the distance of and separate away from the lower slide under the direction of the weight was noted [30].

The spread ability was then calculated from the following formula.

$$\text{Spread ability} = m \times l / t$$

Where, m = weight tied to the upper slide (3g)
l =length of glass slide (5cm)
t =time taken in seconds

3.3.5 Drug content: Each formulation (1g) was accurately weighed and transferred to 100 ml volumetric flask to which about 70 ml of methanol was added. After shaking, the volume was made up to 100 ml with methanol. The content was filtered through a suitable filter paper. 1ml filtrate was taken and suitable diluted and the drug content (extract) was estimated by using UV/Visible spectrophotometer at 265 nm. Results are shown in Table 7.

3.3.6 *In-vitro* diffusion study: The *in-vitro* diffusion studies of gels were performed using a Franz diffusion cell whose diffusion area was 1.59 cm2, and by using dialysis membrane (Sigma Inc. MO, USA; dry, unwashed, pre-cut and open ended; fiat width: 35 mm; inflated diameter, 21mm; Length: 30mm). The membrane soaked in phosphate buffer pH 7.4 for 6-7 h was clamped carefully to one end of the hollow glass tube of dialysis cell (2.3 cm diameter, 4.16 cm2 area). 100 ml of phosphate buffer was taken in a beaker, which was used as receptor compartment for the study. 1gm of each formulation was spreaded uniformly on the diffusion membrane.
The donor compartment was kept in contact with the receptor compartment and the temperature was maintained at 37±0.50C. The solutions on the receptor side were stirred by magnetic stirrer. At pre-determined time intervals, 2 ml of solution from the receptor compartment was pipetted out and immediately replaced with 2 ml fresh phosphate buffer solution. The drug concentration of the receptor fluid was determined spectrophotometrically at 265nm against blank. The amounts of drug permeation of all the formulations were calculated. This experiment was carried out in triplicate and the results are extrapolated in Figure 7 and 8.

3.5.7 Acute skin irritation study: The primary skin irritation test was performed on albino rats and weighing about 150-200 gm. The animals were maintained on standard animal feed and had free access to water ad libitum. The animals were kept under standard laboratory condition. The total mass was divided into four batches, each batch containing seven animals. Two batches of each were used for control and test. Dorsal hairs at the back of the rats were clipped off one day prior to the commencement of the study. Animals showing normal skin texture were housed individually in cages with copography meshes to avoid contact with the bedding. 50 mg of the each formulation of different concentrations were applied over one square centimeter area of intact and abraded skin to different animals.
A 0.8% v/v aqueous solution of formalin was applied as standard skin irritant. The formulation was removed after 24 h and score of erythema was recorded and compared with standard. Score of erythema is read and recorded as: Score 0 for no erythema; Score 1 for Mild erythema (barely perceptible- light pink); Score 2 for Moderate erythema (dark pink); Score3 for Severe erythema (Extreme redness).Results are depicted in Table 10 [31].

3.5.8 Antibacterial activity test Sample Preparations
Solutions of the plant extracts, prepared gel and marketed formulation were prepared using dimethyl sulfoxide (DMSO). Clindamycin (10 mg/ml) was used as a positive control and DMSO as a negative control.
Modified agar well diffusion method was used to detect the antibacterial activities of different extracts and formulations. In this method, each nutrient agar plates were planted with 0.2 ml of 24h broth culture of *S. aureus*, soybean casein digest media plates were seeded with 0.2 ml each of 24 h broth culture of *S. epidermidis* and plates of brain heart infusion media were seeded with 48 h broth culture of *P. acnes*. The plates were dried for 1 h. In each of the plates, four equidistant wells were excavated with a sterile 8 mm borer. Into each plate, 0.5 ml of solutions of extracts, prepared herbal gels, marketed herbal formulation and allopathic Clarithromycin gel were introduced.
The plates of *S. epidermidis* and *S. aureus* were incubated at 37 °C for 24h, and *P. acnes* were incubated for 48h. The diameter of the zones of inhibition (in mm) were measured for evaluating the antibacterial activity. The experiment was repeated three times and the mean was recorded.

3.5.9 Determination of minimum inhibitory concentrations (MIC)
The MIC is defined as the lowest concentration of the compound to inhibit the growth of microorganisms. The extracts were dissolved in DMSO to make a concentration of 100 mg/ml in order to determine the relative minimum inhibitory concentration values. To prepare different concentrations, the extracts were diluted with DMSO. Then, 100µl of these extracts were added to each cup individually. All the tests were repeated in triplicates and the mean was recorded [32].

3.5.10 Stability study
As per ICH guidelines, the stability studies were performed and the formulated gels were filled and stored in collapsible tubes at fixed condition of temperature and humidity *viz*. 40 °C±2 °C/75%±5% RH for a period of three months and the appearance, pH, viscosity and spread ability was studied. Results are depicted in table 9 [33].

4. Results and Discussions

4.1 Extraction of *Luffa acutangula*
The extract of *Luffa acutangula* extracted successfully by using maceration extraction process.

4.2 Phytochemical Investigation

Table 2: Phytochemical investigation of seed extract

Sr. No.	Phytochemicals	Test	Observation	Result
1	Amino acids	Ninhydrine test	Purple colour	+ve
2	Carbohydrates	Molish test	Violet ring	+ve
3	Fixed oils and Fats	Spot test	Oil Stains	+ve
4	Proteins	Biuret test	Pink colour	+ve

4.3 UV- visible spectroscopy
The wavelength of *Luffa acutaangula* seeds extract was found to be 265nm (Table no3) and absorbance at different concentration was given in table no.4 and R^2 is found to be 0.9934.

Table 3: Maximum Wavelength determination

Sr. No.	p/v	Wavelength	Abs
1	↑	265	1.107
2	↓	205	4.00

Table 4: Standard calibration curve of *Luffa acutaangula* seeds extract in Water

Concentration (ug\ml)	Absorbance (265nm)
50	0.205
100	0.355
150	0.494
200	0.633
250	0.877
300	0.992
350	1.123
400	1.393
450	1.404
500	1.480

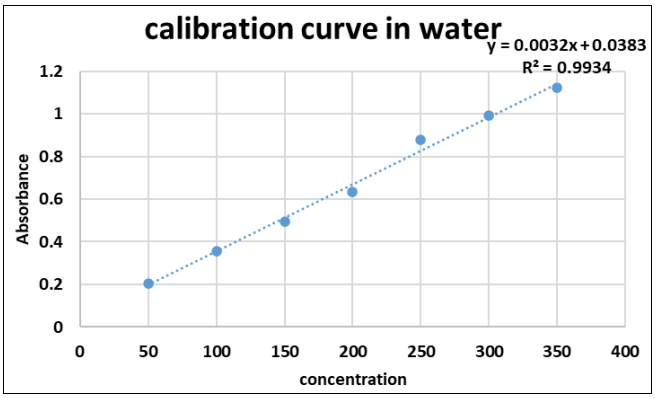

Fig 1: Standard calibration curve of *Luffa acutangula* in water

4.4 Formulation of anti-acne Gel

The herbal gel was prepared by using water based blend method.

The formulated herbal gel containing *Luffa acutangula* seeds extract were evaluated for several physicochemical properties.

4.5 Evaluation of anti-acne Gel

4.5.1 Physical appearance/visual inspection: From the result the physical appearance of all gels were found to be brown in color and consistency found to be good.

Fig 2: Prepared *Luffa acutangula* gel

4.5.2 Determination of pH

The pH of gel is important for minimizing irritation to the skin and the reducing the post-acne hyper pigmentation. pH is one of the ways to minimize damage to the skin. The prepared gel was acid balanced and pH of gels was found that ranged 5-5.5 which is near to the skin pH.

4.5.3 Rheological or Viscosity determination

The results of rheological evaluation showed that the viscosity of the samples changes gradually with the increase in rpm, therefore the gel formulations were time dependent. Secondly as the data showed the viscosity decreases with increase in rpm. The F3 show the good viscosity than the F1 and F2.

Table 5: Viscosity determination of *Luffa acutangula* gel at different rpm

Sr. No.	Formulations	Speed (rpm)	Viscosity (cps)
1	F1	100	1280
2	F2	100	1320
3	F3	100	2200

4.5.4 Spread ability

The spread ability of the prepared herbal gel was done and it was found that F3 has better spread ability than F1 and F2**.**

Table 6: Spread ability determination of *Luffa acutangula* gel

Formulation	Time (In second)	Spread ability (g.cm/sec)
F1	6sec	44.57
F2	6sec	34.57
F3	6sec	54.57

4.5.5 Drug content

The drug content of F1, F2 & F3 gel were found to be 91.33%, 92.07% and 92.78% respectively.

4.5.6 *In-vitro* diffusion study

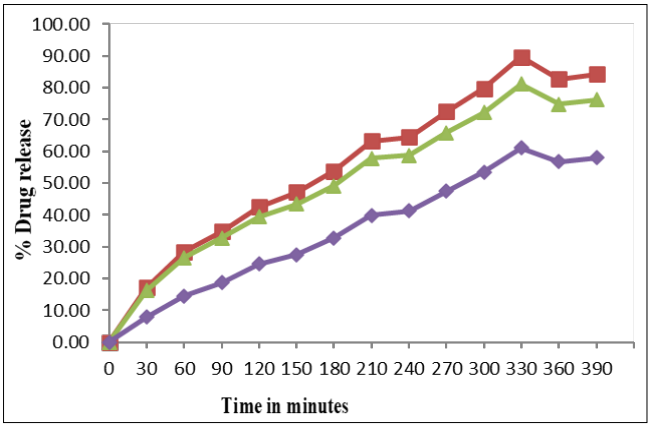

Fig 3: *In-vitro* diffusion study of gel F1, F2 and F3.

From the results it was observed that F3 (indicated by red color) Show better release than other to gel i.e. F1& F2 (indicated by green and purple color respectively)

4.5.7 Skin irritation study of gel: All the formulations did not produce any skin irritation, i.e., no erythema and oedema was seen for about a week when applied over the skin.

Following seven days application of the gel, the results of skin irritation test were tabulated in Table no. 7. Score of erythema is read and recorded as: Score 0 for no erythema; Score 1 for Mild erythema (barely perceptible- light pink); Score 2 for Moderate erythema (dark pink); Score 3 for Severe erythema (Extreme redness). The results indicated that the control preparation (which did not contain any drug), test gel F3 cause slight skin reaction, i.e., erythema and oedema on day 1 and day 2 but did not cause any skin reaction later on. It can be assured that *Luffa acutangula* seed extract and the excipients did not cause any skin irritation and can be used in the gel.

Table 7: Skin irritation study

Formulations	Treatment						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	1	0	0	0	0	0	0
F3 Gel	2	1	0	0	0	0	0

4.5.8 Antimicrobial activity against *Propionibacterium acnes*

The hydroalcoholic seed extracts of *Luffa acutangula* was examined for antimicrobial activities against microorganisms frequently involved in acne inflammation, *Propionibacterium acnes*, *Staphylococcus epidermidis* and *Staphylococcus aureus* are shown in fig.no.4,5 & 6.

From the results below it was observed that extracts showed significant zone of inhibition as compared to standard antibiotic clindamycin.

Table 8: Antimicrobial Activity of seed extracts of *Luffa acutangula*

Zone of inhibition(mm)			
Sr. No.	Microbes	Seed Extract	Clindamycin
1.	*P. acnes*	23	36
2.	*S. epidermidis*	22	33
3.	*S. aureus*	17	31

Concentration of the each extract: 20 mg/ml, Clindamycin: 10 mg/ml

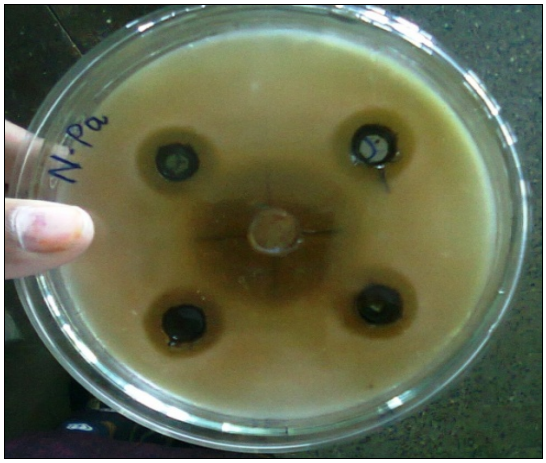

Fig 4: Zone of inhibition of seed extracts of *Luffa acutangula* against *P.acnes*.

Fig 5: Zone of inhibition of seed extract against *S. epidermidis*

Fig 6: Zone of inhibition of seed extract against *S.aureus*

4.5.9 Minimum inhibitory concentration of extracts

The MIC was defined as the lowest concentration of the compound to inhibit the growth of microorganisms. The MIC of all the extracts are as follows.

Table 9: Minimum inhibitory concentration of Seed extract of *Luffa acutangular*

Sr. No.	Bacteria	MIC (mg/ml)
1	*P. acnes*	10.0
2	*S. epidermidis*	3.0
3	*S. aureus*	0.7

4.5.10 Stbility Study

From the results it was concluded that the optimized gel was stable at 40 °C±2 °C/75%±5% RH for a period of three months.

Table 10: Stability study of optimized gel

Sr. No.	Formulations	Appearance	pH	Viscosity (cps)	Spread ability (gm-cm/sec)
1.	F3	Brownish	5.6	2260	57.54

5. Conclusions

The herbal gel was formulated based upon traditional knowledge and emphasis was to formulate a stable and functionally effective. From the all above formulated gels the F3 show the stable and effective formulation. The formulated gel were not only safer than the chemical agents, but also greatly reduce the post-acne hyper-pigmentation. The pH of the gel was adjusted to 5.5 which is safe to use for face. It was found to be harmless, more effective and economical. From the literature survey the prepared gel may be beneficial to anti-acne activity but for the determination phytoconstituents responsible for anti-acne activity, further studies were required.

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