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Formulation and Evaluation of Citrus Aurantifolia Anti-Acne Gel

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ABSTRACT
Acne vulgaris is a chronic inflammatory condition of the pilosebaceous unit that causes comedones, seborrhea, and inflammatory lesions. The idea that natural medicines have fewer adverse effects than synthetic ones makes them more palatable. The demand for herbal formulations is rising on the international market. Citrus aurantifolia is very important (key ingredient) component of cosmetics which is used to treat pigmentation, acne and other skin problem. The goal of the current study is to create and assess an herbal anti-acne gel that contains Citrus aurantifolia. Physical appearance, pH, Spreadability, in-vitro drug diffusion and an effective anti-acne activity test against Cutibacterium acne will all be assessed for the formulation. The in-vitro drug diffusion study of Citrus aurantifolia gel F1 to F5 was found 21.69%, 37.05%, 37.66%, 51.37% and 59.09%. At time 3 hours, F4 and F5 gave a high percentage of drug release. Finally the formulations F4 and F5 can be concluded that are suitable for topical application as Anti-acne Gel. In addition to having a synergistic effect on the bacteria, Citrus aurantifolia may have an impact on acne vulgaris.

I. INTRODUCTION

Hormones, environmental factors furthermore genetic susceptibility may be the cause for acne.

Acne occurs when hair follicles becomes clogged with dead skin cells and a sticky substance called sebum is produced by the sebaceous gland. This excess sebum leads to skin cells to tick together inside the follicle, causing an obstruction leading to comedone. . Bacteria release factors that cause inflammation once they have taken root in the clogged pore or comedone. Comedones become pustules and pimples as a result of this. Nodules can form when an acne lesion ruptures due to extreme inflammation. Nodules form cysts as a result of the affected glands converging, which may cause scarring after they heal (Rohan Londe 2024). Topical delivery is the process of applying a drug-containing formulation to the skin in order to directly treat cutaneous conditions like acne or the cutaneous symptoms of a general disease like psoriasis. The goal is to limit the drug's pharmacological or other effects to the skin's surface or inside the skin. Citrus aurantifolia (Kaghzi nimbu) is very important (key ingredient) component of cosmetics which is used to treat pigmentation, acne and other skin problems. Citrus aurantifolia's or lime oil's , cleansing properties make it effective (efficacious) at treating congested skin, better known as clogged pores. Studies (case history) revealed that the extracts of root and fruit of C. aurantifolia have been found effective(useful) in inhibiting the growth of Staphylococcus aureus, , Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae ,Beta-haemolytic streptococci, Escherichia coli , Neisseria gonorrhoeae (Aibinu et al 2007) and anaerobic facultative bacteria as well (Rahman S et al 2011) .

Materials and method: The mature fresh fruits of C. aurantifolia were collected from home garden, Varanasi. The collected fruits were authenticated (No. Ruta. 2025/01) by Dr. Prof. N.K.Dubey from Department of Botany , Institute of Science , Banaras Hindu Uinversity , Varanasi .The tested bacteria used in this study were Staphylococcus aureus, obtained from Aakar Biotech Technologies , Lucknow. The growth medium used was Mueller Hinton Agar (MHA-OXOID) and Mueller Hinton Broth (MHBOXOID). The chemicals used were carbopol 940, propylene glycol, methyl paraben, propyl paraben, triethanolamine and distilled water.

Preparation of Anti-acne Gel: A total of five formulation batches were prepared according to the composition of formulations. Carbopol 940 was

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dissolved in distilled water in a separate beaker with continuous stirring to avoid air entrapment (Hin et al., 2022). Paraben was dissolved in distilled water in another beaker using a water bath and cooled (Loizzo et al., 2012) . The plant extract was added to the aforesaid mixture (Boshtam et al., 2011) . To this solution, the carbopol mixture was added and thoroughly mixed. After that, propylene glycol and triethanolamine were added and pH was adjusted to 6.8-7 with continuous stirring (Sahu et al., 2012) . The final volume was made by adding distilled water (Kumar et al., 2020).

Formulation of Anti-acne Gel

S.No.	Name of ingredients	F_1	F_2	F_3	F_4	F_5
1.	Citrus aurantifolia	-	1%	1%	1%	2%
2.	Carbopol 940	1%	1%	1%	1%	1%
3.	Methyl paraben	0.1%	0.1%	0.1%	0.1%	0.1%
4.	Propyl paraben	-	-	-	-	0.1%
5.	Propylene glycol	2%	2%	2%	2%	2%
6.	Triethanolamine	q.s.	q.s.	q.s.	q.s.	q.s.
7.	Water	q.s.	q.s	q.s	q.s	q.s

Evaluation of Herbal Anti-acne Gel

1. **Physical evaluation:** Physical characteristics like color, consistency, and homogeneity were examined visually.
2. **pH:** Utilizing a calibrated digital pH meter at a constant temperature, the pH of the formulation's 1% aqueous solution was measured.
3. **Viscosity:** Using a Brookfield Programmable DV-II + Viscometer with spindle C S93 spinning at 50 rpm and 25°C, the viscosity of the prepared gels was determined.
4. **In-vitro drug diffusion study:** For in vitro release, phosphate buffer with a pH of 6.8 had been used as the receptor media. In the Franz diffusion cell, the pretreatment dialysis sac (Cellophane membrane) was applied. After applying the gel sample to the membrane, it was fixed between the quality diffusion cell's donor and receptor compartments. There was 100 milliliters of pH 6.8 phosphate buffer in the receptor compartment. By enclosing the water in the jacket, the diffusion medium's temperature was thermostatically maintained at 37º ± 0.5ºC. A magnetic stirrer spinning at 600 rpm continually agitated the medium. The sample was taken out and replaced with an equivalent volume of freshly made fluid at predetermined intervals. The samples withdrawn were analyzed using spectrophotometry at 285 nm in comparison to their blank.
5. **Spreadability:** Two sets of standard-sized glass slides were taken. One of the slides was covered with the gel formulation. The other slides were positioned over the gel so that the gel was positioned between the two slides in a space that was 6.0 cm from either side of the slide. In order to create a thin layer of gel between the two slides, a 100g weight was applied to the upper slides. The weight was removed and the excess of gel adhering

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(sticking) to the slides was scrapped off. The slides in position were fixed to a stand without minor disturbance and in such a way that upper slides slip off (remove) freely by the force of weight tried to it. The upper slide was carefully tested with a 20g weight. It was recorded how long it took the upper slide to move 6.0 cm and separate from the bottom slide while the weight was applied. After the experiment was conducted three times, the mean was calculated.

6. **Anti-acne activity studies:** By using a modified agar well diffusion method, the anti-acne activities of several formulations were evaluated. In this method, nutrient agar plates were seeded with 0.2 ml of 24-hour broth culture of Cutibacterium acne. The agar plates were allowed to solidify. A sterile 8 mm borer was used to cut wells of equidistance in each of the plates. 0.5 ml of formulations, the herbal gel was introduced into the wells at randomly. The plates were incubated at 37 degrees Celsius for 24 hours (Ritu et al., 2024). The anti-acne activities were evaluated by measuring the zones of inhibitions (In mm).

II. RESULTS AND DISCUSSION

1. Physical Evaluation:

1.1. APPEARANCE: All developed gels (F1-F5) showed good homogeneity with absence of lumps. The developed preparations were much clear and transparent. The results are shown in [Table 5.1.2].

Table No. 1.1 Appearance Characteristics of the Gel Formulations

Formulations	Color	Consistency	Homogeneity
F1	Shiny White	+	Satisfactory
F2	Greenish	++	Good
F3	Greenish	++	Good
F4	Shiny Greenish	+++	Excellent
F5	Shiny Greenish	+++	Excellent

1.2. pH: All formulations had pH values between 6.07±0.05 and 6.98±0.02. The readings matched the skin pH and were substantially within the permissible limits .The outcomes displayed in Table No. 5.1.2

Table No.1.2 pH of Gel Formulations

Formulation	pH
F_1	6.07±0.05
F_2	6.67±0.01
F_3	6.84±0.02
F_4	6.92±0.02
F_5	6.98±0.02

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1.3.Viscosity: In terms of viscosity, the prepared gel ranged from 7452.33±157.24 to cps. The viscosity of the all formulation was as intended. Table No. 5.1.3 shows the result of the experiment.

Table No. 1.3 Viscosity of Gel Formulations

Formulation	Viscosity (cps)
F_1	7452.33±157.24
F_2	7590.67±300.11
F_3	7356.00±133.24
F_4	7540.00±138.24
F_5	7356.00±162.14

1.4. In-vitro drug diffusion study: In-vitro diffusion study was performed by using Franz diffusion cell.

Table No.1.4 In-vitro drug diffusion study of Citrus aurantifolia Gel Formulations

Time (Hours)	% Drug release of formulation F1	% Drug release of formulation F2	% Drug release of formulation F3	% Drug release of formulation F4	% Drug release of formulation F5
0	0	0	0	0	0
1	8.79	19.37	19.62	32.50	31.40
2	17.29	29.07	25.33	31.75	44.27
3	21.69	37.05	37.66	51.37	59.09

1.5.Spreadibility : The prepared formulations' spreadability was determined to be between 9.02±0.34 and 25.24±0.85 mm/s. All of the formulations were free of many gritty particles and had acceptable spredability. The result is displayed in Table No. 5.1.5

Table No.1.5 Spreadability Study of Citrus aurantifolia Gel Formulations

Formulations	Spreadability (mm/S)
F1	9.02±0.34
F2	9.54±0.45
F3	9.82±0.54
F4	22.94±0.55
F5	25.24±0.85

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1.6.Anti-acne activity studies:

0.5 Mc Farland Standard dilution of microbes to be used for the study. 100 µl diluted log cultures of test organism (P. acnes– MTCC 1951) was added to the micro centrifuge tube and added with 5 µl of prepared treatment dilutions of different concentrations (Mentioned in excel sheet) to the defined tubes and incubated for the 24 hours. After Incubation all content was transferred to the 96 well plate and turbidity reading was taken by Elisa Plate Reader (iMark Biorad) at 630 nm. Clindamycin (150µg- DALACIN C-Pfizer) was used as Positive Control. MIC and NIC values were estimated by using software Graph Pad Prism -6 and by using Gompertz model.

Table No. 1.6 Anti-acne activity study of Citrus aurantifolia Gel

Sample Code	MIC (µg/ml)	NIC (µg/ml)
F4	1217	83.95
F5	1432	20.79

III. CONCLUSION

Citrus aurantifolia extracts were used for this study to formulate an herbal gel that has an aqueous base for the treatment of acne. Five formulations of the gel were prepared by varying the proportions of gelling agents and evaluated for their physicochemical characteristics like pH, spreadability, viscosity, In-vitro drug diffusion study and Anti-acne activity studies. Based on these tests, formulation F4 and F5 was selected as the best formulation. The microbial enumeration studies of all the formulations (F4 and F5) demonstrated better microbial activity against Cutibacterium acnes (P.acnes) microorganisms. It was concluded that the present research might hopefully bring advancement in the treatment of acnes using herbs as well as in developing herbal formulations for safe and effective management of diseases.

REFERENCES

[1]. Aggrawal, A., Kumar, S., Jaffe, R.., Hone, D., Gross, M., 1990. J. Exp. Med, Vol. 172, pp. 1083.

[2]. Albougy, H.A., Naido, S., 2002. A systematic review of the management of oral candidiasis associated with HIV/ AIDS. South Asian Development Journal, pp. 457- 466.

[3]. Allansmith, M.R., Burns, C.A., Arnold, R.R., 1982. Infect. Immunol, Vol 35, pp. 202.

[4]. Anantrnarayan.1997. "Text book of Microbiology", 5th Edn., Orient Longman Ltd., Chennai, pp. 369-71.

[5]. Azarbayjani, A.F., Tan, E.H., Chan, Y.W., Chan, SY., 2009. Transdermal delivery of haloperidol by proniosomal formulations with non-ionic surfactants. Biol. Pharm. Bull, Vol. 32, pp. 1453-1458.

[6]. Baloglu, E., Ozyazici M., Hizarcioglu, S.K., Karavana, H.A., 2003. An in-vitro investigation for vaginal bioadhesive formulations: bioadhesive properties and swelling states of polymer mixtures. Farmaco, Vol. 5, pp. 215-240.

[7]. Barry, B.W., 2001. Novel mechanisms and devices to enable successful transdermal drug delivery. Eur J Pharm Sci, pp. 101.

[8]. Barry, BW., 2001. Novel mechanisms and devices to enable successful transdermal drug delivery. Eur J Pharm, Vol. 14, pp. 101.

[9]. Boushra, M., EI- Houssieneny., Hayam, M., 2010. Formulation and evaluation of clotrimazole from pluronic F127 gels. Drug Discoveries and Therapeutics, Vol. 4, Issue 1, pp. 33-43.

[10]. Aswal, A. and Kalra M, Rout.,2013. A Preparation and evaluation of polyherbal cosmeticcream. Der. Pharmacia. Lettre, Vol 5, Issue 1, pp. 83-88.

[11]. Hapsari, R., Berna, E., Juheini ., 2012. A Formulation and evaluation of antioxidant and tyrosinase inhibitory effect from gel containing the 70% ethanolic Pleurotus ostreatus extract. Int. J. of Med. Arom. Plants, pp. 135-140.

[12]. Mohammad Ali, Shahtalebi ., Gholam Reza, Asghari ., Farideh, Rahmani ., Fatemeh ,Shafiee., and Ali, Jahanian-Najafabadi .,2018.Formulation of Herbal Gel of Antirrhinum majus Extract and Evaluation of its Anti-Propionibacterium acne Effects. Adv Biomed Res,Vol 7, pp. 53.

[13]. Rajasekaran, Aiyalu., Arulkumaran, Govindarjan., Arivukkarasu ,Ramasamy., 2016 .Formulation and evaluation of

topical herbal gel for the treatment of arthritis in animal model . Brazilian Journal of Pharmaceutical Sciences ,Vol 2, Issue 3, pp.494-507.

[14]. Budiman, A., Praditasari, A., Rahayu, D., Aulifa, DL.,2019. Formulation of antioxidant gel from black mulberry fruit extract (Morus nigra L.). J Pharm Bioall Sci ,Vol 11, pp.216-22.

[15]. Patel ,NA.,Patel ,NJ., Patel, RP., 2009. Formulation and evaluation of curcumin gel for topical application. Pharm Dev Technol, Vol 14, Issue1, pp.80-9.