International Journal of Pharmaceutics & Drug Research
ISSN: 2347-6346
Available online at http://ijpdr.com

RESEARCH ARTICLE

# FORMULATION AND EVALUATION OF ANTI-ACNE GEL OF *CALENDULA OFFICINALIS* (LEAVES) FOR THE EFFECTIVE TREATMENT OF ACNE

**Swati Bairagi[1], Dr. Vivekanand Katare*[1], Mrs. Abhilasha Delouri[1], Mr. Prabhat Kumar Jain [2]**

**[1]Vivekanand College of Pharmacy, Bhopal (M.P.)**

**[2]Scan Research Laboratories, Bhopal (M.P.)**

***Correspondence Info:**

**Dr. Vivekanand Katare, Vivekanand College of Pharmacy, Bhopal (M.P.)**

*Email:*

vivekanandkatare@gmail.com

***Article History:**

Received: 13/10/2022
Revised: 22/10/2022
Accepted: 11/11/2022

## ABSTRACT

Anti-acne herbal formulations are used for the treatment of acne vulgaris with the added advantage of not producing adverse effects unlike synthetic drugs. *Calendula officinalis*, the pot marigold, common marigold, ruddles, Mary's gold or Scotch marigold is a flowering plant in the daisy family of Asteraceae.The. The aim of the present work is to carried out phytochemical screening, formulation and evaluation of Anti-Acne gel of *Calendula officinalis* (Leaves) for the effective treatment of Acne and also evaluated the in –vitro anti acne activity on *Propionibacterium acnes.* The Results of extraction and phytochemical screening shows that the % yield was found to be 11.74% and the extract shows the presence of Carbohydrates, Flavonoids, Proteins & Amino acids, Diterpenes and Saponins. Phenols were found to be absent. The total flavonoids was present in higher amount. The Spreadability and viscosity of G5 were found to be 13.13±0.16 and 3588±26 in all gel formulations, respectively. The anti-acne gels could inhibit the growth of the microorganisms that inhabit acnes and the herbal gel exhibited comparatively more efficacy to Clintop marketed gel.

***Key words***: *Calendula officinalis*, phytochemical screening, TFC, herbal gel, In-vitro anti acne activity.

## INTRODUCTION:

Acne, as a family of skin disorders is one of the most prevalent dermatologic diseases in the world. It usually affects almost everybody during the life. The pathogenesis of acne is complex but dependent on four key factors including androgen-mediated stimulation of sebaceous gland activity, follicular hyperkeratinization, colonization of the bacterium Propionibacterium acnes (an anaerobic bacterium as a normal constituent of the skin microbial flora), and inflammation (Toyoda and Morohashi, 2001).

The high levels of sebum elicited by androgen cause proliferation of P. acnes in the pilosebaceous ducts and this proliferation triggers the host inflammatory response with a discharge of the pro-inflammatory cytokines,

interleukin-1b (IL-1 b), IL-8, granulocyte–macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor α (TNF-α) and complement deposition. In addition to *P. acnes*, as the main causative microorganism, *Pityrosporum ovale* and *Staphylococcus epidermidis* are present in acne lesions. There are 3 types of acne: comedonal, nodular, and papulopustular. Comedonal is non-inflammatory while nodular and papulopustular are the inflammatory types (Feldman et al., 2004).

The use of natural remedies, particularly herbal medicine, dates back thousands of years. Over the last decade, in view of increasing resistance to existing anti-microbial agents, side effects and sometimes high cost of treatment, interest in medicinal herbs has been progressively increased.

*Calendula officinalis* Linn used medicinally in Europe, China, US and India. It belongs to the family, Asteraceae, and is commonly known as Zergul (Hindi), African marigold, Calendula, Common Marigold, Garden Marigold, Marigold, Pot Marigold (English), Butterblume (German), Chin Chan Ts'ao (Chinese), Galbinele (Romanian) and Ringblomma (Swedish) (Kirtikar and Basu, 1993; The Wealth of India, 1992).

In Europe, the leaves are considered resolvent and diaphoretic while the flowers are used as a stimulant, antispasmodic and emmenagogue . In England, the decoction of the flowers was used as a posset drink for the treatment of measles and smallpox, and the fresh juice as a remedy for jaundice, costiveness (constipation) and suppression of menstrual flow . In India, the florets are used in ointments for treating wounds, herpes, ulcers, frostbite, skin damage, scars and blood purification. The leaves, in infusion, are used for treating varicose veins externally (Khare, 2004).

The aim of the present work is to carried out phytochemical screening, formulation and evaluation of Anti-Acne gel of *Calendula officinalis* (Leaves) for the effective treatment of Acne and also evaluated the in –vitro anti acne activity on *Propionibacterium acnes***.**

## Material and Methods

### Collection of Plant Drugs

The leaves of selected plant namely *Calendula officinalis* were identified and collected from Govt. Botanical Garden, Bhadbhada Bhopal, (M.P). The entire plant drug was authenticated by expert botanist of Department of Botany Geetanjali Science College Bhopal. The collected plant drug was cleaned, shade dried,

pulverized into moderately coarse powder and stored in airtight container for further use.

**Extraction of Plant Drug by maceration method**

The plant Material (Leaves) was extracted with Hydroalcoholic solvent (methanol: water) for about 24 hrs with randomly shaking. Shaking of the drug during maceration is essential in order to replace the saturated layers around the drug with fresh menstruum.The liquid extract was collected in a tarred conical flask. The solvent removed by evaporating the solvent using hot plate. The dry extract obtained was weighed to calculate the percentage yield (Pandey and Tripathy, 2014).

**Preliminary Phytochemical Screening**

Preliminary phytochemical screening means to investigate the plant material in terms of its active constituents. In order to detect the various constituents, present in the Hydroalcoholic extracts of *Calendula officinalis*, was subjected to the phytochemical tests as per standard methods (Kokate, 1994; Harborne, 1976). Phytochemical screening was revealed for the presence of alkaloids, glycosides, carbohydrates, tannins, resins, flavonoids, steroids, proteins and amino acids.

**Quantification of secondary metabolites**

**Total flavonoids content estimation**

The total flavonoid content was determined using the method of Olufunmiso et al (2011). 1 ml of 2% AlCl3 solution was added to 3 ml of extracts or standard and allowed to stand for 15 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/100mg).

**Preparation of gel base**

Carbopol 934 was dissolved slowly with stirring in 60 mL of demineralized water for 1 h to avoid agglomeration Then disodium edentate and triethanolamine were dissolved in 10 mL of demineralized water separately and stirred for 10 min. Mixed 4.83 mL of propylene glycol in 12 mL of demineralized water with stirring for 10 min. Disodium edetate and triethanolamine solution were added to Carbopol solution and the pH was then adjusted to 7.4 by stirring the solution for 10 min. Then propylene glycol solution was added with stirring for 10 min until a clear consistent gel base was obtained.

**Preparation of Anti-Acne Gel**

Measured quantity of methyl paraben, glycerine, and polyethylene glycol and Hydroalcoholic extract of *Calendula officinalis* was dissolved in about 35 ml of water in beaker and were stirred at high speed using mechanical stirrer (or sonicator). Then carbopol 940 was added slowly to the beaker containing above liquid while stirring. Neutralized the solution by slowly adding triethanolamine solution with constant stirring until the gel is formed. All the samples were allowed to equilibrate for 24 hours at room temperature prior to performing rheological measurements **(**Shukla et al., 2019). (Table 1)

**Table 1 Formulation of herbal Gel**

| Ingredients (%) | HG1 | HG2 | HG3 | HG4 | HG5 | HG6 |
|---|---|---|---|---|---|---|
| *Calendula officinalis* extract | 1gm | 1gm | 1gm | 1gm | 1gm | 1gm |
| Carbopol 940 | 0.25mg | 0.5mg | 0.75mg | 1.0 gm | 1.25 gm | 1.5 gm |
| Polyethylene Glycol | 0.2ml | 0.2ml | 0.2ml | 0.2ml | 0.2ml | 0.2ml |
| Methyl Paraben | 0.08mg | 0.08mg | 0.08mg | 0.08mg | 0.08mg | 0.08mg |
| Triethanolamine | 1.0ml | 1.0ml | 1.0ml | 1.0ml | 1.0ml | 1.0ml |
| Distilled Water (q.s) | 100ml | 100ml | 100ml | 100ml | 100ml | 100ml |

**Evaluation of herbal gel**

**Appearance and consistency**

The physical appearance was visually checked for the texture of herbal gel formulations.

**Washablity**

Formulations were applied on the skin and then ease and extent of washing with water were checked manually.

**Extrudability determination of formulations**

The herbal gel formulations were filled into collapsible metal tubes or aluminum collapsible tubes. The tubes were pressed to extrude the material and the extrudability of the formulation was checked.

**Determination of Spreadability**

A special apparatus has been designed to study the Spreadability of the formulations. Spreadability is expressed in terms of time in seconds taken by two slides to slip off from formulation, placed between, under the application of a certain load. Lesser the time taken for the separation of two slides, better the Spreadability.

**Method:** Two glass slides of standard dimensions (6×2) were selected. The anti-acne gel formulation whose Spreadability had to be determined was placed over one of the slides. The second slide was placed over the slide in such a way that the formulation was sandwiched between them across a length of 6 cms along the slide. 100 grams of weight was placed up on the upper slide so that the herbal gel formulation between the two slides was traced uniformly to form a thin layer. The weight was removed and the excess of the antiacne gel formulation adhering to the slides was scrapped off. The lower slide was fixed on the board of the apparatus and one end of the upper slide was tied to a string to which 20 gram load could be applied 50with the help of a simple pulley. The time taken for the upper slide to travel the distance of 6 cms and separate away from lower slide under the direction of the weight was noted. The experiment was repeated and the average of 6 such determinations was calculated for each herbal gel formulation

$$\textbf{Spredability} = \frac{\mathbf{m.l}}{\mathbf{t}}$$

Where, S=Spreadability (gcm/sec), m = weight tied to the upper slide (20 grams), l= length of glass slide (6cms), t = time taken is seconds.

**Determination of pH**

The pH of the herbal gels was determined by digital pH meter. One gram of gel was dissolved in 25 ml of distilled water and the electrode was then dipped in to gel formulation until constant reading obtained. And constant reading was noted. The measurements of pH of each formulation were replicated two times.

**Drug content**

The drug content was determined by taking 1gm of gel in 10 ml volumetric flask diluted with methanol. 3 ml of stock solution was mixed with 1 ml of 2 % AlCl3. The mixture was vortexed for 15s and allowed to stand for 30min at 40°C for colour development. The absorbance was measured at 420 nm using a spectrophotometer (Barry, 1983; Jain et al., 2007; Lachman et al., 1986; Schoch, 1968).

**In-vitro anti acne activity**

**Preparation of plates**

After sterilization, the nutrient agar in flask was immediately poured (20 ml/ plate) into sterile Petri dishes on plane surface. The poured plates were left at room temperature to solidify and incubate at 37oC overnight to check the sterility of plates. The plates were dried at 50oC for 30 minutes before use.

Revival of the bacterial and fungal cultures The Bacterial cultures used in the study were obtained in lyophilized form. With the help aseptic techniques, the lyophilized cultures are inoculated in sterile nutrient broth than incubated for 24 hours at 37 $^{o}$C. After incubation the growth is observed in the form of turbidity. These broth cultures were further inoculated on to the agar plates with loop full of bacteria and further incubated for next 24 hours at 37$^{o}$C to obtain the pure culture and stored as stocks that are to be used in further research work.

**Antibiogram studies**

The well diffusion method was used to determine the antibacterial activity of the herbal gel prepared from the leaves of *Calendula officinalis* using standard procedure (Bauer et al., 1966). There were 3 concentrations used which are 25, 50 and 100 mg/ml for antibiogram studies. The plates were incubated at 37$^{o}$C for 24 hr. and then examined for clear zones of inhibition around the wells with particular concentration of drug

**RESULTS AND DISCUSSION**

The plant drug (50g) was subjected to extraction by (maceration) using Hydroalcohol as solvent for about 24 hrs. The yields were found to be (11.74 % w/w of crude drug) of Hydroalcoholic extract *Calendula officinalis* leaves. Obtained results were recorded in table 2. The yield was found to higher due to presence of more polar soluble components in the crude drug.

Results of Phytochemical test showed the presence of various bioactive compounds such as Carbohydrates, Flavonoids, Proteins & Amino acids, Diterpenes and Saponins. Phenol s or phenolic compounds were found to absent in Hydroalcoholic extract *Calendula officinalis* leaves. The results of phytochemical revels that the all polar and Methanolic and aqueous soluble compound was found to be present in *Calendula officinalis* leaves extract. The results were shown in table 3

**Table 2: Extractive values obtained from *Calendula officinalis***

| S.N. | Solvent | % Yield |
|---|---|---|
| 1. | Methanol+water (20:80) | 11.74% |

**Table 3: Preliminary phytochemical screening of *Calendula officinalis***

| S.N. | Phytoconstituents | Test Name | Hydroalcoholic Extract |
|---|---|---|---|
| 1 | Alkaloids | Wagner's Test | **+(ve)** |
| 2 | Carbohydrates | Fehling's Test | **+(ve)** |
| 3 | Flavonoids | Lead acetate | **+(ve)** |
| | | Alkaline reagent test | **+(ve)** |
| 4 | Proteins & Amino acids | Precipitation test | **+(ve)** |
| 5 | Phenols | Ferric chloride test | **-(ve)** |
| 6 | Diterpenes | Copper acetate test | **+(ve)** |
| 7 | Saponins | Foam test | **+(ve)** |

**Estimation of total flavonoids content**

Flavonoid content was calculated from the regression equation of the standard plot ($y = 0.020x + 0.019$ $R^2 = 0.989$) and is expressed as quercetin equivalents (QE) (fig.). Total Flavonoid content was 0.823mg/100mg quercetin equivalent in Hydroalcoholic extract *Calendula officinalis***.** Flavonoids are the most common and widely distributed group of plant's phenolic compounds.

**Table 5: Total Flavonoid content of Hydroalcoholic extract of *Calendula officinalis***

| S. N. | Extracts 100µg/ml | Flavonoid content Quercetin equivalent mg/100mg |
|---|---|---|
| 1 | Hydroalcoholic extract (100µg/ml) | 0.832 |

n=3, values are given in SEM

**Evaluation of Herbal gel**

According to formulation studies of psychorheological features, all of them have clear colour, no clogging, acceptable homogeneity, and smooth texture Table 6. Table 7 and figure 2,3 and 4 presents the data for washablity, extrudability, Spreadability, pH, and viscosity.

The Spreadability and viscosity of G5 were found to be 13.13±0.16 and 3588±26 in all gel formulations, respectively. Gel formulations were put into collapsible aluminum tubes for an extrudability investigation; the formulations had an average extrudability. The conducted skin irritation test revealed no sensitivity, erythema, or edema. As a result, the created formulations were regarded as non-irritating.

**Table 6: Results of psycho rheological characteristics**

| Formulation | Colour | Clogging | Homogeneity | Texture |
|---|---|---|---|---|
| G1 | Brown | Absent | Good | Smooth |
| G2 | Brown | Absent | Good | Smooth |
| G3 | Brown | Absent | Good | Smooth |
| G4 | Brown | Absent | Good | Smooth |
| G5 | Brown | Absent | Good | Smooth |
| G6 | Brown | Absent | Good | Smooth |

**Table 7: Results of washablity, extrudability, Spreadability, pH, Viscosity**

| Formulation | Washablity | Extrudability | Spreadability (gcm/sec) | pH | Viscosity (cps) |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| G1 | Good | Average | 15.23±0.12 | 6.72± 0.11 | 2974±06 |
| G2 | Good | Average | 14.65±0.15 | 6.83±0.14 | 2991±15 |
| G3 | Good | Average | 14.17±0.25 | 7.02±0.12 | 3135±17 |
| G4 | Good | Average | 13.65±0.35 | 7.05±0.11 | 3387±20 |
| G5 | Good | Average | 13.13±0.16 | 7.00±0.14 | 3588±26 |
| G6 | Good | Average | 13.27±0.33 | 7.13±0.12 | 3497±23 |

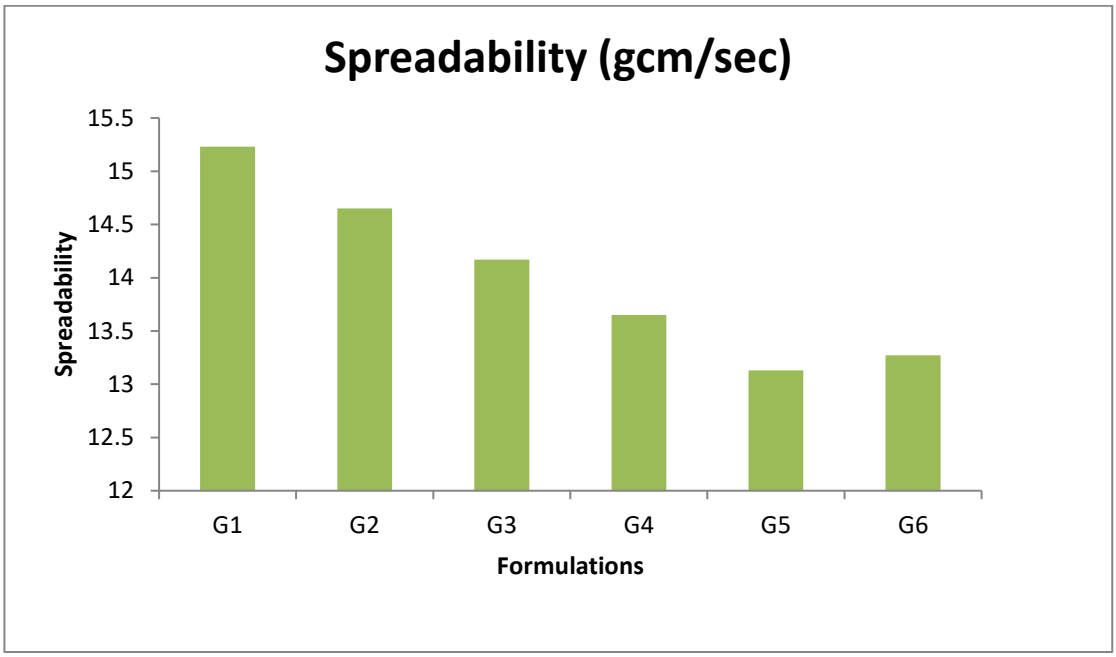


**Figure 2: Spreadability (gcm/sec) of different formulations**

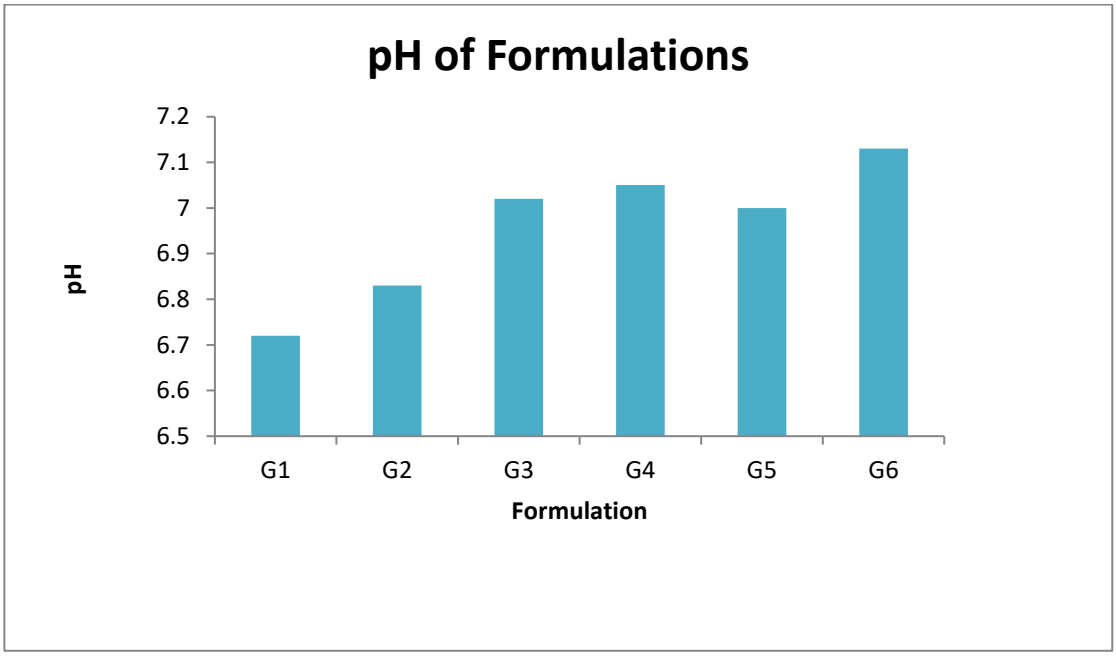


**Figure 3: pH of different formulations**

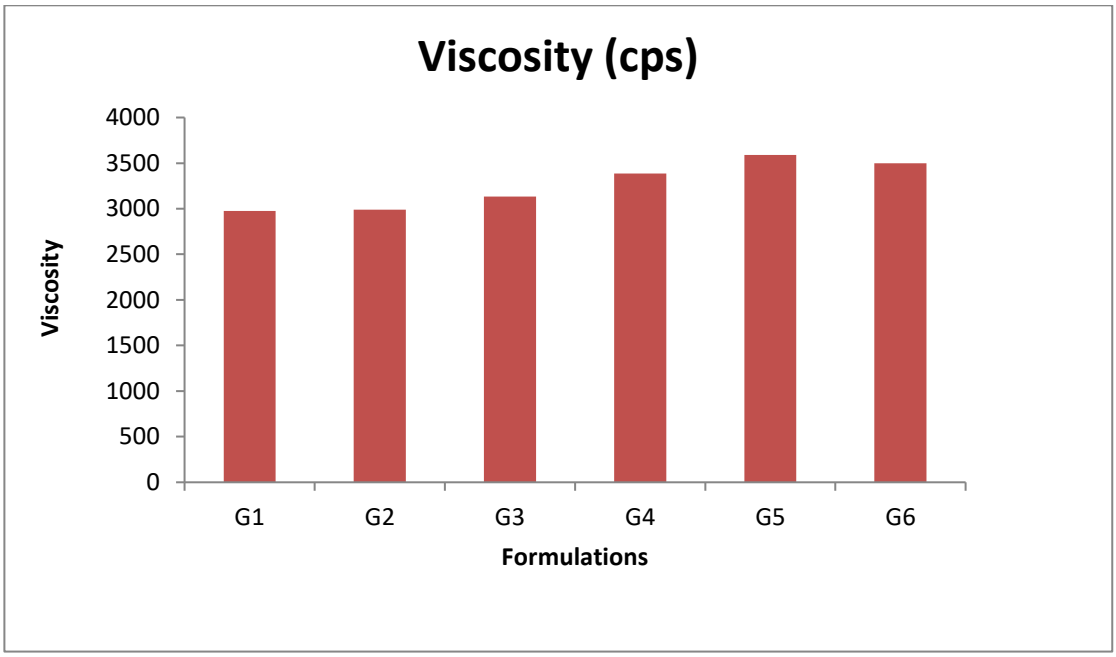


**Figure 4: Viscosity (cps) of different formulations**

**Results of In-vitro anti acne activity**

The efficacy of the anti-acne gels from herbal extracts is shown in Table 8 and figure 5. The anti-acne gels could inhibit the growth of the microorganisms that inhabit acnes and the herbal gel exhibited comparatively more efficacy to Clintop marketed gel.

**Table 8: Anti-acne activity of marketed gel and herbal gel formulation against Propionibacterium acnes**

| S. No. | Formulation | Zone of inhibition | | |
|---|---|---|---|---|
| | | 100mg/ml | 50 mg/ml | 25mg/ml |
| 1. | Clintop (Marketed gel) | 17±0.6 | 15±0.83 | 14±0.46 |
| 2. | Polyherbal gel | 19±0.63 | 16±0.75 | 15±0.4 |

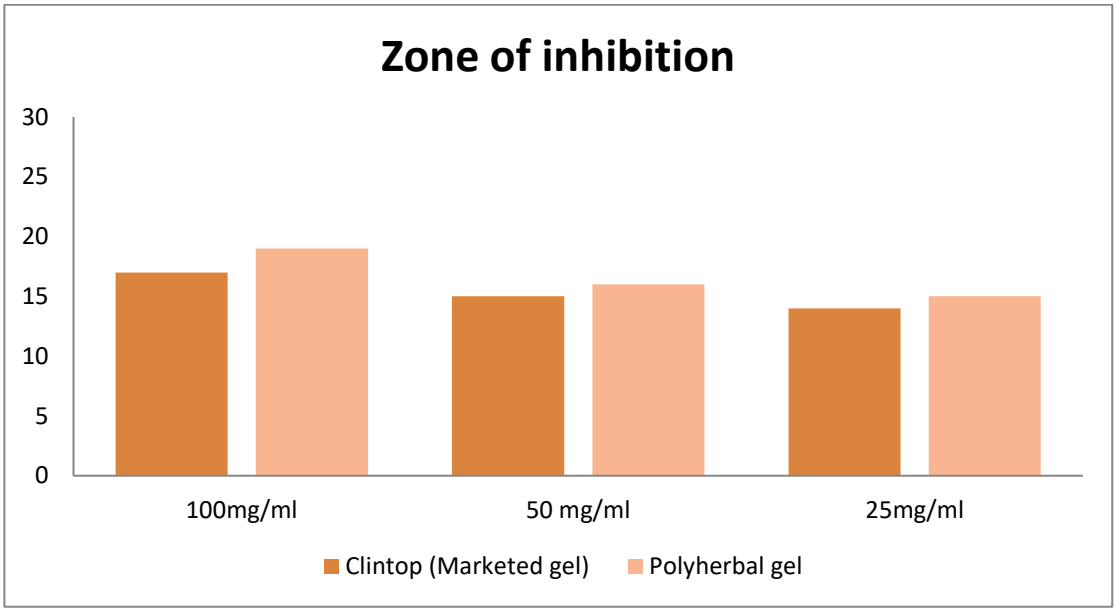


**Figure 5: Anti-acne activity of marketed gel and herbal gel formulation against Propionibacterium acne**

**CONCLUSION**

Using Hydroalcoholic extracts of *Calendula officinalis* leaves in an aqueous-based Carbopol gel system, the current study sought to develop herbal gels for topical application and treatment of Acne Vulgaris. These gels

were then assessed for their physicochemical properties, such as pH and Spreadability. The anti-acne gels could inhibit the growth of the microorganisms that inhabit acnes and the herbal gel exhibited comparatively more efficacy to marketed gel .To identify the active elements that are responsible for the phytochemicals anti-acne effect and to determine whether there is any compound synergism, more phytochemical investigations are also necessary.

**References**


1. Toyoda, M. and Morohashi, M. (2001). Pathogenesis of acne. Med Electron Microsc. 34:29–40.
2. Feldman, S., Careccia, R.E., Barham, K.L. and Hancox, J. (2004). Diagnosis and treatment of acne. Am Fam Physician. 69:2123–30.
3. Kirtikar KR and Basu BD. (1993). Indian Medicinal Plants. Vol II, Deharadun, India, International Book Distributor, pp 1413-1414.
4. The Wealth of India, Raw Materials, A Dictionary of Indian Raw Material & Industrial Products. (1992). Vol 3, New Delhi, Publications & Information Directorate CSIR. pp 55-58.
5. Khare, C.P. (2004). Encyclopedia of Indian Medicinal Plants. Germany, Springer-Verlag Publisher. pp 116-117
6. Pandey, A., and Tripathi, S. (2014). Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. Journal of Pharmacognosy and Phytochemistry, 2(5).
7. Kokate, C.K. (1994). Practical Pharmacognosy. 4th edition. Delhi: Vallabh Prakashan.
8. Harborne, J.B. (1973) Phytochemical methods. London: Chapman and Hall.
9. Olufunmiso, O.O. and Afolayan, A.J. (2011). Phenolic content and antioxidant property of the bark extract of Ziziphus mucronata willd. Subsp. mucronata willd, BMC Complement Alternative Medicine. 11:130
10. Shukla, K. V., Choudhary, N. and Pathak, R. (2019). Formulation and Evaluation of Topical Polyherbal Antiacne Gels Containing Luffa Acutangula, Amaranthus Spinosus and Morus Alba. Journal of Drug Delivery and Therapeutics, 9(4-s), 439-444.
11. Barry BW. (1983). Dermatological Formulations, Marcel Dekker., Inc., New York, Basel. 18: 96-115.
12. Jain, S., Padsalg, B.D., Patel, A.K., Moale, V. (2007). Formulation development and

evaluation of fluconazole gel in various polymer bases. Asian J Pharm. 1(8):63-68.

13. Lachman, L., Lieberman, H.A., Kanig, J.L. (1986). The Theory and practice of Industrial Pharmacy, Varghese publishing House, 3rd edition, 534.
14. Schoch, T.J. (1968). Effects of freezing and cold storage on pasted starches. In: Tressler DK, Van Arsdel WD, Copley MJ, eds. The Freezing Preservation of Foods. Westport: CT. 4: 44-56.
15. Bauer, A.W., Kirby, W.M.M., Sherris, J.C., Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. American J Clin Pathol. 45:493-496