Formulation And Evaluation of Bhringraj Extract Gel for the Treatment of Folliculitis

J.N.B. Indusekhar[1], Dr. Bhaskara Raju Vatchavai[2], Namburi Varshini[3]
[1]*Assistant Professor, Sri Vasavi Institute of Pharmaceutical Sciences, A.P, India*
[2]*Principal and Professor, Sri Vasavi Institute of Pharmaceutical Sciences, A.P, India*
[3]*UG Scholar, Sri Vasavi Institute of Pharmaceutical Sciences, A.P, India*

***Abstract*—Staphylococcus aureus and Klebsiella pneumoniae are the main bacterial infections that produce foliculitis, a common skin ailment that causes inflammation and irritation. The goal of the current study is to create and assess a Carbopol 940 gel based on Bhringraj (Eclipta prostrata) extract utilizing a hydroalcoholic solvent solution in order to determine whether it has anti-folliculitis properties. The extract was made via hydroalcoholic extraction, and its active phytoconstituents were identified by UV spectrophotometry. The antibacterial activity of the prepared gel against S. aureus and K. pneumoniae was assessed using the agar disc diffusion technique, with Ciprofloxacin serving as the standard reference. Its effectiveness against these infections was demonstrated by the findings, which showed strong antibacterial activity with inhibition zones similar to those of the usual medication. Furthermore, the extract's antioxidant ability was evaluated using the DPPH free radical scavenging test, which revealed that it plays a part in counteracting oxidative stress, which fuels skin inflammation. The anti-inflammatory activity of the extract was investigated utilizing the goat blood hemolysis technique in order to further assess its therapeutic effectiveness. Hemolysis was significantly reduced in the results, suggesting strong anti-inflammatory qualities that are essential for reducing folliculitis symptoms. The Bhringraj gel formulation demonstrated high stability, spreadability, and pH appropriateness for topical use. According to the study's findings, the strong antibacterial, antioxidant, and anti-inflammatory qualities of Bhringraj extract-based Carbopol 940 gel make it a promising natural and efficient treatment option for bacterial folliculitis. Its therapeutic potential has to be established by more clinical research**.

***Index Terms*—Bhringraj, Anti-Foliculitis gel, Anti Inflammatory, Anti-Oxidant, Anti-Bacterial**

I. INTRODUCTION

The histological definition of folliculitis is the development of a pustule based on hair follicles due to the presence of inflammatory cells in the ostia and hair follicle wall. The kind of inflammatory cells differs according to the stage at which the biopsy specimen was taken and/or the cause of the folliculitis (Saegeman, 2017). Damage to hair follicles can allow bacteria, fungus, and viruses to infiltrate them and cause illnesses like folliculitis. The top area of the hair follicle and the skin immediately adjacent to it are affected by superficial folliculitis, whereas the deeper region of the follicle and possibly the entire hair follicle are affected by deep folliculitis (Andre, 2015). In addition to being frequently brought on by Staphylococcus aureus, viruses and fungi can also cause foliculitis. Treatments for folliculitis vary depending on the nature and severity of the condition. Follicles are most common on the scalp but can also develop elsewhere on the body except the lips, palms, soles, and mucous membranes (Otberg, 2008) (Sillani, 2010). Both sexes, as well as people of all ages and races, can get foliculitis. When the surrounding skin is not affected, staphylococcal folliculitis, the most superficial type of skin infection, appears as tiny erythematous follicular pustules. Common gram-negative bacteria that induce folliculitis in this context include Pseudomonas (NCIM No.2037) and Klebsiella (NCIM No.2957) matter what causes it, folliculitis lesions might be painful, itchy, or asymptomatic (Sharquie, 2012).

The human body's main defence mechanism is the skin which plays a crucial role in the innate immune system. But it can get a lot of illnesses, from minor allergic responses to dangerous infections. In many

skin conditions, bacteria—especially gram-positive strains like Staphylococcus aureus—play a key role. These bacteria can cause symptoms like pus discharge and, in severe cases, infections like methicillin-resistant Staphylococcus aureus (MRSA) (Kazmi, 2021). The study of alternative remedies is necessary due to the drawbacks of conventional treatments like steroids and antibiotics, which include the possibility of side effects and the emergence of bacterial resistance. Its higher incidence in older adults is associated with alterations in the composition of the skin, such as decreased lipid content and changed keratinisation, which weakens theskin's defences against infection. Herbal extracts from Acanthus ebracteatus, Coccinia grandis, and Clerodendrum inerme have demonstrated synergistic antibacterial and antioxidant qualities, making them viable treatments for xerosis cutis. In terms of their traditional therapeutic uses, these plants are comparable to Eplita prostrata. Because of its strong antibacterial and antioxidant properties, E. prostrata is well-known for its ability to cure infections and control oxidative stress in skin disorders (Pratoomsoot, 2020). Its chemical makeup and potent antimicrobial capabilities have been extensively studied (Batch, 2022). False daisy, or E. prostrata, has been used extensively in traditional Asian medicine to treat skin disorders.

Numerous investigations have demonstrated its potent antimicrobial properties shown that the Against uropathogenic bacteria, E. prostrata ethanolic extract shows moderate to high anti-biofilm action against some strains of Escherichia coli (Bhandari, 2021). In a mouse model of atopic dermatitis, it was discovered that E. prostrata reduces allergic skin inflammation and repairs the skin barrier, indicating that it may be used as a treatment for this ailment (Kang, 2022). E. prostrata exhibits a number of biological properties, including as hepatoprotective, neuroprotective, antibacterial, anticancer, and effects that promote hair growth (Timalsina, 2021). Eclipta prostrata (L.) is a source of antioxidants (Minh, 2019). E. prostrate leaf extract has antibacterial and antioxidant properties (Nahid, 2017). Phytochemical analysis of the ethanol extract of E. prostrate leaves, antioxidant activity, and antibacterial activity against Staphylococcus aureus and Klebsiella pneumoniae are the objectives of the current study.

II. MATERIALS AND METHODS

1.1. Collection and Sample Preparation

Common name: Bhringraj
Scientific name: Eclipta prostrata
Family: Asteraceae
Genus: Eclipta
Species: E. prostrata

Eclipta prostrata leaves were collected from rural areas near Tadepalligudem, Andhra Pradesh. Since some plant constituents are photosensitive, the leaves were thoroughly cleaned under running water, then with distilled water to get rid of all the dirt and debris from the surface, and then shade-dried. After being further incubated for an additional 48 hours at 36°C, the dried leaves were ground into a fine powder using a mixer.

1.2. Extraction Method

The first step in utilizing ethanol (40%) in a Soxhlet apparatus to extract bioactive chemicals from Eclipta prostrata is to put 50 g of dried and powdered plant material into a cellulose thimble within the Soxhlet extractor. Fill a 500 mL round-bottom flask with 500 mL of 40% ethanol, then put the Soxhlet apparatus together, making that the condenser is linked to a constant supply of cooling water. Using a heating mantle, heat the flask to 78°C. This will allow the ethanol to evaporate, condense, and seep through the plant material, releasing its active ingredients. The siphon liquid will turn colorless after 6–8 hours of this operation, signifying full extraction. To get rid of any solid leftovers, use Whatman filter paper to filter the ethanol extract after extraction.Use a water bath or a rotary evaporator (40–50°C) to evaporate the ethanol. The extract can be further dried in a vacuum dryer if needed. In order to preserve the semi-solid ethanol extract for later use, weigh it and store it in a airtight container at 4°C.

1.3. Phytochemical analysis

The semisolid extract of E. prostrata was reconstituted in ethanol for the phytochemical analysis, which looked for various constituents like alkaloids, carbohydrates, tannins, terpenoids, glycosides, steroids, saponins, flavonoids, glycoprotein, volatile oil, and phenols using standard protocols (Riazunnisa, 2013).

1.4. Antibacterial activity assay

Both the Staphylococcus aureus culture and Klebsiella pneumonia bacterial isolates utilised in this investigation were obtained from the National Collection of Industrial Microorganisms (NCIM) at the National Chemical Laboratory in Pune and Microbiology Department of Sri Vasavi Institute of Pharmaceutical Sciences. The disc diffusion experiment was utilised to evaluate the antibacterial efficacy of the ethanol extract (Valgas C., 2007).

Ethanol was added to the extract to achieve a concentration of 1–2 mg/ml.

The Soya Bean Casein Digested (SBCD) agar plates were transferred onto 90 mm Petri dishes after being previously sterilised. K. pneumoniae/S. aureus was injected onto (SBCD) agar plates with a pre-adjusted cell density of 2×10^6cells/ml following solidification. Additionally, the plates were pierced to create open wells at each of the Petri plate's four borders using a sterile cork borer (6 mm diameter). Ciprofloxacin standard (0.1 mg/ml) and E. prostrata extract (1 or 2 mg/ml) were added to open wells. 50% ethanol-added wells act as a control. The plates that were treated were incubated for 24 to 36 hours at 37°C. The antibacterial activity of the plates was assessed after the incubation time by using a millimetre ruler to measure the zones of inhibition of microbe growth surrounding the extract. Three duplicates and three repetitions of the antibacterial assay were performed under meticulous aseptic conditions (Musselman, 1948) (Sabh, 1974) (Bergeron, 1973).

Ingredient	Function	Formula 1	Formula 2	Formula 3
Bringraj Extract	Active intergradient	1 %	2%	3%
Carbomer 940	Gelling agent	0.5 %	1%	2%
Glycerin	Humectant	3ml	4ml	5ml
Di sodium EDTA	Preservative	1mg	2mg	3mg
Buffer Solution	pH adjuster	1ml	1.5ml	2ml
Rose oil	Fragrance	1ml	1.5ml	2ml
Distilled Water	Diluent	100ml	100ml	100ml

1.5. FORMULATION OF GEL

Carbopol 940 should first be dissolved in distilled water, stirring constantly to guarantee uniform dispersion, before making the Bhringraj gel. To ensure that the polymer swells completely, let the mixture hydrate for two to three hours, or better still, overnight. Stir in glycerin to improve texture and moisture retention when completely hydrated.

After that, slowly add the Bhringraj extract to the mixture, stirring constantly to ensure even distribution. After that, progressively raise the pH to the ideal range of 5.5–7.0 by adding Triethanolamine (TEA) drop wise while stirring to neutralize the Carbopol. This will cause gel formation. Use an appropriate preservative to avoid microbial contamination if a longer shelf life is needed. Lastly, thoroughly stir the mixture until a uniformly smooth gel is produced. The produced gel should be stored at room temperature for stability and extended efficacy after being transferred into an airtight container.

1.6. PRELIMINARY SCREENING TEST FOR FORMULATION

1.6.1. Physical Appearance:

Color & Transparency: Observe for uniformity.

Homogeneity: Ensure no phase separation.

1.6.2. pH Measurement:

Use a pH meter or pH paper to check if it falls within the required range for the intended application.

1.6.3. Spreadability Test:

Apply a fixed amount on a glass plate and measure how far it spreads under a fixed weight.

1.6.4. Viscosity & Rheology Testing:

Use a Brookfield viscometer to determine viscosity at different shear rates. Perform a flow test to check pseudoplasticity or thixotropy.

1.6.5. Grittiness Test:

Rub between fingers or apply on skin to ensure smoothness without particulate matter.

1.6.6. Gel Strength / Rigidity Test:

Measure the force required to deform or break the gel using a texture analyzer.

1.6.7. Stability & Phase Separation:

Subject the gel to centrifugation (3000 rpm for 10 min) to check for separation.

Perform an accelerated stability test (40°C, 75% RH for weeks/months).

1.6.8. Swelling Index:

Weigh the gel before and after immersion in a liquid to determine its swelling capacity.

1.7. UV-Vis spectrum

To obtain the UV spectrum of Bhringraj extract, first prepare the extract by extracting the plant material using a suitable solvent, such as ethanol or methanol. Filter the extract to remove any particulate matter. Next, dilute the extract to an appropriate concentration, typically 10-50 µg/mL, using the same solvent. Using a UV-Vis spectrophotometer, measure the absorbance of the extract in the wavelength range of 190-800 nm, ensuring the instrument is properly calibrated with a blank (solvent) before each measurement. Record the spectrum and note the absorbance peaks, which correspond to the bioactive compounds present in the extract. For accurate results, perform the measurements in a quartz cuvette and ensure that the path length is consistent throughout the analysis. The UV spectrum can then be analyzed to identify the characteristic absorption wavelengths associated with compounds such as flavonoids, tannins, alkaloids, and terpenoids.

1.8. In vitro antioxidant studies

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) test protects against free radicals. Standard ascorbic acid and sample were prepared in methanol in different concentrations: 25, 50, 100, 200, 500, and 1000 mg/ml. DPPH at 0.1 mM in methanol was used as a free radical. The control was made similarly using an equal volume of methanol and DPPH, and the tubes were incubated at room temperature in the dark for half an hour. Equal volumes of various concentrations of standards and DPPH were mixed in separate, clean, and labeled test tubes. The absorbance was determined using a UV-Vis spectrophotometer at 517 nm. The degree of consistent purple DPPH decolorization to yellow DPPHH indicated the sample's scavenging effectiveness. To determine the sample's scavenging activity against the stable DPPH, the following formula was used:

Scavenging activity (%) = $\left(\frac{A-B}{A}\right) \times 100$

Where A is absorbance of control and

B is absorbance of sample / standard

1.9. In vitro anti-inflammatory activity

Keep the goat blood below 20°C (don't freeze it) in a sterile, airtight container with an anticoagulant, such as EDTA, already added. Place all of the blood in the centrifuge tubes, centrifuge for 10 minutes at 3000 rpm, remove the supernatant fluid, wash the tubes with 0.9% normal saline, centrifuge again, decant the supernatant fluid, and repeat two more times. RBC can be re-suspended in 0.9% normal saline to obtain a 10% formulation. Serially dilute the pomegranate peel extract sample into 25, 50, 100, 200, 400, 500, 800, and 1000 µg/ml. Similarly, make standard dilutions of the medication Diclofenac at 200 µg/ml.

1.9.1. Hypotonicity-Induced Hemolysis Method

Put the same amount of 10% RBC suspension and 1 mL sample of each dilution into sterile test tubes, label them appropriately, and prepare the standard tubes and control tube in the same manner by adding the same amount of 10% RBC suspension and hypotonic solution (50 mM NaCl solution). Then, incubate everything for 10 minutes at 37 °C. Centrifuge the sample above at 3000 rpm for 10 minutes after incubation, then collect the supernatant fluid. Then, use a UV-Vis spectrophotometer to measure the absorbance at 540 nm and record the absorbance results. Additionally, figure out the percentage of inhibition and plot the number of inhibitions against the concentration.

%Inhibition = $\frac{A-B}{A} \times 100$

Where A is absorbance of control and

B is absorbance of sample / standard

The higher the %inhibition the better the anti-inflammatory activity and vice versa.

III. RESULTS AND DISCUSSION

2.1. Determination of minimum inhibitory concentration (MIC)

Sample	Anti-bacterial activity [zone of inhibition{mm}	
	Staphylococcus aureus	Klebsiella pneumonia

Formula1	16±0.5	14±0.5
Formula2	18±0.5	21±0.5
Formula3	24±0.5	25±0.5
Distilled Ethanol	0.5	0.5
Ciprofloxacin(standard)	20±0.5	22±0.5

The formula 3 gel and ciprofloxacin shows the comparable zone of inhibition, which indicates the potent anti-microbial activity of the bhringraj due to the presence of the chemical constituents like alkaloids, tannins, glycosides and flavanoids. Which can act as the best alternative treatment for the folliculitis

2.2. PRELIMINARY SCREENING TEST FOR FORMULATION

2.2.1. Physical Appearance- Light Orange to Brown gel, smooth texture, homogeneous

2.2.2. pH Measurement- 5.5 - 6.5 (suitable for skin application)

2.2.3. Spreadability- Good spreadability (5 cm under 100 g weight)

2.2.4. Viscosity & Rheology-Moderate viscosity (1000-5000 cP)

2.2.5. Grittiness Test-No grittiness, smooth on application

2.2.6. Gel Strength / Rigidity-Moderate gel strength (depends on gelling agent, ~30-50 g force required for deformation)

2.2.7. Stability & Phase Separation-Stable at 40°C, 75% RH for 3 months; no phase separation

2.2.8. Swelling Index-120-150% swelling in water over 24 hours

2.3. Phytochemical analysis

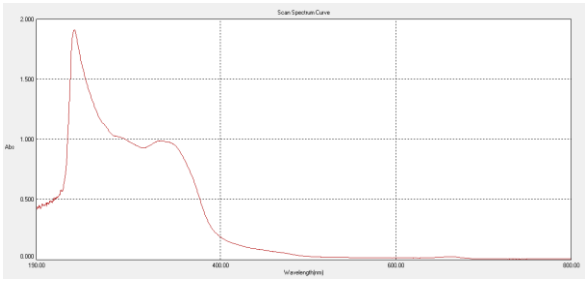
According to the qualitative phytochemical examination, alkaloids, tannins, glycosides, steroids, saponins, glycoproteins, and volatile oil were present in the methanolic extract of E. prostrata.

Table: Phytochemical analysis of ecliptaprostrata.

Alkaloid	Positive
Carbohydrates	Negative
Tannin	Positive
Terpenoids	Negative
Glycoside	Positive
Steroid	Positive
Saponin	Positive
Flavonoids	Negative
Proteins	Present
Glycoprotein test	Positive
Volatile oil	positive

2.4. UV spectrum

Characteristic absorption peaks in the UV-visible (UV-Vis) spectrum of Bhringraj extract, which is made from Eclipta prostrata, match the bioactive substances found there, including terpenoids, alkaloids, flavonoids, and tannins. Alkaloids and phenolic substances like tannins are responsible for the notable absorption that is seen in the UV range (200–400 nm), with peaks usually occurring between 220-270 nm. With noticeable peaks at 320 nm, flavonoids like luteolin and quercetin aid in absorption in the 280–350 nm range. Since terpenoids include conjugated double bonds, they may exhibit reduced absorbance at 200–250 nm.



The visible spectrum (400–800 nm) typically exhibits minimal absorption, while the presence of pigments such as chlorophyll may cause small peaks to show up. In general, Bhringraj extract's bioactive components may be identified and described using the UV-Vis spectrum, which also sheds light on the extract's possible medicinal uses.

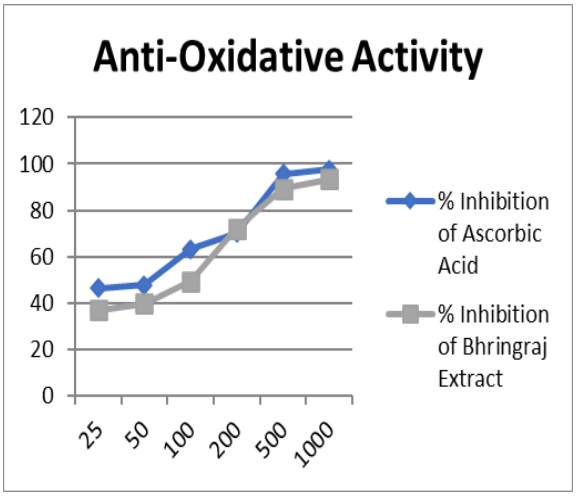
2.5. In vitro antioxidant studies

The ability of DPPH to scavenge free radicals. The table displays the results of the antioxidant activity of the reference (ascorbic acid) and samples at various concentrations. Through their ability to scavenge DPPH (free radical) and transform it into DPPHH, the samples demonstrated notable antioxidant activity. There was a dose-dependent radical scavenging effect. And ascorbic acid's scavenging activity was higher than all nine of the gel samples combined. The F2 sample had the highest level of antioxidant activity among them. The DPPH radical

gains one additional electron and its absorbance drops when an antioxidant is present.

The scavenging activity of the F2 sample was shown to be dose dependent in this investigation; as the concentration increased, so did the scavenging activity. While the samples' DPPH radical scavenging capabilities were not as strong as those of ascorbic acid, the study demonstrated that the samples had the capacity to donate protons and might potentially function as primary antioxidants by inhibiting or scavenging free radicals.

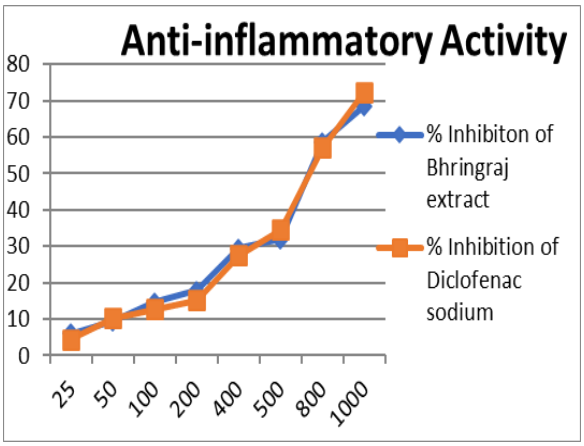
Concentration ($\mu g/ml$)	% Inhibition of Ascorbic acid	% Inhibition of Bhringraj
25	46.57534247	37.18199609
50	47.74951076	39.92172211
100	63.40508806	49.51076321
200	70.25440313	72.21135029
500	95.69471624	89.23679061
1000	97.65166341	93.34637965



2.6. In vitro anti-inflammatory activity

Bhringraj extracts anti-inflammatory properties were assessed by the hypotonicity induced hemolysis test. Calculated for various extract concentrations, the percentage inhibition of hemolysis was compared to that of the standard medication, Diclofenac. The finding show that the extract protects against RBC membrane lysis in a concentration-depedent manner. Increased inhibition was seen at higher extract doses, indicating strong membrane stabilization action. To ascertain how well the extract prevented hypotonicity-induced hemolysis, the absorbance values at 540nm and the associated percentage inhibition were examined and reported in the **table.** To show the extract's effectiveness in comparison to Diclofenac, a percent inhibition vs Concentration graph was created.

Concentration ($\mu g/ml$)	% Inhibition of Diclofenac Sodium	% Inhibition of Bhringraj
25	4.42	5.99
50	10.24	9.35
100	12.69	14.65
200	15.18	17.84
400	27.46	29.53
500	34.56	31.85
800	57.15	58.59
1000	72.45	68.46



IV. CONCLUSION

In conclusion, the research on Bhringraj extract gel has successfully demonstrated its significant pharmacological potential, particularly in its anti-bacterial, anti-inflammatory, and anti-oxidant activities. The gel has shown promising results in inhibiting bacterial growth, making it a potential candidate for addressing various bacterial infections. Additionally, its anti-inflammatory properties were evident, indicating that the gel could be effective in reducing inflammation, a key factor in many skin conditions and diseases. Furthermore, the strong anti-oxidant activity observed in the Bhringraj extract gel suggests its potential in neutralizing free radicals,

which could help in preventing oxidative stress-related damage to skin cells and tissues.

These findings validate the traditional use of Bhringraj in herbal medicine and support its therapeutic applications in modern dermatology and medicine. The gel's natural composition offers a safe and effective alternative to conventional treatments, with fewer side effects. However, while the results from in vitro testing are promising, further clinical studies are essential to assess its safety, efficacy, and long-term effects in human subjects. Overall, Bhringraj extract gel shows great promise as a multifunctional therapeutic agent, with potential for development into a widely used product for skin health and infection control.

REFERENCES

[1] Andre, M. a. (2015). Effective Treatment of Decalvans: Azithromycin in Monotherapy. Hair: Transplantation, 5(1), 1-4.

[2] Batch, L. N. (2022). Chemical composition and antibacterial property of Eclipta prostrata (L.). Can. Tho. Univ. J. Sci., 15, 79-83.

[3] Bergeron, B. B. (1973). Bactericidal activity and pharmacology of cefazolin antimicrobial agentschemother. Antimicrob Agents Chemotherapy, 4, 396-401.

[4] Bhandari, S. K. (2021). Phytochemical analysis of medicinal plants of Nepal and their antibacterial and antibiofilm activities against uropathogenic Escherichia coli. BMC complement Med.Ther., 21, 116.

[5] Kang, Y. K. (2022). Anti-inflammatory effects of Eclipta prostrata Linné on house dust mite-induced atopic dermatitis in vivo and in vitro. J. Ethnopharmacol., 292, 115-233.

[6] Kazmi, S. K. (2021). Evaluation of the antibacterial potential of various plant extracts against clinically important skin infectious bacterial strains. Anti-Infect Agents, 19, 57-63.

[7] Minh. (2019). Dried herbal tea production from Eclipta prostrata. Journal of Pharmaceutical Sciences and Research, 11(3), 684-687.

[8] Musselman, E. a. (1948). The rate of bactericidal action of penicillin in vitro as a function of its concentration and its paradoxically reduced activity at high concentrations against certain organisms. Journal of experimental medicine, 88(1), 99-131.

[9] Nahid, N. a. (2017). Evaluation of antioxidant and antimicrobial potentials of Eclipta prostrata collected from the Nepal region. The Pharma Innovation Journal., 6(11), 04-07.

[10] Otberg, N. K. (2008). Follicullitis decalvans. Dermatologic Therapy, 21, 238-244.

[11] Pratoomsoot, C. W. (2020). Synergistic antimicrobial and antioxidant properties of Coccinia grandis (L.) Voigt, Clerodendrum inerme (L.) Gaertn., and Acanthus ebracteatus Vahl. Extracts and their potential as a treatment for xerosis cutis complement, 27, 410-420.

[12] Riazunnisa, C. S. (2013). In-vitro Antibacterial activity and Phytochemical studies of some medicinal plants. International Journal of Pharmaceutical sciences Review and Research, 23(2), 77-80.

[13] Sabh, B. a. (1974). Special Tests: Bactericidal Bctivity and Activity of Antimicrobials in Combination. InIE. H. Lennette, E. H.Spaulding and J. P. Truant (Ed.), Manual of Clinical Microbiology, 2nd Edition. American Society for Microbiology, Washington, 431-435.

[14] Saegeman, M. V. (2017). Aeromonas Associated with Swimming Pool Folliculitis. Pediatric Infectious Disease Journal, 35(1), 118-129.

[15] Sharquie, K. N. (2012). Chronic Scalp Folliculitis versus Acne Vulgaris (Observational Case Series Study). J ClinExpDermatol Res, 3(3), 53-56.

[16] Sillani, C. B. (2010). Effective treatment of folliculitis decalvans using selected antimicrobial agents. J Trichol, 2, 20-30.

[17] Timalsina, D. D. (2021). Eclipta prostrata (L.) L. (Asteraceae): ethnomedicinal uses, chemical constituents, and biological activities. Biomolecules, 11, 17-38.

[18] Valgas C., D. S. (2007). Screening methods to determine antibacterial activity of natural products. Brazilian Journal Microbiolology, 38, 369-380.