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Formulation and Evaluation of *piper betle L.* Based Gel Dosage Form Against *Propionibacterium Acne*

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Abstract: ***Now a day the majority of Indians are affected by the persistent skin illness know as Acne. Sebaceous glands are involved in the chronic inflammatory skin condition. Acne is an Inflammatory skin disease that occurs due to blockages In polysebaseous and inflammation that occurs by bacteria such as Propionibacterium acne. Ethanolic extract of piper betle L. inhibit the growth of Propionibacterium acne bacteria and show the antiacne activity. It contains additional qualities including anti-ageing, anti-inflammatory, antibacterial, etc. and it typically used to treat acne. Piper betle L. was collected and authenticated and then extracted with maceration method by using Ethanol (95%) as a solvent. The phytoconstituent identification done by phytochemical screening test. We formulated the antiacne gel using ingredient like ethanolic extract of piper betle L. Certain evaluation parameter we tested to check whether cream is suitable for human skin and its antibacterial activity was checked on the micro-organism Propionibacterium acne which is responsible for causing acne. Propionibacterium acne is a bacteria isolated from the acne swab from the person who has acne and cultivate in ASLA agar. We measure the zone of inhibition by agar well diffusion method. The agar well diffusion method is a simple and widely used technique to assess the antimicrobial activity of antibiotic, plant extract or other antimicrobial agent against Propionibacterium acne.***
Keywords: Piper betle L., Maceration, Phytochemical screening, Antiacne gel, Propionibacterium acne, Agar well diffusion method, Zone of inhibition.

I. INTRODUCTION

Acne, black heads, pimples, dark circle are common among youngster and person who suffer from it. Many people refer the herbal product over the synthetic one because herbal product having less side effect. We formulated herbal anti-acne cream they have minimal side effect. Acne it is a skin disease affecting approximately 80-85% of young people the age range of 1225 years. There is a high prevalence of acne in female. Various anti-acne drug particularly those made of herbal ingredients have been developed to improve the therapy. [1] Acne is multifactorial chronic inflammatory disease of polysebaseous units. Propionibacterium acne and staphylococcus aureus are considered as the major skin bacteria that causes the formation of Acne.[3] The many plants that are effective to overcoming acne problems one of these is the betel leaf. which contained Chavicol a substance with a potent bactericidal effect. Ethanolic extract of *piper betle L.* inhibit the acne causing bacteria *Propionibacterium acne*. *Propionibacterium acne* is a gram-positive bacteria human skin commensal that prefers anaerobic growth conditions and is involved in the pathogenesis of acne. ASLA Agar is used for selective isolation and cultivation media of *propionibacterium acne.* Ethanolic extract of *piper betle L.* is inhibit the growth of *propionibacterium acne* and It's antiacne activity. Propionibacterium acne an anaerobic pathogen plays an important role in the pathogenesis of Acne.[8] The piper betle L. show the activity against the propionibacterium acne. To check this antiacne activity we performed microbial assay such as agar well diffusion method to confirm the antiacne activity of piper betle L.[8] The gel is very suitable for formulation and use for patient. Gel is a semisolid dosage form and it is water in oil type emulsion. Due to their versatility of texture and uses. Gel is a most frequent dosage form for skin drug delivery. Betel leaves show antiacne properties and for this gel dosage form is best for patient use. This gel is completely made from natural ingredients and has passed all the evaluation parameter so it has very less side effect

II. MATERIAL AND METHOD

A. Material

The material used in this study consist *piper betle L.* obtained from Shivraj green house, Gadhinglaj .Ethanol (95%), methyl paraben, propyl paraben, Triethanolamine , Carbopol, rose water, mineral oil and distilled water. *propionibacterium acne* was obtained from the Microbiology laboratory, Shivraj college, Gadhinglaj.

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B. Methods

1) Collection and Authentication

The *piper betle L.* collected from the Shivraj green house, Gadhinglaj and this herb authenticated by the Botany Department, Shivraj college, Gadhinglaj.

2) Extraction

The extraction of *piper betle L.* was done by Maceration process. Collect betel leaf and wash it with distilled water. Dry the betel leaf at 60^0c for 48 hours. Grind the dried betel leaf and prepare it's fine powder.10 gm Fine powder take into the container and pour the solvent such as 1000 ml Ethanol (95%) in the betel leaf powder. Closed the container at least three days Stir the content periodically. Filter the extract and store it at 2^0 c.[3]



Figure1- extract of piper betle L.

3) Phytochemical Screening

The Ethanolic extract of *Piper betle L.* was passes following chemical test for identification of phytoconstituents. Chemical nature of the constituents can be used as a tool to device a method for analysis of active constituents.[12]

Table 1. – Phytochemical screening

Sr.no.	Test	Observation	Inference
1.	Test for alkaloids- Mayer's test – Extract of test sample + Mayer's reagent	Pale yellow colour produced	Alkaloids are present.
2.	Wagner's test- Extract of test sample + Wagner's reagents	Brownish red colour ppt. observed	Alkaloids are present.
3.	Test for flavonoids Shinoda test Extract of test sample+ pinch of Mg. turning + 2 drops of conc. Hcl.	Pink colour observe	Flavonoids are present
4.	Test for Tannins Ferric chloride test Dilute extract of test sample+2 ml of ferric chloride.	Dark blue colour	Tannins are present
5.	Gelatin test Test sample extract in water+ 2 ml of NaOH solution + 2 drop of 10% $cuso_4$ solution	Precipitation observes	Gelatin present
6.	Test for phenol Extract of test sample+ 2 ml of potassium paramagnet solution	Disappear the colour of Potassium paramagnet	Polyphenols are present.

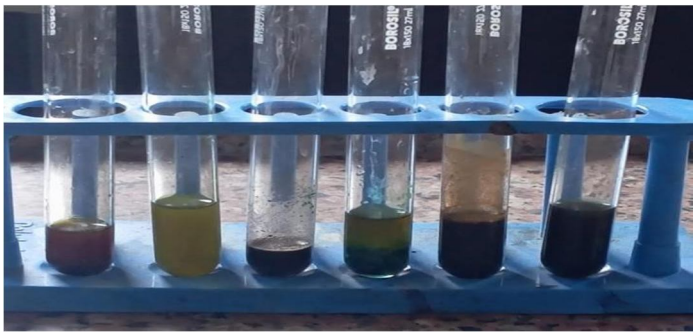


Figure 2. - Chemical identification test
a) Shinoda test b) Mayer's test c) Wagner's test d) Gelatine test e) Ferric chloride test f) Test for phenol

Table 2. Formulation of antiacne gel from ethanolic extract of *piper betle L.*

Sr. No.	Ingredients	Roll of ingredients	F1	F2	F3
1.	Carbopol	Gelling agent	5gm	5gm	5gm
2.	Ethanolic extract of piper betle L.	Antiacne, antibacterial agent	1ml	1.5ml	2ml
3.	Triethanolamine	neutralizer	1ml	1ml	1ml
4.	Glycerine	Viscosity enhancer	0.5ml	0.5ml	0.5ml
5.	Propyl paraben	Preservative	0.5ml	0.5ml	0.5ml
6.	Methyl paraben	Preservative	0.5ml	0.5ml	0.5ml
7.	Rose water	Perfume	Q.S	Q.S	Q.S
8.	Clitoriaternatea (Gokarn flowers)	Colouring agent	Q.S	Q.S	Q.S
8.	Distilled water	Vehicle	Q.S	Q.S	Q.S

4) Procedure for formulation of gel

Weight and dissolved Carbopol in distilled water. Add Triethanolamine drop by drop with stirring until the gel form. Add ethanolic extract of piper betle L. to the Carbopol mixture.

Add glycerine and preservative to the above mixture. Adjust the pH to 6.8 to 7.4. Fill the formulated gel into container.[7]



Figure3. formulated antiane gel

III. EVALUATION OF FORMULATED ANTIACNE GEL

1) Physical evaluation: In physical evaluation we evaluate the external appearance of prepared gel such as a colour, odour, texture, state, etc.

2) Ph: We determined pH by using pH meter. Take 1 gm prepared gel and mixed in 100 ml distilled water. Keep aside 2 hours without disturb.[5]

3) Viscosity: Viscosity of gel was done by using Brooked field viscometer at a temperature 25-100^{0}C using spindle no.63 at 2.5 RPM. According to the result all the three formulations show adequate viscosity.[5]

4) Spreadability: 1gm gel spread on the centre of one slide place another slide on the top of the gel to form sandwich. Ensure the gel spread evenly between the slide. Record the time taken for the separation of two slide. The less time taken for separation of the two slides better the Spreadability. [5]

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5) Washability: Washability test was carried out by applying small amount of gel on the hand and then washing it with tap water. All the formulation were easily washable.[5]

6) Greasiness: Here the gel was applied on the skin surface in the form of smear and checked if the smear was oily or grease like.[5]

IV. EVALUATION OF ANTIACNE ACTIVITY ON *PROPIONIBACTERIUM ACNE*

Propionibacterium acne is a gram-positive bacterium. It looks like rod shaped and slightly curve. It is major skin associated bacterium that was long considered commensal, until several studies related it to been opportunistic pathogen. Propionibacterium acne is a gram-positive human skin commensal that prefers anaerobic bacterium residing in the sebaceous glands. Propionibacterium acne produced free fatty acids within sebaceous gland. Which can irritate the follicular wall and induce inflammation leading to cutaneous infection and caused acne.[9]

A. Isolation And Cultivation Of Propionibacterium Acne

Sample collected from human skin mainly infected sites using sterile cotton swab or skin scraping. We isolated from deep infections by using biopsy and pus aspirates. Sample store at 4^0 C.

B. Culture Media for Isolation

The suitable culture media for Propionibacterium acne is ASLA based agar media.

C. Composition of ASLA Agar Base

Table 3. - Formulation of ASLA agar

Sr.no.	Ingredients	Quantity (gm/liter)
1.	Ammonium sulphate	1gm
2.	Disodium phosphate	1.2 gm
3.	Monopotassium phosphate	1.2 gm
4.	Manganese sulphate	0.05 gm
5.	Magnesium sulphate	0.2 gm
6.	Ferric sulphate	0.04 gm
7.	L-Cysteine hydrochloride	0.5 gm
8.	Agar	10 gm

pH = 6.5 at 25^0 C Sample loaded into the prepared ASLA Agar plates and keep into the incubator at 37 ^{0}c. temperature for 3 to 7 days. (slowly growing microorganism).[11]

D. Zone of Inhibition

The zone of inhibition is a clear area around an antibiotic sample where bacterial growth is prevented. It is used to determine the antimicrobial susceptibility of Propionibacterium acne through agar well diffusion method. [(10)]

E. Agar well Diffusion Method

The agar well diffusion method is a simple and widely used technique to assess the antimicrobial activity of antibiotic, plant extract or another antimicrobial agent against
Propionibacterium acne.[11]

F. Procedure

Prepared and pour the agar medium into sterile petri dishes. Allow the agar to solidify and reach room temperature.Bacteria suspension loaded into the agar plate and using cell spreader evenly spread the bacterial suspension on the agar surface. Let the plate sit for 5-10 minutes to allow absorption Use a sterile cork borer to create wells in the agar.

Space well at least 2 cm apart to prevent overlapping zones.Pipette 50-100 microliter of the test compound into each well. Allow the solution to diffuse for 10-15 minutes at room temperature. Plate place into the Incubator. Incubated at 37^0 C for 48-72 hours. After incubation period observed clear zones around the wells. Measure the diameter(mm) of inhibition zones using ruler.

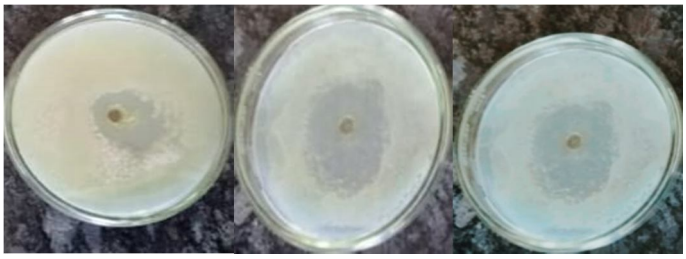


Figure 4- Zone of Inhibition of formulated Antiacne gel (F1, F2, F3)

V. RESULT AND DISCUSSION

The collection of the Pipe betle L. from the Green house, Shivraj college, Gadhinglaj and authenticated from the Botany Department, Shivraj college, Gadhinglaj.

The collected Pipe betle L. dried under sunlight. Dried Pipe betle L. extracted by the

Maceration using 95% ethanol give the 46 % extraction yield.

% extraction yield = weight of extracted substance / weight of original substances *100

= 2.3 / 5 *100

= 46%

Extracted piper betle L. phytoconstituent screening done through phytochemical identification test. After the performing of phytochemical identification test revealed that the flavonoids, Alkaloids, tannins, Phenols and gelatin these secondary metabolites are present the piper betle L. extract.

Table 4. - Phytochemical screening

Sr.no.	Phytochemical test	Result
1.	Test for alkaloid	+ve
2.	Test for flavonoid	+ve
3.	Test for tannins	+ve
4.	Test for phenol	+ve
5.	Test for gelatin	+ve

The obtained extract was used for the Antiacne gel formulation. Various quality control tests were performed to see if the formulated antiacne gel was suitable or not such as physical appearance, pH, viscosity, washability, Spreadability and greasiness. Formulated antiacne gel passes all the quality control parameters within limit as per Indian pharmacopeia.

Table 5.- Evaluation parameters

Sr.no	Evaluation parameter	F_1	F 2	F 3
1.	Physical evaluation			
a	Colour	Pale blue	Pale blue	Pale blue
b	Odour	Pleasant	Pleasant	Pleasant
c	Texture	Smooth	Smooth	Smooth
d	State	Semisolid	Semisolid	Semisolid
2.	pH	7	7.4	7.5
3.	Viscosity (Cps)	2567cp	2587cp	2345cp
4.	Spreadability (gm x cm/sec)	5 cm/10sec	6.3cm/10sec.	6cm/10sec
5.	Washability	Easily wash	Easily wash	Easily wash
6.	Greasiness	Non greasy	Non greasy	Non greasy

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Piper betle L. based antiacne gel activity check on the Propionibacterium acne by agar well diffusion method using ASLA agar base. Inhibition zone of piper betle L. extract and formulated antiacne gel as following

Table 6.-Zone of inhibition of piper betle L. extraction

Sr. No.	Concentration	Zone of inhibition(mm)
1.	1	1.6 mm
2.	2	1.5mm
3.	3	1.8 mm
4.	4	2mm
5.	5	2.2mm

Table 7.-Zone of inhibition of formulated gel

Sr. No.	Concentration (ug/ml)	F1	F2	F3
1.	1	1.9mm	1.8mm	2.1mm
2.	2	2mm	2.2mm	1.8mm
3.	3	2.2mm	2mm	1.9mm
4.	4	1.9mm	2.1mm	2mm
5.	5	1.8mm	1.9mm	2mm

VI. CONCLUSION

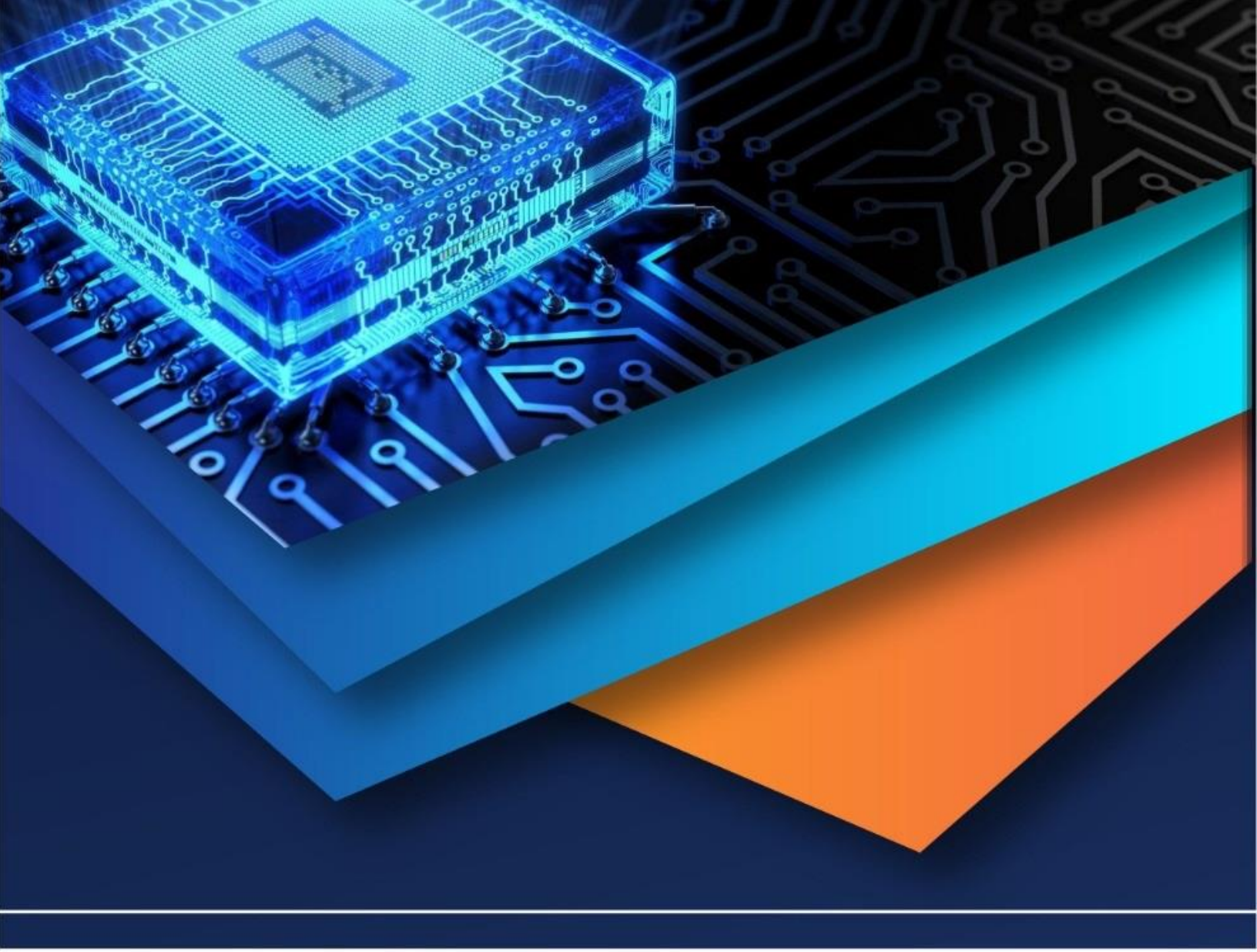
In this study we formulated and evaluated antiacne gel from ethanolic extract of piper betle L. In this project we collect and authenticate piper betle L. from the Botany department, Shivraj college, Gadhinglaj. The piper betle L. dried under the sunlight and extracted through Maceration process by using ethanol (95%) as a solvent In ethanol the piper betle L. give 46% extraction value.(figure-1) The presence of secondary metabolites such as Flavonoids, Alkaloids, Tannins, Phenol and gelatin phytoconstituents was found in Ethanolic extract of piper betle L (figure-2,Table1,4). Formulated antiacne gel passes all the quality control parameter. (figure 3,Table 5.) Check the antiacne activity of piper betle L. by performing agar well diffusion method (figure 4, table 6,7). It provide the protection against Propionibacterium acne which is responsible for the causing acne It is herbal based antiacne gel that's why it having minimum side effect.

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