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FORMULATION AND PHARMACOLOGICAL EVALUATION OF TOPICAL POLYHERBAL ANTIACNE GELS

Prateek Yadav*, Satyawan Dangi, Dr. Satkar Prasad and Dr. Shailesh Kumar Ghatuary

RKDF School of Pharmaceutical Sciences, NH-12, Jatkhedi, Hoshangabad Road, Bhopal- 462026.

***Corresponding Author: Prateek Yadav**
RKDF School of Pharmaceutical Sciences, NH-12, Jatkhedi, Hoshangabad Road, Bhopal- 462026.

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ABSTRACT
India is known as the "Emporium of Medicinal plants" due to availability of several thousands of medicinal plants in the different bioclimatic zones. Medicinal plants continue to provide valuable therapeutic agents, both in modern medicine and in traditional systems of medicine. 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. Acne is a common but serious skin disease, which affects approximately 80%adolescents and young adults in 11-30 age groups. 42.5% of men and 50.9% of women continue to suffer from this disease into their twenties. Acne vulgaris (acne) is a cutaneous pleomorphic disorder of the pilosebaceous unit involving abnormalities in sebum production and is characterized by both inflammatory (papules, pustules and nodules) and non- inflammatory (comedones, open and closed) lesions. Propionibacterium acnes are common pus-forming microbes responsible for the development of various forms of acne. In the present study anti-acne gels were prepared using polymer carbopol 940 along with the hydroalcoholic extracts of plants *Hibiscus Rosa*-Sinesis Linn, *Embelia Ribes* and *Allium Cepa* evaluated for their physicochemical properties, like pH, washability, extrudability, spreadability and viscosity. The formulations (PHF1- PHF6) were tested for the anti acne activity by well diffusion method against Propionibacterium acnes. Results showed that the gels were non-irritant, stable and posses anti-acne activity. The efficacy when tested with a standard was almost same to that of Clindamycin. This suggests that *Hibiscus Rosa*-Sinesis Linn, *Embelia Ribes* and *Allium Cepa* has 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: *Hibiscus Rosa*-Sinesis Linn, *Embelia Ribes*, *Allium Cepa*, Carbopol 940, Anti-acne activity, Well diffusion method, Clindamycin.

1.0. INTRODUCTION
Skin disease normally arises due to accumulating toxins in blood due to impurities in blood, improper food habits and lifestyle. Acne vulgaris is a disorder of the skin which affects most of the adolescents during puberty stage due to hormonal changes which changes path-physiologic factors.[1] This condition is related to obstruction in follicular distention with activation of inflammatory response with open or close comedones, inflammatory papules and nodules. Gram-positive bacterium such as Staphylococcus, Propionibacterium and Escherichia species are linked to the skin condition of acne. Acne vulgaris normally affects the skin areas with dens sebaceous follicles such as the face, upper chest, back. Symptoms of acne vulgaris include pain, tenderness, or erythema.[2] various synthetic antibiotic drugs such as clindamycin, doxycycline, and minocycline are used to overcome this problem. Most of the time topical therapy is the first-line treatment with benzyl peroxide and retinoid. However, increasing frequency of antibiotics and its side effects there should be necessary to focus on the exploration of herbal drugs. Its reported safety with minimal adverse effect, in recent years there is a gradual development of interest in the use of the medicinal plant. Several Indian medicinal plants certified with various pharmacological activities due to diversified classes of phytoconstituents. Literature also proved that medicinal plant with varying potency when combined, it produces the synergistic therapeutic effect with improving patient's complian.[3]

Plant such as *Hibiscus Rosa*-Sinesis Linn, *Embelia Ribes* and *Allium Cepa* possess many potential therapeutic activities due to individually presence of rich phytoconstituents. For many years, antibiotics have been used to treat acne vulgaris. However, antibiotic resistance has been increasing in prevalence within the dermatologic setting. The development of antibiotic resistance including the specific nature of the relationship of bacteria to antibiotics, how the antibacterial is used, host characteristics, and environmental factors. To overcome the problem of antibiotic resistance, medicinal plants have been extensively studied as alternative treatments for diseases. So our aim and objective to develop safe and effective polyherbal formulation for effective management of acne.[4]

2.0. MATERIAL AND METHODS

2.1. Plant material collection

Leaves of *Hibiscus rosa-sinesis* Linn., seeds of *Embelia ribes* and *Alliumcepa* was collected from local area of Bhopal (M.P.) in the month of January, 2018.

2.2. Extraction of plant material

Dried powdered leaves of *Hibiscus rosa-sinesis* Linn., seeds of *Embelia ribes* and *Allium cepa* has been extracted with hydroalcoholic using maceration process for 48 hrs, filtered and dried using vaccum evaporator at 40^0C.

2.3. Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

2.4. Phytochemical Screening[5]

The chemical tests were performed for testing different chemical groups present inextracts.

Alkaloids To the extract dilute hydrochloric acid was added. Then it wasboiled and filtered.

Mayer's test:-To 2-3 ml of filtrate, few drops of the Mayer's reagent were added. Formation of cream precipitate indicated the presence of alkaloids.

Dragendorff's test:-To 2-3 ml of filtrate, few drops of the Dragendorff's reagent was added. Formation of orange brown precipitate indicated the presence of alkaloids.

Hager's test:-To 2-3 ml of filtrate, few drops of Hager's reagent were added. Formation of yellow precipitate indicated the presence of alkaloids.

Wagner's test:-To 2-3 ml of filtrate, few drops of Wagner's reagent were added. Formation of reddish brown precipitate indicated the presence of alkaloids.

Carbohydrates

Molisch's test (General test):-In a test tube containing 2 ml of extract, 2 drops of freshly prepared 10 per cent alcoholic solution of α- naphthol was added. Then it was shaken and 2 ml of Conc.sulphuric acid was added from sides of the test tube. So the violet ring was formedat the junction of two liquids, indicated the presence of carbohydrates.

Fehling's test (Reducing sugars):-To 2 ml of extract, equal volume of mixture of equal parts of Fehling's solution Aand B were added and boiled for few minutes in boiling water bath. Formation of red or brick red coloured precipitate indicated the presence of reducing sugars.

Benedict's test (Reducing sugars):-Equal volume of Benedict's reagent and test solution were added in a test tube andboiled for 5 min in a water bath. Formation of green, yellow or red coloured precipitate depending on amount of reducing sugar present in test solution indicated the presence of reducing sugar.

Flavonoids

Ferric-chloride test: Test solution with few drops of ferric chloride solution shows intense green colour.

Alkaline reagent test: To 2 ml of test solution add 2 ml alkali, gives yellow color, which disappears on addition of dil. HCl it disappears, which indicates presence of flavonoids.

Shinoda's test: In a test tube containing 0.5 ml of the extract, a small piece of magnesium was added. Then few drops of conc. hydrochloric acid were added. Formation of pink colour indicated the presence of flavonoids.

Proteins

Biuret's test (General test): To 1 ml of test extract, 4% of sodium hydroxide solution and few drops of 1% copper sulphate solution were added. Formation of a violet red colour indicated thepresence of proteins.

Saponins

Foam test: The extract was shaken vigorously with water in a test tube. Formation ofpersistent foam indicated the presence of saponins.

Haemolytic test: Few drop of extract solution was mixed with Blood, which indicateshaemolysis, shows presence of saponin.

Salkowaski tes: Concentrated sulphuric acid (2 ml) was added to 2 ml of test solution. The solution was shaken and allowed to stand. The colour of lower layer changed to yellow indicating presence of triterpenoids**.**

Steroids

Salkowski test: To 2 ml of extract, 2 ml of chloroform and 2 ml of concentrated sulphuric acid were added and shaken, red color at lower layer indicated the presence of steroids**.**

Liebermann-burchard reaction**:** T.S 2 ml was mixed with chloroform (2 ml). To the solution, 2 ml of acetic anhydride and 2 drops of conc. Sulphuric acid from the side of test tube were added. Change in colour first red, then blue and finally green indicated presence ofsteroids.

Amino acid

Ninhydrin test (General test):3 ml of test solution and 3 drops of 5% ninhydrin solution in a test tube were heated in boiling water bath for 10 minutes. Formation of Purple or bluish colourindicated the presence of amino acid.

Millons test: T.S (3 ml) and Million's reagent (5 ml) were mixed in a testtube. The appearance of white precipitate changing to brick red or dissolved and gave red color to solution on heating indicated presence of proteins.

Xanthoprotic test: To the test tube containing T.S (3 ml), 1 ml of conc. Sulphuric acid was added. Appearance of white precipitate which turnsyellowonboiling and orange on addition of NH4OH indicated presence of tyrosin and/or tryptophan containing proteins.

Glycosides

Test A: 200 mg of extract were diluted with 5 ml of dilute sulphuric acid by warming on a water bath and filtered it. Then the acid extract was neutralized with5% solution of sodium hydroxide. Then 0.1 ml of Fehling's solution A and B wereadded until it became alkaline (test with pH paper) and heated on a water bath for 2 minutes. Noted the quantity of red precipitate formed and compared with that of formed in test B.

Test B: 200 mg of extract was diluted with 5 ml of water instead of sulphuric acid.Then equal amount of water (as used for sodium hydroxide in the above test) after boiling was added. Then 0.1 ml of Fehling's solution A and B were added until it became alkaline (test with pH paper) and heated on a water bath for 2 minutes.

Noted the quantity of red precipitate formed. The quantity of precipitate formed in test B was compared with that formed in test A. If the precipitate in test A was greater than in test B then glycoside may be present. Since test B represents the amount of free reducing sugar already present in the crude drug, whereas test A represents free reducing sugar plus those related on acid hydrolysis of any glycoside in the crude drug.

Baljet test: 2 ml of the test solution was treated with 2 ml of sodium picrate solution. The development of yellow to orange colour indicatedpresence of cardiac glycosides.

Legals test: To 2 ml of test solution, 1 ml of pyridine and 1 ml of sodiumnitroprusside was added. Change in color to pink or red indicated presence of cardiac glycosides. Glacial acetic acid (3-5 drops), one dropof 5% FeCl3 and conc. Sulphuric acid were added to the test tube containing 2 ml of T.S. Appearance of reddish-brown color at the junction of two layers and bluish green in the upper layer indicated presence of glycosides.

Tannins

Ferric chloride test: Extract solutions were treated with 5% ferric chloride solution. Formation of blue colours indicated the presence of hydrolysable tannins and formation of green colour indicated the presence of condensed tannins.

Lead acetate test: Extract solutions were treated with 5% lead acetate solution. Formation of white precipitate indicated the presence of hydrolysable tannins.

Gelatin test:3 ml of test solution when treated with gelatin solution (3ml) gave white precipitate.

2.5. Qualitative chromatographic Analysis[6]

Thin Layer Chromatography

Thin layer chromatography: T.L.C. is based on the adsorption phenomenon. In this type of chromatography mobile phase containing the dissolved solutespasses over the surface of stationary phase.

Steps involved in T.L.C.

1) Preparation of plates
2) Activation of plates
3) Preparation and saturation of chamber
4) Sample application and development
5) Detection and calculation of Rf Value TLC of extract was performed and reported.

Preparation of Plates

1. Silica gel, the most frequently used stationary phases, was employed as such for adsorption T.L.C.
2. To reduce the band broadening the stationary phase should consist of small particles of uniform size so as to provide a large surface area for interaction and a small void volume.
3. Silica Gel was mixed with water and made into slurry.
4. Coated the slurry by spreading the slurry on the plate uniformly.
5. Firstly air dried the plate for some time and then kept for activation.

Activation of Plates

1. By heating the plates in an oven at 100 to 110^{o}c for 30minutes
2. Activation is necessary for linear movement of solutes over stationary phase.

Preparation and Saturation of Chamber

1. Prepared the solvent system.

Poured it into the chamber and saturated the chamber by lining the chamber with a piece of filter paper that has been wet with the mobile phase

Sample application and development

1. After plates were activated, the sample, which may be range from a few µgto mg, was applied on the plates with the help of capillary tube.
2. Plates were placed in the chamber that contains developing solvent to adepth of about 0.5 cm.
3. Plates were then removed from the chamber, the

mobile phase front is marked by scratching the surface, and the solvent was evaporated in an oven.

Detection and Calculation of Rf. Value

Once the chromatogram was developed the Rf Value of the spot was calculated using the formula.

$$Rf = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

2.6. Estimation of total Phenolic and flavonoid Content

Total Phenolic content estimation

Principle: The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method.

Preparation of Standard: 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5- 25µg/ml was prepared in methanol.

Preparation of Extract: 10 mg of dried extracted dissolve in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenols.

Procedure: 2 ml of extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (75g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Total flavonoids content estimation

Principle: Determination of total flavonoids content was based on aluminum chloride method.

Preparation of standard: 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol.

Preparation of extract: 10 mg of extract dissolved in 10 ml methanol and filter. Three (1mg/ml) of this extract was for the estimation of flavonoid.

Procedure: 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

2.7. Formulation development of Polyherbal gel

Method of preparation

Measured quantity of Methyl Paraben, Glycerin, Polyethylene glycol and hydroalcoholic extract of *Hibiscus rosa-sinesis*, *Embelia ribes* Linn, and *Allium cepa* were 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.

Carbopol 940 – Gelling Polymer
Triethanolamine- gelling agent, pH Adjusting agent, Neutralizer
Methyl Paraben – Preservative
Distilled Water, Glycerin and Polyethylene Glycol-**solvents**

Table 1: Formulation of Polyherbal Gel.

Ingredients (%)	PHF1	PHF 2	PHF3	PHF4	PHF5	PHF6
Hibiscus rosa-sinesis Linn.,extract	1gm	1gm	1gm	1gm	1gm	1gm
Embelia ribes extract	1gm	1gm	1gm	1gm	1gm	1gm
Allium cepa extract	1gm	1gm	1gm	1gm	1gm	1gm
Carbopol 940	0.25mg	0.5mg	0.75mg	1.0 gm	1.25gm	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

2.8. Evaluation of polyherbal Gel

2.8.1. Appearance and consistency[8]

The physical appearance was visually checked for the texture of Polyherbal gel formulations and observations may be like stated in Table 8.

2.8.2. Washability[9]

Formulations were applied on the skin and then ease and extent of washing with water were checked manually and observations may be like stated in table 9.

2.8.3. Extrudability determination of formulations

The polyherbal gel formulations were filled into collapsible metal tubes or Aluminium collapsible tubes. The tubes were pressed to extrude the material and the extrudability of the formulation was checked.(table no 9).

2.8.4. Determination of Spreadability

Principle

An important criterion for anti-acne gels is that it must possess good spreadability. Spreadability is a term expressed to denote the extent of area to which the gel readily spreads on application to skin. The therapeutic efficacy of a formulation also depends on its spreading value.

A special apparatus has been designed to study the spreadability of the formulations. Spreadability is expressed in terms of time in seconds taken by twoslides 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 anti-acne gel formulation between the two slides was traced uniformly to form a thin layer. The weight was removed and the excess of the anti-acne 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 anti-acne gel formulation.

$$\text{Spreadability} = \frac{m.l}{t}$$

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.

2.8.5. Determination of pH[10]

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

2.8.6. Drug content[11]

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 % $AlCl_3$. 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.

2.8.7. Anti-acne activity of Polyherbal gel Pathogenic microbes used[11]

The pathogenic microbes used in the current study are three bacteria obtained fromMicrobial Culture collection, National Centre forcell science, Pune, Maharashtra, India.

Media preparation (broth and agar media) Composition of nutrient agar media;

Agar	-	1.5 gms.
Beef extract	-	0.3 gms.
Peptone	-	0.5 gms.
Sodium chloride	-	0.55 gms.
Distilled water	-	to make100 ml.

pH – 7

Method of preparation

This agar medium was dissolved in distilled water and boiled in conical flask of sufficient capacity. Dry ingredients are transferred to flask containing required quantity of distilled water and heat to dissolve the medium completely.

Sterilization culture media

The flask containing medium was cotton plugged and was placed in autoclave for sterilization at 15 lbs /$inch^2$ (121^{o}C) for 15 minutes.

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 37^{o}C overnight to check the sterility of plates. The plates were dried at 50^{o}C for 30 minutes before use.

Revival of the bacterial 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 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.

2.8.8. Antibiogram studies[12]

Broth cultures of the pure culture isolates of those test microorganisms which are sensitive towards the 100 mg/ml concentration of phyto extract used in present study were prepared by transferring a loop of culture into sterile nutrient broth and incubated at 37^{o}C for 24-48hours. A loop full was taken from these broths and seeded onto sterile nutrient agar plates through sterile cotton swab to develop diffused heavy lawn culture.The well diffusion method was used to determine the antibacterial activity of the extracts prepared from the powdered leaves of *Hibiscus rosa-sinesis* Linn., seedsof *Embelia ribes* and *Allium cepa* using standard procedure. There were 3 concentration 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.

2.9. RESULTS AND DISCUSSION

Determination of Percentage Yield

Yield of Extraction: The crude extracts so obtained after the maceration process, each extracts were further concentrated on water bath evaporation the solvents completely to obtain the actual yield of extraction. To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from different samples using Pet. ether, hydroalcoholic as solvents are depicted in the table 2.

Table No. 2: % Yield of hydroalcoholic extract.

S. No.	Solvent	% Yield (w/w)
1	*Hibiscus rosa-sinesis* Linn	4.5%
2	*Embelia ribes*	6.4%
3	*Allium cepa*	6.2%

Phytochemical screening of extract

A small portion of the dried extracts were subjected to the phytochemical test using Kokate (1994) methods to test for alkaloids, glycosides, tannins, saponins, flavonoids and steroids separately for extracts of all samples. Small amount of each extract is suitably resuspended into the sterile distilled water to make the concentration of 1 mg per ml. The outcomes of the results are discussed separately in the table 3.

Table No.3: Result of Phytochemical screening of hydroalcoholic extracts.

S. No.	Constituents	*Hibiscus rosa-sinesis*	*Embelia ribes*	*Allium cepa*
1.	Alkaloids	-ve	+ve	+ve
2.	Glycosides	-ve	-ve	-ve
3.	Flavonoids	+ve	+ve	+ve
4.	Phenolics	+ve	-ve	-ve
5.	Amino Acids	-ve	+ve	-ve
6.	Carbohydrate	+ve	+ve	+ve
7.	Proteins	-ve	+ve	-ve
8.	Saponins	+ve	+ve	-ve

Table No.4: Results of Comparative Thin Layer Chromatography of hydroalcoholicextract

S. No.	Toluene: Ethyl acetate: Formic acid(5:4:1) Quercetin (Rf value)	Toluene: Ethyl acetate: Formic acid(7:5:1) Gallic acid (Rf value)
1	0.714	0.678

From the Rf value it was confirmed the presence of Quercetin as Flavanoids and Gallic acid as phenol compound in the extract.

Table No.5 Results of Estimation of Total Phenolic and flavanoid content estimation Total Phenolic content estimation (TPC)

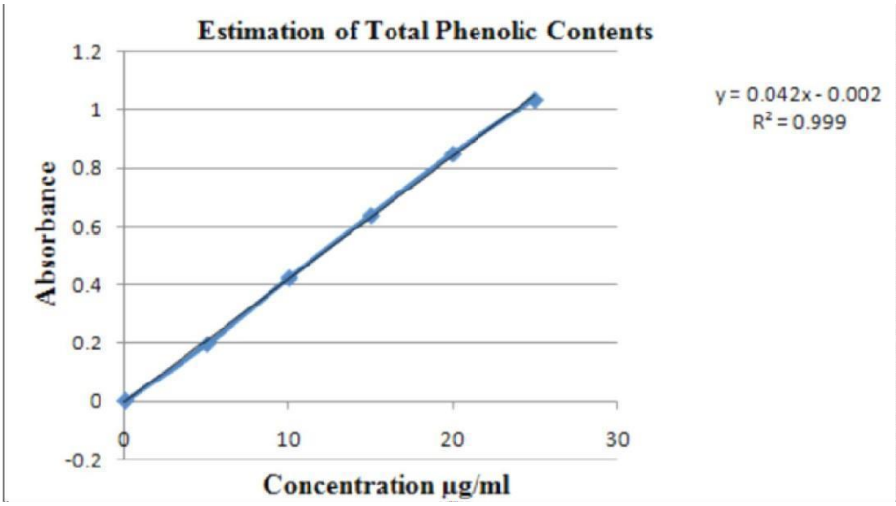
The content of total phenolic compounds (TPC) content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve.

Y = 0.042X-0.002, R^2= 0.999, where X is thegallic acid equivalent (GAE) and Y is the absorbance.

Calibration Curve of Gallic acid.

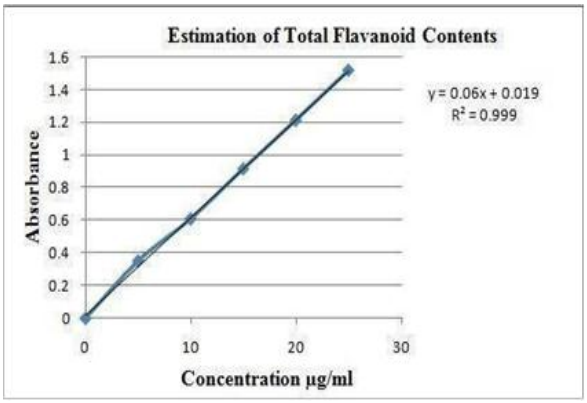
Table No. 5: Preparation of calibration curve of Gallic acid.

S. No.	Concentration	Absorbance
0	0	0
1	5	0.194
2	10	0.422
3	15	0.637
4	20	0.848
5	25	1.035



Graph 1: Graph of Estimation of Total Phenolic contentTotal flavanoid content estimation (TFC)

The content of total flavanoid compounds (TFC) content was expressed as mg/100mg of quercetin equivalent of dry extract sample using the equation obtained from the calibration curve: **Y = 0.06X+0.019, R^2= 0.999,** where X is the quercetin equivalent (QE) and Y is the absorbance.



Graph 2: Graph of Estimation of Total flavanoid content.

Calibration Curve of Quarcetin

Table No. 6: Preparation of calibration curve of Quarcetin.

S. No.	Concentration	Absorbance
0	0	0
1	5	0.352
2	10	0.61
3	15	0.917
4	20	1.215
5	25	1.521

Table No. 7: Total Phenolic and Total flavanoid content.

S. No.	Solvents→ Bioactivecompound↓	Hydroalcoholic extracts		
		Hibiscus rosa-sinesis	***Embelia ribes***	***Allium cepa***
1.	Total Phenol (Gallic acid equivalent (GAE) mg/100mg)	0.805	-	-
2.	Total flavanoid (Quercetin equivalent (QE) mg/100mg)	0.314	0.656	0.605

Results of Formulation and evaluation Development of polyherbal Gel

Table No. 8: Results of Psycho Rheological Characteristists.

Formulation	Colour	Clogging	Homogeneity	Texture
PHF1	Brown	Absent	Good	Smooth
PHF2	Brown	Absent	Good	Smooth
PHF3	Brown	Absent	Good	Smooth
PHF4	Brown	Absent	Good	Smooth
PHF5	Brown	Absent	Good	Smooth
PHF6	Brown	Absent	Good	Smooth

Results: In the above formulations of gels, it has been noted that all of them hasclear colour, No clogging, good homogeneity and smooth texture**.**

Results of washability and Extrudability

Table No. 9: Results of washability and Extrudability.

Formulation	Washability	Extrudability
PHF1	Good	Average
PHF2	Good	Average
PHF3	Good	Average
PHF4	Good	Average
PHF5	Good	Average
PHF6	Good	Average

Results: In the above formulations of gels, they have good washability as well as extrudability.

Results of spreadability

Table No. 10: Results of spreadability.

Formulation	Spreadability (gcm/sec)
PHF1	15.23±0.12
PHF2	14.65±0.15
PHF3	14.15±0.25
PHF4	13.65±0.35
PHF5	13.12±0.15
PHF6	13.25±0.33

(n=3)

Results: In all above formulations of gel the spreadibility of PHF5 is good.

Determination of pH

Table No. 11: Determination of pH.

Formulation	pH
PHF1	6.82± 0.11
PHF2	6.95±0.15
PHF3	7.02±0.11
PHF4	7.05±0.14
PHF5	7.00±0.12
PHF6	7.15±0.13

(n=3)

Results: The above formulation of topical gels has different pH value for different formulation.

Results of Viscosity

Table No. 12: Results of Viscosity.

Formulation	Viscosity (cps)
PHF1	3150±10
PHF2	3256±15
PHF3	3365±18
PHF4	3458±20
PHF5	3654±25
PHF6	3562±22

(n=3)

Results: In the above formulations the viscosity of different sample of gel were determined and found that there is increase in viscosity. The formulation PHF5 has good viscosity.

Results of Flavonoid Content

Table No. 13: Results of Flavanoid Content using AlCl3 method.

Formulation	% Flavonoid Content
PHF1	88.25
PHF2	90.25
PHF3	89.98
PHF4	90.25
PHF5	95.56
PHF6	92.25

Results: In the above formulation of different gels the percentage of drug content was found that PHF5 has maximum percentage of drug content.

Results of antimicrobial activity of optimized formulation Antibiogram studies

The present investigation in this research work, the Anti-acne activity of polyherbal gel of leaves of *Hibiscus rosa-sinesis* Linn., seeds of *Embelia ribes* and *Allium cepa* was evaluated against *Propionibacterium acnes* pathogens used under present study. The polyherbal gel obtained from plant used to suitably dilute upto the concentrations of 100, 50 and 25 microgram per ml and applied on to the test organism using well diffusion method. Results of the experiment are being concluded in the Table, which clearly shows the anti-acne activity of Hydroalcoholic extracts of leaves of *Hibiscus rosa-sinesis* Linn., seeds of *Embelia ribes* and *Allium cepa* against *Propionibacterium acnes* bacterial strain used in present work.

Table No. 14: Anti-acne activity of standard and polyherbal gel formulation against Propionibacterium acnes.

S. No.	Formulation	Zone of inhibition		
		100µg/ml	**50 µg/ml**	**25µg/ml**
1.	Clindamycin (STD)	31±0.5	28±0.74	22±0.86
2.	Polyherbal gel	25±0.76	21±0.5	18±0.57

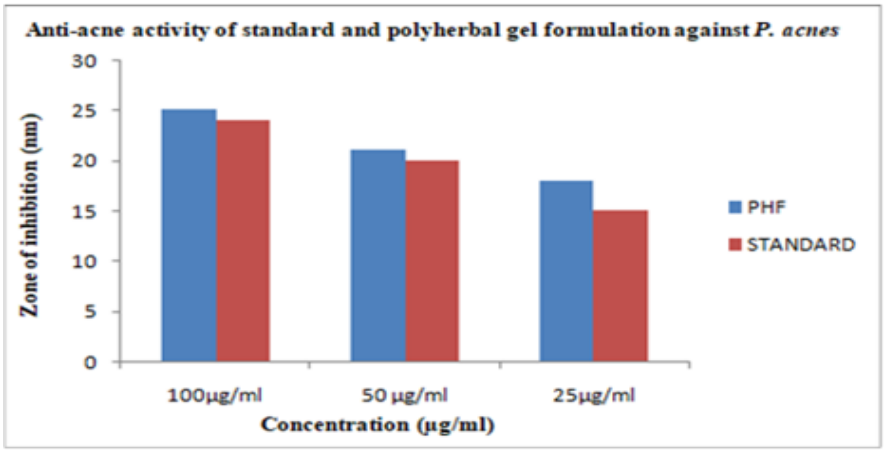


Figure 7.3: Anti-acne activity of standard and polyherbal gel formulation against Propionibacterium acnes.

CONCLUSION

Acne vulgaris is a most common skin disorder of pilosebaceous unit that affect areas containing the largest oil glands, including the face, back, and trunk. It is generally characterized by formation of seborrhea, comedone, inflammatory lesions. Propionibacterium acnes have been recognized as pus-forming bacteria triggering an inflammation in acne. The present research work deals with formulation and evaluation of herbal gels against this etiologic agent of acne vulgaris. The developed formulations were evaluated for their in vitro antibacterial activity against P. acnes. The Zones of inhibitions for the antibacterial activity were compared with the standard Clindamycin, active ingredients used in the formulation Hydroalcoholicextracts). The prepared Polyherbal formulation showed antimicrobial activity against P. acnes.

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