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FORMULATION, DEVELOPMENT AND EVALUATION OF POLYHERBAL GEL FOR EFFECTIVE TREATMENT OF ACNE

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ABSTRACT

Medicinal plants play an important role in the development of potent therapeutic agents. Plant based drugs provide outstanding contribution to modern therapeutics as a source of many valuable secondary metabolites which serves as plant defence 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* and 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.

K**eywords:** *Propionibacterium acnes, Hibiscus Rosa-Sinesis* Linn, *Embelia Ribes, Allium Cepa* Carbopol 940, Anti-acne activity, Well diffusion method, Clindamycin.

INTRODUCTION:

Medication and cosmetic measures to overcome skin problems continue to be a foremost research and development initiates by pharmaceutical and personal care industries. Herbal medicines with the history

of use from ancient time have entered the growing cosmeceuticals market for combating various skin problems [1]. It is attracting renewed attention from both practical and scientific view even though the mode of action of phytoconstituents from herbal origin is more complex than mechanisms of one bioactive factor. Ancient records show that the varieties of herbal approaches are proven to be effective for primary health care and treatment of various diseases[2]. Skin is most important and sensitive part of the human body. The external environmental exposure leads to many kinds of skin problems and disorders like acne, sunburn and pigmentation [1]. Acne is one of the most common multifactorial chronic inflammatory diseases of the pilosebaceous follicles involving altered follicular keratinization, androgen induced sebaceous hyperplasia, hormonal imbalance, immune hypersensitivity and bacteria (*Propionibacterium acnes*) colonization [3-4]. P. acnes is an anaerobic Gram-positive bacterium that produces propionic and acetic acid [5]. These bacteria are involved in the development of inflammatory acne by activating complements and metabolizing sebaceous triglycerides into fatty acids that irritate the follicular wall and surrounding dermis. It also produces exoenzymes and chemotactically attracts neutrophils [6]. The sustained antibiotic application entails the antibiotic resistance, which involving the specific nature of the relationship of bacteria to antibiotics [7]. It is sufficient purposes for searching alternative remedies to resolve these problems, including the medicinal plants. Onion (*Allium cepa L*.) is a multipurpose food plant that is used as traditional Indian spices. It has great health significance and is consumed for its putative nutritional and health benefits for centuries [8]. Traditionally, onions and plants belonging to the Allium genus and Allium is the largest and important representative genus of the family Liliaceae which have been used as an herbal remedy for a wide range of ailments, due to their association with many pharmacological effects [8-9]. Biological effects attributed to onions have been commonly ascribed to the volatile sulfurcontaining compounds, such as thiosulfinates, mainly responsible for the characteristic taste, aroma and lachrymatory effects [10]. However, these volatile products are highly unstable and recently attention has been focused on the effects of phenolic compounds, such as flavonoids, which are more stable [11]. Onion is known for being a good natural source of flavonoids mainly represented by the flavonols - quercetin and kaempferol, which are present as their glycosides [12]. In recent years, many publications have reported evidence of beneficial health effects attributed to flavonoids including antiallergenic, anti-inflammatory, cardioprotective, vasodilatory, anticarcenogenic and antioxidant properties [13]. Dried fruits of *Embelia ribes* belong to family Myrsinaceae is one of the most significant plants used from the prehistoric time in the form of the drug Baibidanga or Vidanga [14]. It has been used as an ingredient in most of the Ayurvedic formulation for the treatment of various ailments. Various formulation of *Embelia ribes* are used in ayurvedic system of medicine like asava, aristha, lauha and taila [15]. Commonly it is known as false black pepper. It is listed in red book as

threatened species. In various literatures, it is found that the fruits of that plant used as an anthelmintic, diuretic, carminative, contraceptive, anti-bacterial, anti-inflammatory astringent, antioxidant, anticancer agents and seed possessed antibiotic and antitubercular properties [16]. *Hibiscus rosa-sinensis* belongs to the family Malvaceae. Traditionally the flowers can be used as anti asthmatic agents [17-18]. Many chemical constituents such as cyanidin, quercetin, hentriacontane, calcium oxalate, thiamine, riboflavin, niacin and ascorbic acids have been isolated from this plant. The genus *Hibiscus* (Malvaceae) comprises about 275 species in the tropics and sub-tropics [19]. Flowers of *Hibiscus tiliaceus* L. are widely used for birth control and for treating skin infections [20]. Leaves and flowers of selected Hibiscus species are used in traditional medicine. Information on their antioxidant, antityrosinase and antibacterial activities is meagre [21]. Therefore, an attempt was made to evaluate the anti acne activity of *Hibiscus Rosa-Sinesis* Linn, *Embelia Ribes and Allium Cepa* extracts against *P. acnes.*

Materials and Methods

Plant materials

Leaves of *Hibiscus rosa-sinesis* Linn., seeds of *Embelia ribes* and seeds of *Allium cepa* was collected from local area of Bhopal (M.P.) in the month of January, 2018. Plant material (Leaves and Seeds) selected for the study were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time at room temperature. Then the plant material was shade dried without any contamination for about 3 to 4 weeks. Dried plant material was grinded using electronic grinder. Powdered plant material was observed for their colour, odour, taste and texture.

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals and solvent used in this study were of analytical grade. The pathogenic microbes used in the current study are obtained from Microbial Culture collection, National Centre Forcell Science, Pune, Maharashtra, India.

Extraction

Dried powdered of Leaves of *Hibiscus rosa-sinesis* Linn., seeds of *Embelia ribes* and *Allium cepa* has been extracted with hydroalcoholic solvent using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40^0C and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts [22].

Qualitative phytochemical analysis of plant extract

The *Hibiscus rosa-sinesis* Linn, *Embelia ribes* and *Allium cepa* extracts obtained was subjected to the preliminary phytochemical analysis following standard methods by Kokate and Khandelwal [23-24]. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavanoids, glycosides, saponins, alkaloids, protein and amino acid.

Quantification of secondary metabolites

Quantitative analysis is an important tool for the determination of quantity of phytoconstituents present in plant extracts. For this TPC and TFC are determined. Hydroalcoholic extract obtained from *Hibiscus rosa-sinesis* Linn, *Embelia ribes* and *Allium cepa* plant material of subjected to estimate the presence of TPC and TFC by standard procedure.

Total Phenolic content estimation

The total phenolic content was determined using the method of Olufunmiso *et al* [25]. A volume of 2 ml of extracts or standard was mixed with 1ml of Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 1ml (7.5g/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 UV/visible spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/100mg).

Total flavonoids content estimation

The total flavonoid content was determined using the method of Olufunmiso *et al* [25]. 1 ml of 2% $AlCl_3$ 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).

Formulating anti-acne gel

Measured quantity of methyl paraben, glycerine, polyethylene glycol and hydroalcoholic extract of *Hibiscus rosa-sinesis, Embelia ribes* 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. All the samples were allowed to equilibrate for 24 hours at room temperature prior to performing rheological measurements (Table 1).

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	1.25 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

Comparative evaluation of prepared gels

The psychorheological characteristics were studied for topical gel formulations like colour, clogging, homogeneity and texture etc. Consistency or hardness of gel was measured by Penetrometer. Three containers were filled carefully and completely with formulation, without forming air bubbles and stored at 25±0.5°C for 24 hrs. Test samples were placed on Penetrometer and position of spindle was adjusted as such that, its tip just touches the surface of sample. Penetrating object was released for 5 sec. Depth of penetration was measured. Same was repeated with remaining formulation. Extrudability study was performed by gel formulations were filled into aluminum collapsible tubes. The tubes were pressed by applying weight to extrude the material. Weight was measured which required to extrude the gel from collapsible tubes. An important criterion for gel 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 taken to slip a movable slides from another fixed slide placed in a frame with formulation under the application of a certain load. Lesser the time taken for the separation of two slides, better the spreadability. The experiment was repeated and the average of 6 such determinations was calculated for each gel formulation.

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

Where, S=Spreadability (gcm/sec)

m = weight tied to the upper slide (20 g)

l= length of glass slide (6 cm).

t = time taken is seconds.

pH of gel was determined by digital pH meter. 10 gram of gel was taken and the electrode was then dipped in to gel solution for 30 min until constant reading obtained. And constant reading was noted. The measurements of pH of each formulation were replicated three times. The viscosity of the prepared gel was determined using Brookfield digital viscometer. The viscosity was measured using spindle no. 6 at 10 rpm at ambient room temperature 25-30°C. The sufficient quantity of gel was filled in appropriate wide mouth container. Wide mouth container use to allow spindle of the Viscometer inside of the container. Viscosity value was noted down after stable of reading. Samples of the gel were allowed to settle over 30 min at the constant room temperature before the measurements. The stability of the gels was tested using freeze thaw cycling method. The gels were subjected to a temperature of 4°C for 7 days, 25°C for 7 days and then at 40°C for 7 days. The gels were exposed to the ambient room temperature after each step and noted for synerisis, viscosity, and pH changes [26-29]. 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 for colour development. The absorbance was measured at 420 nm using a spectrophotometer.

***In-vitro* anti acne activity**

The well diffusion method was used to determine the antibacterial activity of the polyherbal gel prepared from the leaves of *Hibiscus rosa-sinesis* Linn., seeds of *Embelia ribes and Allium cepa* using standard procedure [30]. There were 3 concentration used which are 25, 50 and 100 mg/ml for each formulation in antibiogram studies. Its essential feature is the placing of wells with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted overnight broth cultures should never be used as an inoculum. The plates were incubated at 37^{o}C for 24 hr. and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug.

Results and discussion

The colour of the formulations was brown and the intensity of the colour increased with the increase in concentration of the extract in the gel. This might be due to the brown colour of the combined extracts. The crude extracts so obtained after the maceration extraction process, extracts was further concentrated

on water bath for evaporate 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 hydroalcoholic extracts of *Hibiscus rosa-sinesis* Linn, *Embelia ribes and Allium cepa* were obtained 4.5, 6.6, 6.2 w/w respectively. Phytochemical analysis of hydroalcoholic extracts of plants showed the presence of alkaloid, flavonoid, phenols, amino acid, protein, canbohydrate and diterpines while, glycosides and oils and fats were reported to be absent Table 2.

Table 2 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.	Diterpenes	-ve	+ve	+ve
5.	Phenolics	+ve	-ve	-ve
6.	Amino Acids	-ve	+ve	-ve
7.	Carbohydrate	+ve	+ve	+ve
8.	Proteins	-ve	+ve	-ve
9.	Saponins	+ve	+ve	-ve
10.	Oils and fats	-ve	-ve	-ve

The determination of the total phenolic content, expressed as mg gallic acid equivalents and per 100 mg dry weight of sample. TPC of hydroalcoholic extract of *Hibiscus rosa-sinesis* showed the content values of 0.805. The total flavonoid content of the extracts of was expressed as percentage of quercetin equivalent per 100 mg dry weight of sample. The total flavonoids estimation of Hydroalcoholic extract of *Hibiscus rosa-sinesis* Linn, *Embelia ribes and Allium cepa* showed the content values of 0.314, 0.656, 0.605 respectively. Results are provided in (Table 3 and Fig. 1 & 2).

Table 3 Total Phenolic and Total flavanoid content

S. No.	Solvents→ Bioactive compound↓	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

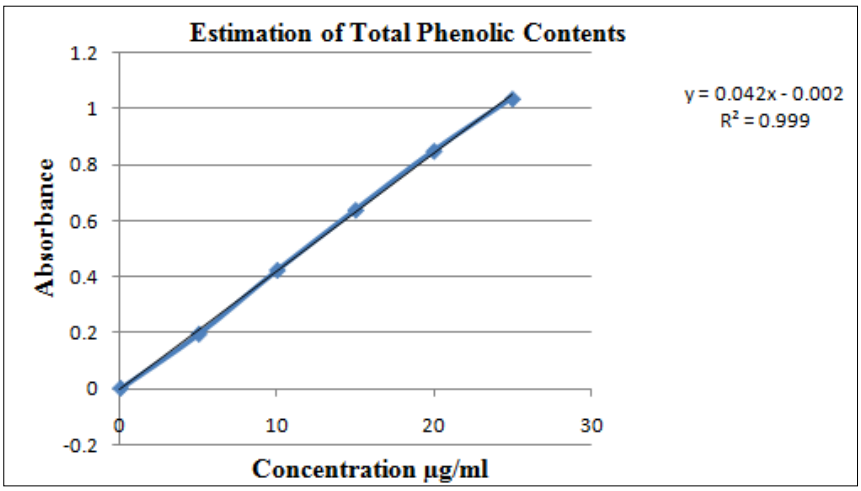

Fig.1Graph of Estimation of Total Phenolic content

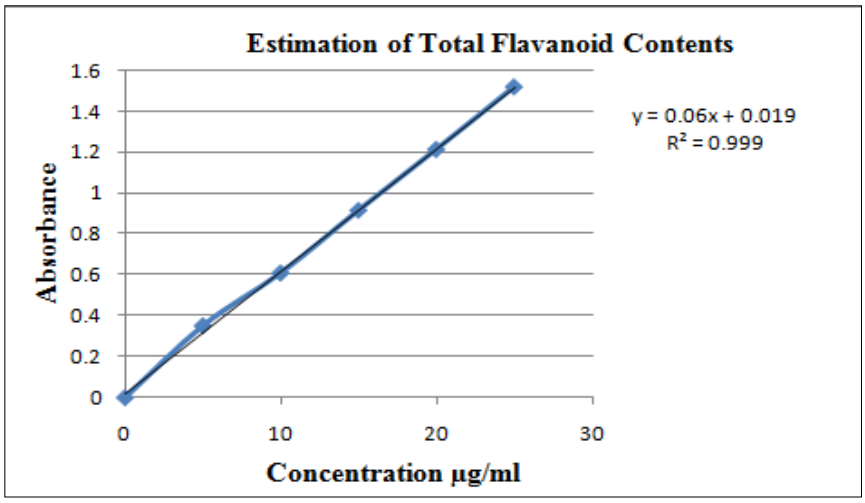

Fig. 2Graph of Estimation of Total flavanoid content

From the psychorheological characteristics studies of formulation showed that all of them have clear colour, No clogging, good homogeneity and smooth texture Table 4**.**

Table 4 Results of Psychorheological 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

The results of washability, extrudability, spreadability, pH, viscosity was given in table 5. In all formulations of gel the spreadibility and viscocity of PHF5 is good was found to be 13.12±0.15 and 3654±25. Extrudability study was performed by gel formulations were filled into aluminium collapsible tubes, the formulation have average extrudability. The skin irritation test performed showed no signs of sensitivity, erythema and edema. So the prepared formulations were considered to be non-irritant. In the all formulation of different gels the percentage of drug content was found maximum in PHF5 Table 6.

Table 5 Results of washability, extrudability, spreadability, pH, Viscosity

Formulation	Washability	Extrudability	Spreadability (gcm/sec)	pH	Viscosity (cps)
PHF1	Good	Average	15.23±0.12	6.82± 0.11	3150±10
PHF2	Good	Average	14.65±0.15	6.95±0.15	3256±15
PHF3	Good	Average	14.15±0.25	7.02±0.11	3365±18
PHF4	Good	Average	13.65±0.35	7.05±0.14	3458±20
PHF5	Good	Average	13.12±0.15	7.00±0.12	3654±25
PHF6	Good	Average	13.25±0.33	7.15±0.13	3562±22

Table 6 Results of flavanoid content using $AlCl_3$ method

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

The efficacy of the anti-acne gels from herbal extracts is shown in Table 7. The anti-acne gels could inhibit the growth of the microorganisms that inhabit acnes and the herbal gel exhibited comparatively less efficacy to standard drug Fig 3.

Table 7 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

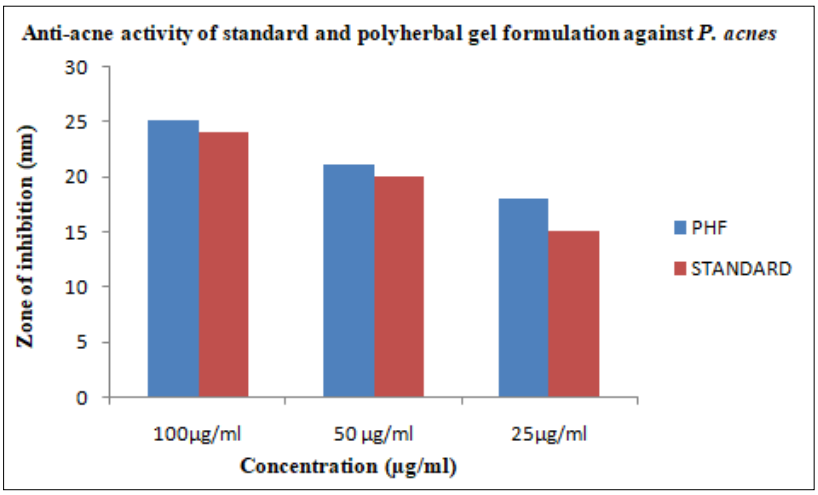

Fig. 3 Anti-acne activity of standard and polyherbal gel formulation against *Propionibacterium acnes*

Conclusion

The present study was aimed to developed polyherbal gel for anti acne treatment using hydroalcoholic extracts of *Hibiscus rosa-sinesis* Linn, *Embelia ribes and Allium cepa* an aqueous based carbopol gel system and evaluated for their physicochemical properties, like pH, spreadability, viscosity and microbial assay. The anti acne activities of the mentioned gel were lower than standard drug, this needs to be fully clarified by further assay methods and using additional concentrations of extracts. Further phytochemical studies are also required to isolate and characterize active ingredients that are responsible for its anti acne activity and to explore the existence of synergism if any, among the compounds.

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