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FORMULATION AND EVALUATION OF TOPICAL POLYHERBAL ANTIACNE GELS CONTAINING *EMBELIA RIBES, ACACIA NILOTICA* AND *CHENOPODIUM ALBUM*

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ABSTRACT

Anti-acne herbal formulations are used for the treatment of acne vulgaris with the added advantage of not producing adverse effects unlike synthetic drugs. Acne is an inflammatory skin disease that occurs due to blockages in polysebase and inflammation that are caused by bacteria. Topical and systemic antibiotics are always used for treatment of acne, but the gradual resistance to antibiotics can affect the success rate of acne cure. 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 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. *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 methanol extracts of plants Seeds of *Embelia ribes*, bark of *Acacia nilotica* and leaves of *Chenopodium album* and evaluated for their physicochemical properties, like pH, washability, extrudability, spreadability and viscosity. The formulations (PHG1-PHG6) 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 Clintop (Marketed gel). This suggests that seeds of *Embelia ribes*, bark of *Acacia nilotica* and leaves of *Chenopodium album* have 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: *Embelia ribes, Acacia nilotica, Chenopodium album, Propionibacterium acnes, Acne vulgaris,* Carbopol, Physicochemical properties.

INTRODUCTION

The herbal drug industry in India is probably the oldest medical care system in the world. The history of herbs in ancient India is so old that the ancient form of herbal healing has even been mentioned in the Vedas, an ancient religious work of the Indians. The ancient herbal healing methods of Ayurveda and Unani deal with the use of herbs and natural products to tackle health conditions. Although herbal medicines would appear to be new for western healers and medical practitioners, the truth is that most prescribed medicines even today contain plant extracts. At present, the countries across the world appreciate this ancient form of medicine and Indian herbal drugs is in good demand resulting in its rapid growth and witnessing almost a thirty percent growth rate annually.[1] A great increase in the worldwide demand for herbal cures, herbal skin care products and even herbal cosmetics were observed in the recent years. Skin, being the most exposed part of our body to the pathogens, requires protection from skin diseases, especially acne causing bacteria. Acnes are found to be the most common skin problem that 85% of the teenagers face today. They may continue to even adulthood and mostly affect the areas with largest oil glands like face and neck. Acnes are generally characterized by the presence of seborrhea, inflammatory lesions, comedone, excessive sebum production and host to bacteria such as *Propionibacterium acnes*, *Staphylococcus epidermidis*, and *Malassezia furfur* in the follicles. So these microorganisms can be targeted for the potential acne treatment. The usage of the long-term antibiotics for the treatment makes the organisms develop resistance to the drugs. This adaptation is multi-factorial and depends upon the organism susceptibility to the treatment and host factors like hormones, stress conditions etc. To overcome this problem, the herbal alternatives for the treatment have been studied. As the herbal extracts cannot be directly used for the treatment,

they were modulated and were formulated as poly herbal anti-acne gel.[2] *Embelia ribes Burm F* a medicinal woody climber belongs to the Myrsinaceae family. It is also commonly known as false black pepper or vidanga. *E. ribes* is one of the 32 medicinal plant species identified by the Medicinal Board, Govt. of India, New Delhi, as being important for large-scale cultivation because of its commercial use.[3] *E. ribes* grows in semi-evergreen and deciduous forests at an altitude of 1,500m found in central and lower Himalayas, Arunachal Pradesh, Assam, Bengal, Orissa, Andhra Pradesh and Madhya Pradesh[4] throughout India. The fruits, leaves and roots are used to cure various diseases Embelin reported as aqueous extract of the fruits showed antibacterial[5] and antifertility[6] activities. It has the anti-bacterial and antiprotozoal properties.[7] Also in abdominal disorders, lung diseases, constipation, indigestion, fungus infections, mouth ulcer, sore throat, pneumonia, heart disease and obesity, antifertility[8], analgesic, anti-inflammatory, antioxidant.[9] The bark of *Acacia nilotica* (Family: Mimosaceae), commonly known as black babool or babbula, has been traditionally used as an astringent, acrid, cooling, styptic, emollient, anthelmintic, constipating, depurative, aphrodisiac, diuretic, expectorant, emetic and nutritive ageat. It is useful in vitiated conditions of *kapha and pitta*, haemorrhages, wounds, ulcers, helminthiasis, ascites, chronic dysentery, diarrhoea, leprosy, leucoderma, skin diseases, burning sensation, cough, bronchitis, leucorrhoea, haemorrhoids, seminal weakness, oral ulcers and odontopathy. The gum is sweet, astringent, cooling, emollient, expectorant, constipating, liver tonic, aphrodisiac, haemostatic, antipyretic and tonic.[10] *Chenopodium album* (L.) of the family *Chenopodiaceae* (Goosefoot family) belongs to the genus *Chenopodium.* It is also known as fat-hen, bathua, vastukah, chakvit. This weedy plant has various medicinal applications. It is a polymorphous, mealy white and erect herb which is 3.5m in height, and found wield in altitude of 4,700m. The herb is a common weed during summer and winter in waste places and in the field of wheat, barley, mustard and gram, and reduces their yield. The tender shoots are eaten raw in salad or with curd; they are also cooked as a vegetable or used as an ingredient in paratha. The dehydrated leaves of bathua can also be incorporated in various conventional food items as it can improve the nutritional quality of the product as well as add variety in the diet.[11] The dried herb is stored for future use. It is also used as fodder; pigeons consume the plant in large quantities.[12] Studies carried out in different parts of the world indicate that *C. album* is a rich source of nutrients, antioxidants and important dietary elements.[13,14] In this study, the gels were formulated using Carbopol with varying concentrations of the herbal extracts and were tested for their anti-acne efficacy and were examined for the antimicrobial activity against the acne causing microorganisms.

MATERIALS AND METHODS

Plant materials

Seeds of *Embelia ribes*, bark of *Acacia nilotica* and leaves of *Chenopodium album* were collected from local area of Bhopal (M.P.) in the month of March, 2019. Plant material selected for the study was washed thoroughly under running tap water and then was 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. 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 seeds of *Embelia ribes*, bark of *Acacia nilotica* and leaves of *Chenopodium album* has been extracted with methanol 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.[15]

Qualitative phytochemical analysis of plant extract

The *Embelia ribes, Acacia nilotica* and *Chenopodium album* extracts obtained was subjected to the preliminary phytochemical analysis following standard methods by Kokate and Khandelwal.[16,17] 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.

Total Phenolic content estimation

The total phenolic content was determined using the method of Olufunmiso *et al.*[18] 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.*[18] 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, polyethylene glycol and methanolic extract of *Embelia ribes, Acacia nilotica* and *Chenopodium album* 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 (%)	PHG1	PHG2	PHG3	PHG4	PHG5	PHG6
Embelia ribes extract	1gm	1gm	1gm	1gm	1gm	1gm
Acacia nilotica extract	1gm	1gm	1gm	1gm	1gm	1gm
Chenopodium album extract	1gm	1gm	1gm	1gm	1gm	1gm
Carbopol 940	0.5	1.0	1.5	2.0	2.5	3.0
Polyethylene Glycol	0.1	0.1	0.1	0.1	0.1	0.1
Methyl Paraben	0.02	0.02	0.02	0.02	0.02	0.02
Triethanolamine	qs	qs	qs	qs	qs	qs
Distilled Water (q.s)	100ml	100ml	100ml	100ml	100ml	100ml

Evaluation of polyherbal gel

Appearance and consistency

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

Washability

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

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.

Determination of spreadability

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

METHOD

Two glass slides of standard dimensions (6×2) were selected. The anti-acne gel formulation whose spreadability had to be determined was placed over one of the slides. The second slide was placed over the slide in such a way that the formulation was sandwiched between them across a length of 6 cms along the slide. 100 grams of weight was placed up on the upper slide so that the 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.

Determination of pH

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

Drug content

The drug content was determined by taking 1gm of gel in 10 ml volumetric flask diluted with methanol. 3 ml of stock solution was mixed with 1 ml of 2% $AlCl_3$. 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.[19-22]

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

Preparation of plates

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

Revival of the bacterial and fungal cultures

The Bacterial cultures used in the study were obtained in lyophilized form. With the help aseptic techniques the lyophilized cultures are inoculated in sterile nutrient broth than incubated for 24 hours at 37°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°C to obtain the pure culture and stored as stocks that are to be used in further research work.

Antibiogram studies

The well diffusion method was used to determine the antibacterial activity of the polyherbal gel prepared from the *Embelia ribes*, *Acacia nilotica* and *Chenopodium album* using standard procedure.[23] There were 3 concentration used which are 25, 50 and 100 mg/ml for antibiogram studies. The plates were incubated at 37°C for 24 hr. and then examined for clear zones of inhibition around the wells with particular concentration of drug.

RESULTS AND DISCUSSION

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. The percentage yield of extract was given in Table 2. Phytochemical analysis of methanolic extracts of plants showed the presence of flavonoid, phenol, alkaloids, carbohydrate and saponins while, protein, glycosides and oils and fats were reported to be absent Table 3. Quantitative phytochemical assay was performed by calculating total phenolic content (TPC) and total flavonoid content (TFC) Table 4. From the psychorheological characteristics studies of formulation showed that all of them have clear colour, No clogging, good homogeneity and smooth texture Table 5**.** The results of washability, extrudability, spreadability, pH, viscosity was given in Table 6. 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 phenol content was found maximum in PHG4 Table 7.

Table 2: % Yield of methanolic extract.

S. No.	Extracts	% Yield (w/w)
1	*Embelia ribes* extract	6.98
2	*Acacia nilotica* extract	5.77
3	*Chenopodium album* extract	7.23

Table 3: Result of phytochemical screening of methanol extracts.

S. No.	Constituents	*Embelia ribes*	*Acacia nilotica*	*Chenopodium album*
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

Table 4: Total phenolic and Total flavonoid content.

S. No.	Solvents→ Bioactive compound↓	Methanolic extracts		
		Embelia ribes	***Acacia nilotica***	***Chenopodium album***
1.	Total Phenol (Gallic acid equivalent (GAE) mg/100mg)	0.657	0.916	0.788
2.	Total flavonoid (Quercetin equivalent (QE) mg/100mg)	0.421	0.213	-

Table 5: Results of Psycho Rheological Characteristics.

Formulation	Colour	Clogging	Homogeneity	Texture
PHG1	Brown	Absent	Good	Smooth
PHG2	Brown	Absent	Good	Smooth
PHG3	Brown	Absent	Good	Smooth
PHG4	Brown	Absent	Good	Smooth
PHG5	Brown	Absent	Good	Smooth
PHG6	Brown	Absent	Good	Smooth

Table 6: Results of washability, extrudability, spreadability, pH, Viscosity.

Formulation	Washability	Extrudability	Spreadability (gcm/sec)	pH	Viscosity (cps)
PHG1	Good	Good	13.25±1.25	6.92± 0.11	2865±12
PHG2	Good	Good	12.25±1.23	6.95±0.15	2750±15
PHG3	Good	Good	11.23±1.45	7.02±0.11	2655±14
PHG4	Good	Good	10.23±2.36	7.02±0.14	2610±10
PHG5	Average	Good	9.85±2.32	7.08±0.12	2545±11
PHG6	Average	Good	9.25±2.10	7.15±0.13	2415±14

Table 7: Results of phenol content using Folin-Ciocalteu method.

Formulation	% Phenol content (equivalent to gallic acid mg/100mg)
PHG1	2.45
PHG2	2.12
PHG3	2.19
PHG4	2.65
PHG5	2.31
PHG6	2.05

The efficacy of the anti-acne gels from polyherbal extracts is shown in Table 8 & Fig 1. The anti-acne gels could inhibit the growth of the microorganisms that inhabit acnes and the polyherbal gel exhibited comparatively more efficacy to Clintop marketed gel.

Table 8: Anti-acne activity of standard and polyherbal gel formulation against *Propionibacterium acnes.*

S. No.	Formulation	Zone of inhibition		
		100mg/ml	50 mg/ml	25mg/ml
1.	Clintop (Marketed gel)	18±0.5	16±0.94	15±0.57
2.	Polyherbal gel	20±0.74	17±0.5	16±0.57

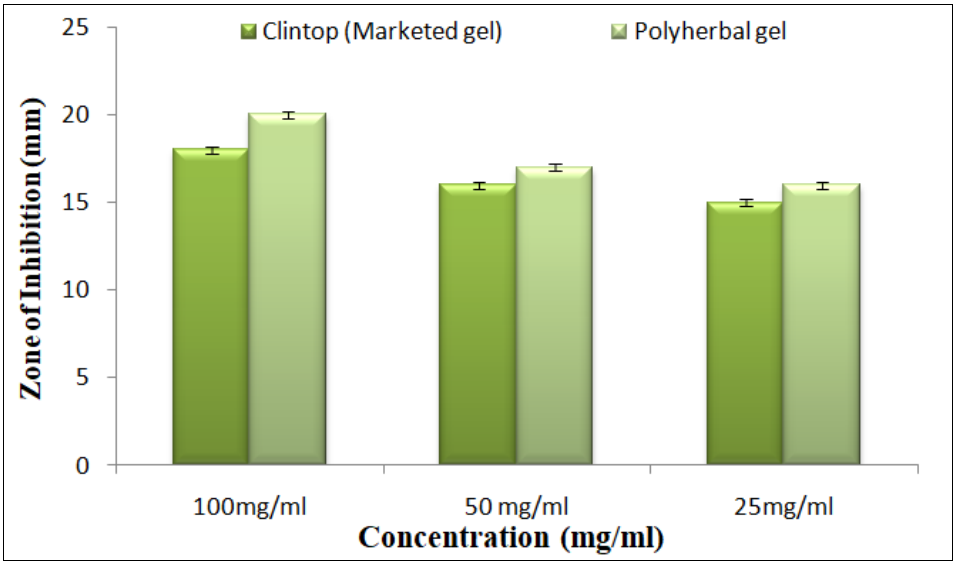

Figure 1: Anti-acne activity of marketed gel and polyherbal gel formulation against *Propionibacterium acnes.*

CONCLUSION

The present study was aimed to developed polyherbal gels for anti acne treatment using methanolic extracts of *Embelia ribes*, *Acacia nilotica* and *Chenopodium album a*n 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 more than marketed gel, 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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