Development and Evaluation of Anti-Acne Gel Containing *Embelia Ribes, Hibiscus Rosa-Allium Cape,* and *Sinesis* Linn against *Propionibacterium Acnes*

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ABSTRACT
Medicinal plants are crucial to the creation of powerful therapeutic substances. As a source of several useful secondary metabolites that act as plant defence mechanisms against predators including microorganisms, insects, and herbivores—compounds that have been shown to have potential for actionplant-based medications make an exceptional addition to contemporary treatments. The microorganism's resistance to antibiotics, or, to put it another way, the short effective life span of any antibiotic, has led to a huge surge in the hunt for antimicrobial plant extracts. A common yet dangerous skin condition, acne affects over 80% of teenagers and young adults between the ages of 11 and 30. In their twenties, 50.9% of women and 42.5% of men still have this illness. Both inflammatory (papules, pustules, and nodules) and non-inflammatory (comedones, open and closed) lesions are characteristics of acne vulgaris, also known as acne, a cutaneous pleomorphic condition of the pilosebaceous unit that involves irregularities in sebum production. Numerous types of acne are caused by the common pus-forming bacteria Propi-onibacterium acnes. The polymer carbopol 940 was used in this study to create anti-acne gels, which were then tested for physicochemical characteristics such as pH, washability, extrudability, spreadability, and viscosity using hydroalcoholic extracts of the plants Hibiscus Rosa-Sinesis Linn, Embelia Ribes, and Allium Cepa. Using the well diffusion method, the anti-acne activity of the formulations (PHF1–PHF6) against Propionibacterium acnes was evaluated. The gels were shown to be stable, non-irritating, and to have anti-acne properties. When tested using a standard, the efficacy was nearly identical to that of Clindamycin. This implies that Allium Cepa, Hibiscus Rosa-Sinesis Linn, and Embelia Ribes have the ability to combat acne-causing bacteria; as a result, they can be utilised in topical anti-acne treatments and may help treat the bacteria's resistance to antibiotics.
Keywords: *Propionibacterium acnes, Hibiscus Rosa-Sinesis* Linn, *Embelia Ribes, Allium Cepa* Carbopol 940, Anti-acne activity, Well diffusion method, Clindamycin

Introduction

Pharmaceutical and personal care companies are still at the forefront of research and development when it comes to medication and cosmetic solutions for skin issues. Since ancient times, herbal remedies have been used to treat a variety of skin conditions, and they are now part of the expanding cosmeceuticals sector [1]. Even though the mechanisms of action of phytoconstituents derived from herbs are more intricate than those of a single bioactive component, it is receiving fresh interest from both a practical and scientific standpoint. Numerous herbal techniques have been shown to be successful in treating a wide range of illnesses and providing primary healthcare, according to historical sources [2]. The most vital and delicate organ in the human body is the skin. Numerous skin conditions, including acne, sunburn, and pigmentation, are brought on by exposure to the external environment [1]. One of the most prevalent multifactorial chronic inflammatory illnesses of the pilosebaceous follicles, acne is caused by bacteria (Propionibacterium acnes), hormonal imbalance, immunological hypersensitivity, and altered follicular keratinisation [3, 4]. Propionic and acetic acids are produced by the anaerobic Gram-positive bacteria P. acnes [5]. By triggering complements and converting sebum triglycerides into fatty acids that irritate the follicular wall and adjacent dermis, these bacteria contribute to the development of inflammatory acne. Additionally, it chemotactically draws neutrophils and generates exoenzymes [6]. Antibiotic resistance, which involves the unique character of bacteria's response to antibiotics, is a result of prolonged antibiotic use [7]. It is enough justification to look for alternate solutions, such as medicinal plants, to address these issues. A versatile food plant, onions (Allium cepa L.) are utilised as traditional Indian spices. It has been used for millennia for its alleged nutritional and health benefits, and it has significant health relevance [8]. Due to their numerous pharmacological effects, onions and plants in the Allium genus the largest and most significant representative genus of the Liliaceae family have long been utilised as herbal remedies for a variety of illnesses [8,9]. Onions' distinctive flavour, scent, and lachrymatory effects are mostly caused by volatile sulfur-containing chemicals called thiosulfinates, which are sometimes blamed for the biological consequences associated with onions [10]. The impacts of phenolic compounds, including flavonoids, which are more stable, have recently drawn attention due to the extreme instability of these volatile products [11]. Flavonols, primarily quercetin and kaempferol, which are found in onions as their glycosides, are well-known for being a good natural source of flavonoids [12].

Numerous studies have documented positive health effects linked to flavonoids in recent years, including their antiallergenic, anti-inflammatory, cardioprotective, vasodilatory, anticarcenogenic, and antioxidant qualities [13]. Embelia ribes, a member of the Myrsinaceae family, is one of the most important herbs that has been utilised from prehistoric times as the medicine Baibidanga or Vidanga [14]. For the treatment of numerous illnesses, it has been a component of the majority of Ayurvedic formulations. Ayurvedic medicine uses Embelia ribes in a variety of forms, including asava, aristha, lauha, and taila [15]. It's commonly referred to as fake black pepper. The Red Book lists it as an Endangered species. The plant's seeds have antibiotic and antitubercular qualities, and its fruits were employed as anthelmintic, diuretic, carminative, contraceptive, antibacterial, anti-inflammatory, astringent, antioxidant, and anticancer agents, according to a number of publications [16]. Hibiscus rosa-sinensis is a member of the Malvaceae family. The flowers have historically been employed as anti-asthmatics [17, 18]. Numerous chemical components have been identified in this plant, including cyanidin, quercetin, hentriacontane, calcium oxalate, thiamine, riboflavin, niacin, and ascorbic acids. There are over 275 species of Hibiscus (Malvaceae) throughout the tropics and subtropics [19]. Hibiscus tiliaceus L. flowers are frequently used to treat skin diseases and as a birth control method [20]. Traditional medicine makes use of the leaves and blooms of certain hibiscus species. There is little information available about their antibacterial, antityrosinase, and antioxidant properties [21]. Thus, an effort was made to assess the anti-acne properties of extracts from Hibiscus Rosa-Sinesis Linn, Embelia Ribes, and Allium Cepa against P. acnes.

Materials and Methods

Plant materials

Hibiscus rosa-sinesis Linn. leaves, Embelia ribes seeds, and Allium cepa seeds were gathered from the Bhopal (M.P.) area.By comparing it to the voucher specimen, expert botanist Dr. Pradeep Tiwari of Doctor Hari Singh Gour of Vishwavidyalaya (M.P.) was able to identify the sample. After being carefully cleaned under running tap water and then rinsed with distilled water, the plant material (leaves and seeds) chosen for the study was left to dry at room temperature for a while. The plant material was then allowed to dry in the shade for three to four weeks without any contamination. An electronic grinder was used to ground the dried plant material. The colour, taste,

texture, and odour of the powdered plant material were evaluated. For phytochemical and biological research, dried plant material was maintained in an airtight container.

Chemical reagents

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) provided all of the chemicals employed in this investigation.Every chemical and solvent utilised in this investigation was of analytical quality. The pathogenic microorganisms utilised in this investigation were acquired from the National Centre for Forcell Science's Microbial Culture Collection in Pune, Maharashtra, India.

Extraction

After being extracted with a hydroalcoholic solvent through a 48-hour maceration process, dried powdered Hibiscus rosa-sinesis Linn. leaves, Embelia ribes seeds, and Allium cepa seeds were filtered and dried at 400 degrees Celsius using a vacuum evaporator. They were then kept in an airtight container free from contamination until they were used. Lastly, the dried extracts' yield percentages were computed [22].

Qualitative phytochemical analysis of plant extract

Kokate and Khandelwal conducted a preliminary phytochemical analysis using normal procedures on the extracted Hibiscus rosa-sinesis Linn, Embelia ribes, and Allium cepa [23, 24]. A variety of active principles, including phenolic chemicals, carbohydrates, flavanoids, glycosides, saponins, alkaloids, fats or fixed oils, protein, amino acids, and tannins, were screened for in the extract.

Quantification of secondary metabolites

One crucial method for figuring out how much phytoconstituent is in plant extracts is quantitative analysis. TPC and TFC are established for this. Using a conventional process, a hydroalcoholic extract from plant material of Hibiscus rosa-sinesis Linn, Embelia ribes, and Allium cepa was used to determine the presence of TPC and TFC.

Total Phenolic content estimation

The Olufunmiso et al. technique was used to calculate the total phenolic content [25]. One millilitre of Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and one millilitre (7.5g/l) of sodium carbonate were combined with two millilitres of extracts or standard. To develop the colour, the mixture was vortexed for 15 seconds and then left to stand at 40°C for 15

minutes. The UV/visible spectrophotometer was used to measure the absorbance at 765 nm. Gallic acid equivalent (mg/g) was used to convey the findings of the calculation of the total phenolic content using the standard graph of gallic acid.

Total flavonoids content estimation

The Olufunmiso et al. technique was used to calculate the total flavonoid content [25]. A UV/visible spectrophotometer was used to measure the absorbance of the reaction mixture at 420 nm after 1 ml of a 2% AlCl3 methanolic solution was added to 1 ml of extracts or standard and left to stand for 60 minutes at room temperature. Using the standard quercetin graph, the flavonoid content was determined and the results were reported as quercetin equivalent (mg/g).

Formulating anti-acne gel

About 35 millilitres of water were used to dissolve measured amounts of methyl paraben, glycerine, polyethylene glycol, and hydroalcoholic extracts of Hibiscus rosa-sinesis, Embelia ribes, and Allium cepa. The mixture was then vigorously agitated using a mechanical stirrer (or sonicator). Then, while stirring, carbopol 940 was gradually added to the beaker containing the liquid above. Triethanolamine solution was added gradually while being constantly stirred to neutralise the mixture and create a gel. Before conducting rheological tests, all of the samples were given a full day to acclimatise to room temperature (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

Topical gel formulations' psychorheological properties, such as colour, clogging, homogeneity, and texture, were examined. A penetrometer was used to measure the gel's consistency or hardness. The formulation was carefully and thoroughly put into three containers, preventing air bubbles,

and they were then kept at 25±0.50C for a full day. Test samples were set up on the penetrometer, and the spindle's position was changed so that the tip of the spindle barely touched the sample's surface. For five seconds, the penetrating item was released. Penetration depth was measured. The leftover formulation was used in the same way. Gel compositions were put into aluminium collapsable tubes to conduct an extrudability research. To extrude the material, weight was applied to press the tubes. The weight needed to extrude the gel from collapsable tubes was measured. The capacity to spread easily is a crucial requirement for gel. The term "spreadability" refers to the size of the region that the gel easily covers when applied to the skin. The spreading value of a formulation also affects its medicinal efficacy. A specialised tool has been created to investigate the formulations' spreadability. The time it takes to slip a movable slide from a stationary slide that is positioned in a frame with formulation when a specific load is applied is the measure of spreadability. Better spreadability results from separating two slides in less time. For each gel formulation, the average of six such measurements was computed after the experiment was repeated.

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

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.

A digital pH meter was used to measure the gel's pH. The electrode was dipped in ten grammes of gel solution for thirty minutes until a steady reading was achieved. Additionally, continuous reading was observed. Three duplicates of each formulation's pH measurements were made. The Brookfield digital viscometer was used to measure the created gel's viscosity. Spindle number six was used to determine the viscosity at 10 rpm and room temperature (between 25 and 300C). A suitable wide-mouth container was filled with an adequate amount of gel. A container with a wide mouth is used to accommodate the viscometer's spindle inside. After the measurement stabilised, the viscosity value was recorded.

Prior to the measurements, gel samples were let to settle for 30 minutes at room temperature. The freeze-thaw cycling method was used to test the gels' stability. The gels were heated to 4°C for seven days, 25°C for seven days, and 40°C for seven days. Following each stage, the gels were allowed to come to room temperature and observations were made regarding pH, viscosity, and synerisis [26–29]. By putting 1g of gel into a 10 ml volumetric flask that had been diluted with methanol, the drug content was ascertained. One millilitre of 2% AlCl3 was combined with three millilitres of stock solution. For colour development, the mixture was vortexed for 15 seconds and then left to stand at 40°C for 30 minutes. The absorbance was measured at 420 nm using a spectrophotometer.

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

Preparation of plates

Following sterilisation, the flask's nutritional agar was promptly transferred into sterile Petri plates on a flat surface (20 ml/plate). To verify the sterility of the plates, they were incubated at 37°C for an entire night after being allowed to harden at room temperature. Before being used, the plates were dried for 30 minutes at 50 degrees Celsius.

Revival of the bacterial and fungal cultures

The study's bacterial cultures were acquired in lyophilised form. The lyophilised cultures are inoculated in sterile nutrient broth using aseptic procedures, and they are then incubated for 24 hours at 37°C. Following incubation, the growth manifests as turbidity. In order to get a pure culture, these broth cultures were further inoculated onto agar plates with loop full of bacteria. They were then incubated for the following 24 hours at 37°C and preserved as stocks for use in future study.

Antibiogram Studies

Using conventional methods, the antibacterial activity of the herbal gel made from Hibiscus rosa-sinesis Linn. leaves, Embelia ribes seeds, and Allium cepa seeds was assessed using the well diffusion method [30].In antibiogram studies, three concentrations 25, 50, and 100 mg/ml were employed for each formulation. Its key component is the placement of antibiotic-containing wells on the agar surfaces as soon as the organism being tested is inoculated. It is never advisable to utilise undiluted overnight broth cultures as inoculums. After 24 hours of incubation at 37°C, the

plates were checked for distinct zones of inhibition surrounding the wells that had been impregnated with a specific drug concentration.

Results and discussion

The formulations were brown in colour, and as the extract content in the gel rose, so did the color's intensity. The brown hue of the mixed extracts may be the cause of this. Following the maceration extraction method, the crude extracts were concentrated further on a water bath to completely evaporate the solvents and determine the extraction yield. In phytochemical extraction, determining the extraction yield as a percentage is a crucial phenomenon for assessing the standard extraction efficiency for a given plant, different plant sections, or other solvents. Phytochemical analysis of hydroalcoholic plant extracts revealed the presence of alkaloids, flavonoids, phenols, amino acids, proteins, carbohydrates, and diterpines, while glycosides, oils, and fats were reported to be absent (Table 2). The percentage yield of hydroalcoholic extracts of Hibiscus rosa-sinesis Linn, Embelia ribes, and Allium cepa was 4.4, 6.5, and 5.2 w/w, respectively.

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

calculating the total phenolic content and expressing it as milligrammes of gallic acid equivalents per 100 milligrammes of sample dry weight. Hibiscus rosa-sinesis hydroalcoholic extract's TPC revealed content values of 0.702. Quercetin equivalent per 100 mg dry weight of sample was used to express the extracts' total flavonoid concentration. The hydroalcoholic extracts of Hibiscus rosa-sinesis Linn, Embelia ribes, and Allium cepa were estimated to contain 0.299, 0.546, and 0.623 total flavonoids, respectively. The findings are shown in Figs. 1 and 2 and Table 3.

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)	0.702	-	-
2.	Total flavanoid (Quercetin equivalent (QE) mg/100mg)	0.299	0.546	0.623

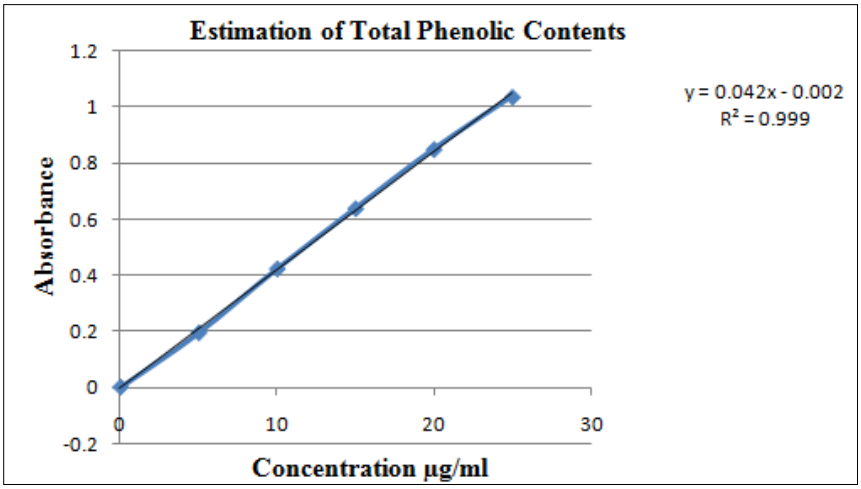

Fig.1Graph of Estimation of Total Phenolic content

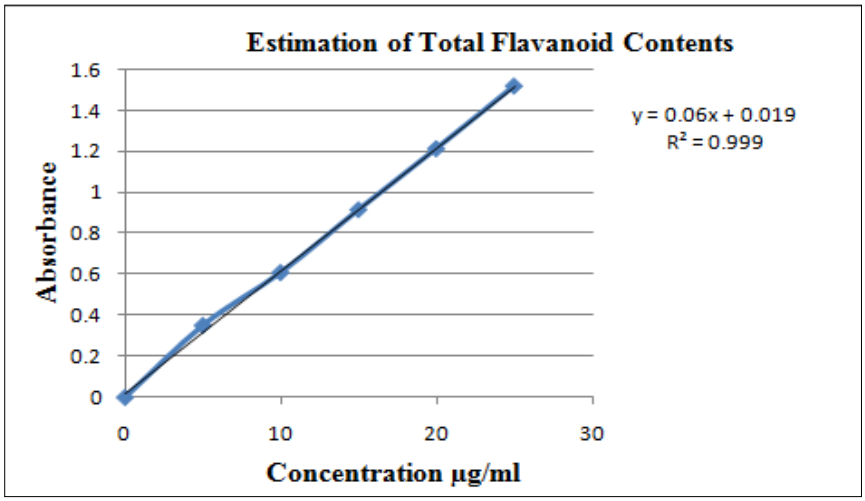

Fig. 2Graph of Estimation of Total flavanoids content

According to formulation psychorheological investigations, each one has a smooth texture, good homogeneity, no clogging, and a clear colour (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

Table 5 displays the findings for washability, extrudability, spreadability, pH, and viscosity. The spreadibility and viscocity of PHF5 were reported to be 13.12±0.15 and 3654±25 in all gel formulations. Gel formulations with medium extrudability were used in an extrudability trial that involved filling them into aluminium collapsable tubes. There were no indications of sensitivity, erythema, or oedema in the skin irritation test. As a result, the created formulations were deemed non-irritating. The highest proportion of medication content was found in PHF5 Table 6 across all gel formulations.

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

Table 7 displays the effectiveness of the anti-acne gels made from herbal extracts. The gels were shown to be less effective than standard medications in Fig. 3 and to be able to stop the growth of the microorganisms that cause acne.

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

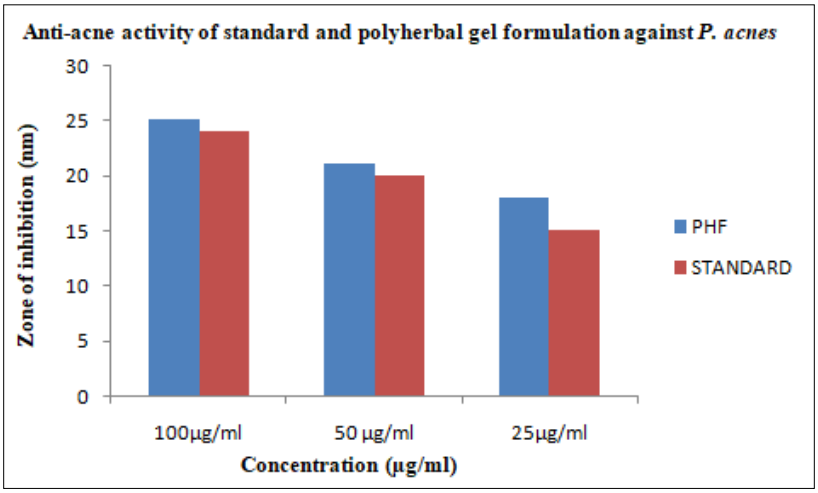

Fig. 3 Anti-acne activity of standard and polyherbal gel formulation against *Propionibacterium acnes*

Conclusion

Using hydroalcoholic extracts of Hibiscus rosa-sinesis Linn, Embelia ribes, and Allium cepa in an aqueous-based carbopol gel system, the current study sought to create herbal gels for the treatment of acne. These gels were then assessed for their physicochemical characteristics, including pH, spreadability, viscosity, and microbial assay. Further test techniques and the use of higher extract concentrations are required to completely clarify the fact that the anti-acne properties of the aforementioned gel were less than those of a typical medication. To identify and describe the active

components that give it its anti-acne properties and to determine whether there is any synergism between the compounds, more phytochemical research is also necessary.

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