

DEVELOPMENT AND CHARACTERIZATION OF POLYHERBAL ANTIMICROBIAL GEL FOR ACNE TREATMENT

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ABSTRACT

In this study development and characterization of polyherbal gel of leaves of *Garcinia mangostana*, *Mesua ferrea* and Peel of *Punica granatum* L. Acne vulgaris is collective disorder of the skin affects approximately 80% adolescents during their puberty stage. Medicinal plants are used all over the world to treat various diseases due to its variety of phytochemical constituents. Ideally, topical therapy is the first line treatment for many skin diseases. Among the various topical formulations, gels have been considered as a potential vehicle due to its non-sticky nature, stable and greater aesthetic value. The hydroalcoholic extract of *Mesua ferrea* had revealed the presence of alkaloids, flavonoids, glycosides and phenol. Phytochemical screening of peel extract of *Punica granatum* L showed the present of glycosides, flavonoids, diterpenes, phenol carbohydrate and saponins. The formulation PHG5 has good viscosity. In the above formulation of different gels the percentage of drug content was found that PHG5 has maximum percentage of drug content. Acne is the most common disorder treated by dermatologists. The present study was conducted to investigate the *in vitro* anti-acne activity of three selected medicinal plant extracts. Activity of *Garcinia mangostana*, *Mesua ferrea* and *Punica granatum* L. was further evaluated so that they can be developed into formulations and used commercially for treatment of acne.

Keywords: *Garcinia mangostana*, *Mesua ferrea*, *Punica granatum*, Polyherbal Antimicrobial gel, Acne treatment.

INTRODUCTION

Ayurveda is one of the traditional medicinal systems with an established history of many centuries. Furthermore, known as Ayurvedic Medicine, this ancient Vedic knowledge is considered to be one of the oldest healing sciences and has survived until the present generation over many centuries of tradition. Originated in India thousands of years ago, Ayurveda is known as the "Mother of All Healing"¹. Etymologically speaking, it is the combination of the Sanskrit words ayur (life) and veda (science or knowledge), which means "the science of life," focusing on bringing harmony and balance in all areas of life including mind, body and spirit ².

In Ayurveda, Panchamahabhutas or the five elements: Vayu (air), Teja (fire), Aap (water), Prithvi (earth) and Akasha (aether) are believed to build up the living microcosm (human beings) and the macrocosm (external universe). When combined in pairs, the Panchamahabhutas form Tridosha or the three humors namely Vata (responsible for body movement), Pitta (responsible for bodily chemical reactions such as metabolism and temperature) and Kapha (responsible for growth, protection, lubrication and sustenance). All these present the constitution or Prakriti of an individual, which determines the physical as well as mental characteristic of human. The concept is that health is achieved when there is a balance between these three fundamental doshas, whereas imbalance causes diseases. Based on these Panchamahabhutas and Tridosha, the Prakriti of an individual is determined and a distinctive treatment plan can be prescribed according to their unique constitution ³.

The philosophy behind Ayurveda is preventing unnecessary suffering and living a long healthy life. Unlike the allopathic medicines which uses mainly synthetic chemicals designed for specific target receptors and primarily give symptomatic relief, Ayurveda involves the use of natural means such as diet, herbs, spices, minerals, exercise, meditation, yoga, mental hygiene, sounds, smells and mechano-procedures to eliminate the root cause of the disease by restoring balance, at the same time create a healthy life-style to prevent the reoccurrence of imbalance. Ayurveda is said to be holistic as it aims to integrate and balance body, mind and spirit to prevent illness and promote wellness, longevity, vitality and happiness.

In terms of literature, the fourth Veda written during Indian Civilization, Atharvaveda serves as the earliest authentic text discussing on the nature of existence, health and disease, pathogenesis and principles of treatment. Here in Atharvaveda, the healing verses of Ayurveda can be primarily found, in which more than a hundred hymns were mentioned as the cures for diseases, including fever, leprosy, consumption, heart diseases, wounds, headaches, parasites, eye and ear diseases, poisoning, rheumatism and epilepsy. The uniqueness of this ancient medical system lies behind the vast variety of healing method used: Charms, plant and animal juices, natural forces (sun and water) as well as human contrivances ⁴. The eight branches of treatment, Ashtanga was mentioned here as well: Kaya Chikitsa (Internal medicine), Shalya Tantra (Surgery), Shalakya Tantra (Ear, nose, throat and eye diseases), Kaumarbhritya (Pediatrics), Agada Tantra (Toxicology), Bhuta Vidya (Psychiatry), Rasayana (Rejuvenation therapy) and Vajeeekarana (Aphrodisiac therapy).

From the knowledge in Atharva-veda, early texts of Ayurveda such as Chakara Samhita and Sushruta Samhita were developed. Although the former focuses on the causes of diseases and the constitution of a person, the later emphasizes on Ayurvedic surgery and the details of its techniques ⁵.

In Chakara Samhita, it was stated that the Ayurvedic teaching is transferred by Indra to Bhardwaj, who in turn taught this to Atreya⁷. The disciples of Atreya wrote their own samhitas, with Agnivesha Samhita being the one well-accepted. It is then revised, edited and supplemented by Chakara about 800 years later. On the other hand, Sushruta Samhita mentioned the transfer of knowledge from Indra to Dhanvantari, along with Bhardwaj. The disciples in this school such as Sushruta wrote Sushruta samhita, compiling Dhanvantari's teaching and his additional findings⁸.

The history of Ayurveda can be traced back to the period between the pre-vedic periods (4000 B. C.-1500 B. C.). According to Ayurvedavatarana (the descent of Ayurveda), Lord Brahma, the Hindu God of Creation passed on his "knowledge of life" to Daksha Prajapati and Ashwins, subsequently to Indra. This knowledge is then transferred to different rishis (sages), in which these disciples of Ayurveda wrote different treatises based on their interpretations. Here, both Bhardwaj and Dhanvantari received the knowledge from Indra. They later developed school of medicine and school of surgery respectively⁶.

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. Various synthetic antibiotic drugs such as clindamycin, doxycycline, minocycline are used to overcome this problem. Most of the time topical therapy is the first-line treatment with benzyl peroxide and retinoids. 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 compliance.

Acne vulgaris is a chronic inflammatory disorder of the skin which affects approximately 80% adolescent during puberty stage. The increasing frequency of intake of antibiotics to overcome this problem explores several side effects. Therefore it needs to focus on the herbal formulation as a topical first-line treatment. In this study development and characterization of polyherbal gel of leaves of *Garcinia mangostana*, *Mesua ferrea* and Peel of *Punica granatum* L.

MATERIAL AND METHODS

Collection of plant material

Every parts of the plant like bark, leaves, flowers, roots, fruits and seeds may contain active secondary metabolites. Fresh & healthy plant materials, free from diseases of *Garcinia mangostana*, *Mesua ferrea* and *Punica granatum* L were collected from ruler area of Bhopal (M.P.) in the month of January, 2022.

Extraction procedure

Extraction is an essential step in phytochemical processing for the finding of bioactive secondary metabolite from plant materials. For the standardization of herbal products, selection of a suitable extraction technique is also important. Extraction is used in the removal of desirable soluble constituents, exclusion those not required with the help of the selected solvents. The collected plant materials were thoroughly washed in tap water and rinsed in distilled water. The cleaned, healthy collected plant samples were cut into small pieces and dried under shade for 3 to 4 weeks. Following procedure will be adopted for the preparation of extract from the shade dried material.

Defatting of plant material

44 gram of *Garcinia mangostana*, 40 gram of *Mesua ferrea* and 50 gram of *Punica granatum* L shade dried plant material were coarsely powdered and subjected to extraction with petroleum ether (60-80°C) in a maceration method. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

Defatted plant materials of *Garcinia mangostana*, *Mesua ferrea* and *Punica granatum* L were exhaustively extracted with hydroalcoholic solvent (ethanol: aqueous: 70:30v/v) by maceration method. The extract was evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extracts⁹⁻¹¹.

Determination of percentage yield

The extraction yield is evaluate of the solvent's efficiency to extracts bioactive components from the selected natural plant samples and it was defined as quantity of plant extracts recovered in mass after solvent extraction compared with the initial quantity of plant samples. After extraction, yield of the plant extracts obtained were calculated in grams and then converted it into percentage. Following formula was adopted for determination of percentage yield of selected plant materials. The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage Yield} = \frac{\text{Weight of Extract}}{\text{Weight of Powder drug taken}} \times 100$$

Phytochemical screening

Medicinal plants are resources of traditional medicines and many of the modern medicines are produced indirectly from plants. Phytochemical constituents are of two type primary bioactive constituents (chlorophyll, proteins, amino acids, sugar etc.) and secondary bioactive constituents include (alkaloids,

terpenoids, phenols, flavonoids etc.). Phytochemical examinations were carried out for all the extracts as per the standard methods.

1. Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

b) Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

c) Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

d) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

c) Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

b) Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

4. Detection of saponins

a) Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

b) Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. Detection of phenols

a) Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of flavonoids

a) Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

b) Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

7. Detection of proteins

a) Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

8. Detection of diterpenes

a) Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes¹²⁻¹³.

Quantitative estimation of bioactive compounds

Total phenolic content estimation

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer¹⁴.

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 10-50µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of 2% AlCl₃ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm¹⁵.

Formulation development of polyherbal gel

Method of preparation

Measured quantity of methyl paraben, glycerin, polyethylene glycol and hydroalcoholic extract of *Garcinia mangostana*, *Mesua ferrea* and *Punica granatum* L 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 ¹⁶⁻¹⁷.

Carbopol 940 – Gelling Polymer

Triethanolamine- gelling agent, pH Adjusting agent, Neutralizer

Methyl Paraben - Preservative

Distilled Water, Glycerin and Polyethylene Glycol-solvent

Table 1: Formulation of polyherbal gel

Ingredients	PHG1	PHG 2	PHG3	PHG4	PHG5	PHG6
<i>Garcinia mangostana</i> extract	1gm	1gm	1gm	1gm	1gm	1gm
<i>Mesua ferrea</i> extract	1gm	1gm	1gm	1gm	1gm	1gm
<i>Punica granatum</i> L 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

Evaluation of polyherbal Gel¹⁸⁻¹⁹

A. Appearance and consistency:

The physical appearance was visually checked for the texture of polyherbal gel formulations and observations may be like stated in Table 7.

B. Washability

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 7.

C. 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.

D. 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 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 in 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 (Phenol 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 Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

In vitro anti-acne and antimicrobial activity of polyherbal gel

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°C for 24hours. 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 *Garcinia mangostana*, *Mesua ferrea* and peel of *Punica granatum* L 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°C for 24 hr. and then examined for clear zones of inhibition around the wells with particular concentration of drug.

RESULTS AND DISCUSSION

Acne vulgaris is collective disorder of the skin affects approximately 80% adolescents during their puberty stage. Medicinal plants are used all over the world to treat various diseases due to its variety of phytochemical constituents. Ideally, topical therapy is the first line treatment for many skin diseases. Among the various topical formulations, gels have been considered as a potential vehicle due to its non-sticky nature, stable and greater aesthetic value. From the table No. 2-4, it could see that, flavonoids, saponins, alkaloids, glycosides, carbohydrate and phenol were present in hydroalcoholic extract of *Garcinia mangostana*. The phytochemical screening of leaves of *Garcinia mangostana* revealed negative results for proteins and diterpenes. The phytochemical tests revealed various bioactive secondary metabolites which might be responsible for their medicinal attributes. The hydroalcoholic extract of *Mesua ferrea* had revealed the presence of alkaloids, flavonoids, glycosides and phenol. Phytochemical screening of peel extract of *Punica granatum* L showed the present of glycosides, flavonoids, diterpenes, phenol carbohydrate and saponins.

In the above formulations of gels, it has been noted that all of them has clear colour, no clogging, good homogeneity and smooth texture. In the above formulations of gels, they have good washability as well as extrudability. In all above formulations of gel the spreadibility of PHG5 is good. The above formulation of topical gels has different pH value for different formulation. In the above formulations the viscosity of different sample of gel were determined and found that there is increase in viscosity. The formulation PHG5 has good viscosity. In the above formulation of different gels the percentage of drug content was found that PHG5 has maximum percentage of drug content. Acne is the most common disorder treated by dermatologists. As many as 80-90% of all adolescents have some type of acne and 30% of them require medical treatment. It is an inflammatory disease of the pilosebaceous unit characterized by the formation of open and closed comedones, papules, pustules, nodules, and cysts. The present study was conducted to investigate the *in vitro* anti-acne activity of three selected medicinal plant extracts. Activity of *Garcinia mangostana*, *Mesua ferrea* and *Punica granatum* L. was further evaluated so that they can be developed into formulations and used commercially for treatment of acne.

Table 2: Results of percentage yield of extract of *Garcinia mangostana*, *Mesua ferrea* and *Punica granatum* L

Hydroalcoholic extracts	Percentage yield (w/w)
<i>Garcinia mangostana</i>	6.68%
<i>Mesua ferrea</i>	5.54%
<i>Punica granatum</i> L	8.41%

Table 3: Result of Phytochemical screening of extract of *Garcinia mangostana*

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids	
	A) Wagner's Test:	-Ve
	B) Hager's Test:	+Ve
2.	Glycosides	
	A) Legal's Test:	-Ve
3.	Flavonoids	
	A) Lead acetate Test:	+Ve
	B) Alkaline Reagent Test:	+Ve
4.	Saponins	
	A) Froth Test:	+Ve
5.	Phenolics	
	A) Ferric Chloride Test:	+Ve
6.	Proteins	
	A) Xanthoproteic Test:	-Ve
7.	Carbohydrate	
	A) Fehling's Test:	+Ve
8.	Diterpenes	
	A) Copper acetate Test:	-Ve

Table 4: Result of Phytochemical screening of extract of *Mesua ferrea*

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids	
	A) Wagner's Test:	-Ve
	B) Hager's Test:	+Ve
2.	Glycosides	
	A) Legal's Test:	-Ve
3.	Flavonoids	
	A) Lead acetate Test:	+Ve
	B) Alkaline Reagent Test:	-Ve
4.	Saponins	
	A) Froth Test:	-Ve
5.	Phenolics	
	A) Ferric Chloride Test:	+Ve
6.	Proteins	
	A) Xanthoproteic Test:	-Ve
7.	Carbohydrate	
	A) Fehling's Test:	-Ve
8.	Diterpenes	
	A) Copper acetate Test:	-Ve

Table 5: Result of Phytochemical screening of extract of *Punica granatum L*

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids	
	A) Wagner's Test:	-Ve
	B) Hager's Test:	-Ve
2.	Glycosides	
	A) Legal's Test:	+Ve
3.	Flavonoids	
	A) Lead acetate Test:	-Ve
	B) Alkaline Reagent Test:	+Ve

4.	Saponins	
	A) Froth Test:	+Ve
5.	Phenolics	
	A) Ferric Chloride Test:	+Ve
6.	Proteins	
	A) Xanthoproteic Test:	+Ve
7.	Carbohydrate	
	A) Fehling's Test:	+Ve
8.	Diterpenes	
	A) Copper acetate Test:	-Ve

Table 6: Estimation of total phenolic and flavonoids content of *Garcinia mangostana*, *Mesua ferrea* and *Punica granatum* L

S. No.	Hydroalcoholic extract	Total phenol content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1.	<i>Garcinia mangostana</i>	0.568	0.414
2.	<i>Mesua ferrea</i>	0.741	0.548
3.	<i>Punica granatum</i> L	0.596	0.352

Table 7: Results of evaluation of gel formulation

Formulation	Spreadability (gcm/sec)	pH	Viscosity (cps)	Phenol content (mg/100mg)
PHG1	21.20±0.52	6.72± 0.11	2456±32	0.563
PHG2	20.18±0.14	6.85±0.15	3265±31	0.745
PHG3	16.67±0.47	7.02±0.11	4125±24	0.713
PHG4	14.52±0.35	7.00±0.14	3021±32	0.654
PHG5	15.36±0.47	7.10±0.12	2985±18	0.869
PHG6	12.74±0.63	7.25±0.13	3125±20	0.745

*Mean±S.D., Average of three determinations

Table 8: Antimicrobial activity of standard drug against selected microbes

S. No.	Standard	Microbes	Zone of inhibition (mm)		
			25mg/ml	50 mg/ml	100mg/ml
1.	Ciprofloxacin	<i>Klebsiella pneumoniae</i>	11±0.86	13±0.94	17±0.47
		<i>Bacillus subtilis</i>	12±0.57	15±0.5	18±0.47

Table 9: Antimicrobial activity of polyherbal gel formulation (PHG5) against selected microbes

S. No.	Microbes	Zone of inhibition (mm)		
		25mg/ml	50 mg/ml	100mg/ml
1.	<i>Klebsiella pneumoniae</i>	6±0	7±0.94	8±0.86
2.	<i>Bacillus subtilis</i>	8±0.47	10±0.5	14±0.57

Table 10: Anti-acne activity of marketed formulation and polyherbal gel (PHG5) against *Propionibacterium acnes*

S. No.	Formulation	Zone of inhibition (mm)		
		25µg/ml	50 µg/ml	100µg/ml
1.	Clintop gel	11±0.86	13±0.94	17±0.47
2.	Polyherbal gel	7±0.86	10±0.57	18±0

CONCLUSION

Herbal plants such as *Garcinia mangostana*, *Mesua ferrea* and *Punica granatum* L are selected and incorporated into a 1.25%w/w carbopol gel base as a non-oily (aqueous) topical polyherbal formulation. The antibacterial study results of the developed polyherbal topical gel showed the antibacterial activity against *Klebsiella pneumonia*, *Bacillus subtilis* and *Propionibacterium acnes*. The study results conclude that the formulated polyherbal gel with methanolic extract of *Garcinia mangostana*, *Mesua ferrea* and *Punica granatum* L can be used for the management of acne vulgaris.

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