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Polyherbal anti acne gel containing extracts of *Mangifera indica* and *Syzygium cumini* seeds: bioassay guided activity against *Propionibacterium acne*

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ABSTRACT

Mangifera indica and *Sygygium cumini* seeds are rich sources of secondary metabolites and a sustainable cost effective biomaterial. An effective herbal therapy for topical affliction, Acne using such material will prove to be a good alternative to costly and clinically significant antibiotics. The aim of the study was to develop topical polyherbal gel from *Mangifera indica* and *Syzygium cumini* for the treatment of *acne vulgaris*. Plant materials were extracted in methanol and fractionated in different polarity solvents. These fraction were evaluated for antibacterial activity against *Propionibacterium acne* (*P. acne*) in-vitro. The most effective fraction was formulated as gel. The formulated gel was evaluated for physico chemical parameters, irritancy, antiacne activity and stability. Methanolic extract was the most active in-vitro against *Propionibacterium acne* (MTCC 1951). The fabricated gel showed non-stickiness and no irritancy. It showed comparable efficacy as that of clindamycin gel as shown by histopathological study. It also showed the comparable antiacne activity as that of clindamycin gel shown in acne induced wistar albino rats. The formulated gel was stable over a period of 6 months at accelerated stability conditions. It was concluded from the study that methanolic extract of *Mangifera indica* and *Syzygium cumini* seeds can be formulated in an aqueous based gel system for topical therapy of mild *Acne vulgaris* to obtained similar efficacy as that of antibacterial gel (clindamycin) taken as reference standard without its side effects.

Keywords: *Syzygiumcumini, Mangiferaindica, antibacterial, anticancer, radiprotective.*

1. INTRODUCTION

World Health Organization defines traditional herbal medicinal products as home-grown medicinal products or plant-derived substances. These pharmaceuticals at first appeared as unrefined medications, for example, tinctures, teas, poultices, powders and other home grown details [1].

Acne is a skin condition that occurs when hair follicles become plugged with oil and dead skin cells. It often causes whiteheads, blackheads or pimples, and usually appears on the face, forehead, chest, upper back and shoulders due to inflamed or injected sebaceous gland & prevalent chiefly among teenagers, though it affects people of all ages [2]**.** *Propionibacterium acne*is the chief causative agent of Acne. It is a Gram-positive, facultative, anaerobic rod that is a major colonizer & inhabitant of the human skin along with *Staphylococcus, Corynebacterium, Streptococcus, Pseudomonas* [3].

Mango (*Mangifera indica* L.) is a juicy fruit which belongs to the family of Anacardiaceae. It is grown in many parts of the world, especially in tropical countries. Mango's seed contains Polyphenols such as gallic acid, tannin, xanthone, quercetin, gallic acid, caffeic acid, catechins, kaempferol, tannins, and also the unique mangoxanthonoid, mangiferin. Various parts of plant are used as a dentrifice, antiseptic, astringent, diaphoretic, stomachic. The seeds are used in asthma, anti-oxidant, anti-inflammatory, antibacterial [4].

Syzygium cumini plant, generally bearing small black-purple drupe is indigenous to Indian subcontinent and has diverse activities. It is also known asJambulandkalaJamun in India. It belongs to the family Myrtaceae is identified as antidiabetic, anti-inflammatory, anti-pyretic. It seeds contains *Syzygiumcumini*has traditionally been used for its rich nutrition and medicinal value [5].

The objective of the study was to evaluate the anti-propionibacterium potential of the extracts from *Mangiferaindica* and *Syzygiumcumini* and development of the aqueous based gel formulation of active composite extract. The formulation was prepared and evaluated for anti-acne activity in rats. Further, to assess the topical effects of the formulation,histopathology of the skin was also performed.

2. MATERIALS AND METHODS

Materials.

Plant collection.

The seeds of *M. indica* &*S. cumini* were collected from local market of Ghaziabad, Uttar Pradesh, India. The seeds were authenticated from a taxonomist Dr. AnamikaTripathi, Associate Professor, Project Co-ordinator, NAMP, Department Of Botany, Hindu College, Moradabad, affiliated to Mahatma JyotibaPhule, Rohilkhand University, Bareilly with Ref. No.-HC/Bot/PERL/209-2018.

Microbial strain.

The acne causing pathogenic bacteria,*Propionibacterium acnes*(MTCC 1951)is obtained from Microbial type culture collection center, Institute of Microbial Technology, Chandigarh, Punjab, India.

Animal.

Wistar rats (150-300g) were obtained from All India Institute of Medical Sciences New Delhi (India) .The animals were housed in animal house of I.T.S College of Pharmacy, Muradnagar, Ghaziabad, India in polycarbonate cages, in a room maintained under controlled room temperature 22±2ºC, relative humidity 60-70% and provided with food and water ad libitum. All the experimental procedures (Project Proposal no-ITS/01/IAEC/2018) and protocols used in the study were reviewed by the Institutional Animal Ethics Committee (Registration Number: 1044/PO/Re/S/07/CPCSEA, 2nd feb 2007) and the care of laboratory animals were taken as per the guidance of CPCSEA, Ministry of Forests and Environment, Government of India. The animals were fasted for 24h before experimentation but allowed free access to water throughout. All studies were carried out by using six animals in one group for Anti-acne activity.

Preparation of crude extract.

The seeds of the plants were dried in the air and finely grounded in coarse powder. Extraction of both the plants was done separately with methanol in Soxhlet apparatus for 24 h. The extract was concentrated to the half of its volume by using Rotatory Vacuum Evaporator. After this, fractionation was done by using Hexane, Chloroform, ethyl acetate three times for each solvent. All the fractions were concentrated in Rotatory Vacuum Evaporator.

***In-vitro* antimicrobial assay by Agar well diffusion method.**

The screening of different fractions was carried out using the Agar well diffusion method. The bacterial strain (*Propionibacterium acnes)* were inoculated in Thioglycolate media and incubated at 37^0C for 48h in anaerobic condition. Freshly sterilized agar media was prepared and sterilized at 15Ibs pressure 121^0C for 20 minutes. Uniform sized wells were made with sterile borer and were filled with plant extracts of various concentrations. Bacterial suspensions were uniformly spread on each agar plates. Plates were then incubated at 37°C for 48 hrs under anaerobic conditions. The anti-microbial agent erythromycin (15 µg/disc) was included as a standard control. Zone of inhibition in mm was measured to compare the anti-microbial activity of plant extracts [6-13].

Determination of Minimum Inhibitory Concentration.

The minimal inhibitory concentration (MIC) values were determined by broth dilution assay. The cultures were prepared at 24 h and 48 h broth cultures of *Propionibacterium acnes*. Six sterile test tubes with 9ml sterile nutrient broth were taken. 1ml of different concentration of drug solution was added and 0.1ml inoculum was also added to the test tube aseptically. A blank with the nutrient broth and the drug solution was also prepared. A positive control, containing media with 0.1ml inoculum was maintained to indicate the growth promotion capacity of the media. Test samples of *Propionibacterium acnes* were incubated under anaerobic condition in an anaerobic jar (Hi-Media) with gas pack for 48h [8, 9-15].

Formulation of Gel.

The gel was formulated using a composite sample of methanolic extracts *M. indica* seeds *& S. cumini* seeds using carbopol 934, Propylene glycol, triethanolamine, sodium benzoate etc. The antiacne gel of methanolic extract of *M. indica & S. cumini* seeds extract was prepared. Sufficient amount of carbopol 934 was dissolved in distilled water with continuous stirring. Then small amount of methanolic extracts of both plant is dissolved in propylene glycol and added in carbopol solution. Sodium benzoate was added as preservative. All solution were mixed together. Then Triethanolamine was added dropwise until the gel consistency and pH is adjusted between 6.8 to 7.4. The final volume was made up to 200g by the addition of distilled water [9-19].

Physical evaluation of gel.

1. Organoleptic parameter

It was determined by sensory evaluation.

2. Viscosity

Viscosity was determined by Brookfield viscometer spindle # 7 and 12 at 25°C of the gels prepared.

3. Stickiness

Stickiness was determined by applying the gel over the skin of rat and checking whether there was the presence or absence of stickiness after application of the gel.

4. **Determination of pH**

pH was determined by using pH meter. In this method, electrode is washed with distilled water, dried with the help of tissue paper and then dipped in 20ml gel formulation[8].

Antiacne Activity.

(a) Propionibacterium acnes induce acne

Animals of either sex (180-220g) are selected. Animals were shaved in the interscapular area. After 24 hours of shaving, the test preparation, control, and standard will be applied to the shaved area.

All animals were divided into four groups in which six animals in each group.

(I) Group: This group was treated as control group.

(II) Group: In this group, Wistar albino rats were given intradermal injection of *Propionibacterium acne.*

(III) Group: In this group, gel of *Mangifera indica* & *Syzygiumcumini seeds* was applied 0.5mg/kg/day, for 21 days after inducing acne.

(IV) Group: In this group, clindamycin was given 0.5 mg/ kg/day for 21 days after inducing acne.

Procedure:

Wistar albino rats were selected. Animals are shaved in the interscapular area. After 24 hours of shaving, the intradermal injection *P.acne*is given in the interscapular region of the rat skin. Acne and papules production happened after 48h of the injection. After induction, the test preparation, control, and standard were applied to the shaved area. Body weight of individual animal was taken daily for each group and record was maintained from the starting day of the study till the last dosing. If the death of any animal occurs in between the study time, its weight was also to be taken. Food intake was measured on each day. All animals were divided into four groups [19-21].

Histopathology Study.

After the treatment, treated rats were sacrificed and subjected to histopathological examination. The interscapular region of skin was aseptically removed and tissue sample was fixed in 10%

buffered formalin and processed with paraffin wax for histopathological examination. Section of 5µm size is made and stained with hematoxylin and eosin. The extent and depth of change and alterations were evaluated.and mean thickness was evaluated by microscopy.

Data Analaysis (Anova Test).

Analysis of variance (ANOVA) is a statistical method used to test differences between two or more means.

3. RESULTS

Antibacterial activities of extracts.

Methanolic extract of *M. indica* seeds and *S. cumini* seeds were evaluated in-vitro against *Propionibacterium acne* using agar well diffusion method. Figure 1 shows the effect of different fractions at 3 different concentrations along with control, for *M. indica* seeds and Fig No. 2 shows the effect of different fractions of *S. cumini* seeds. The zone of inhibition for the methanolic extract was highest for *S. cumini* as well as *S. cumini* (Table 1).

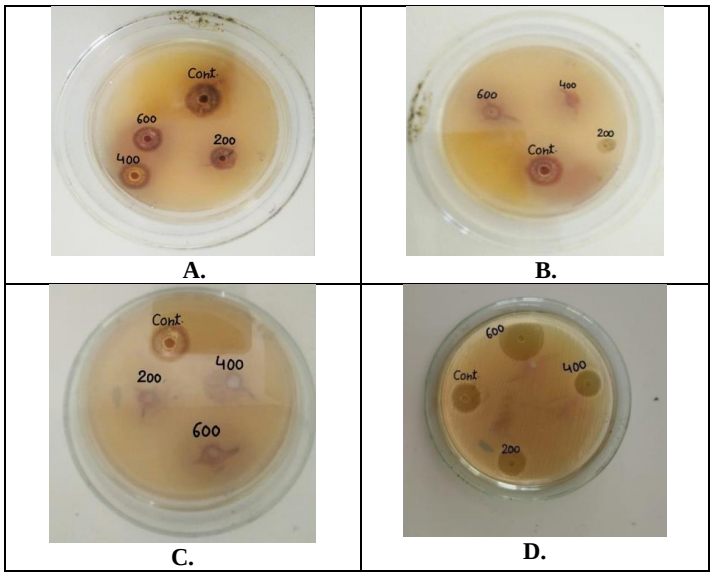

Figure 1. Agar well diffusion assay for *S. cumini* seeds extracts(A) Hexane extract (B) Chloroform extract (c) Ethyl acetate extract (d) Methanol extract.

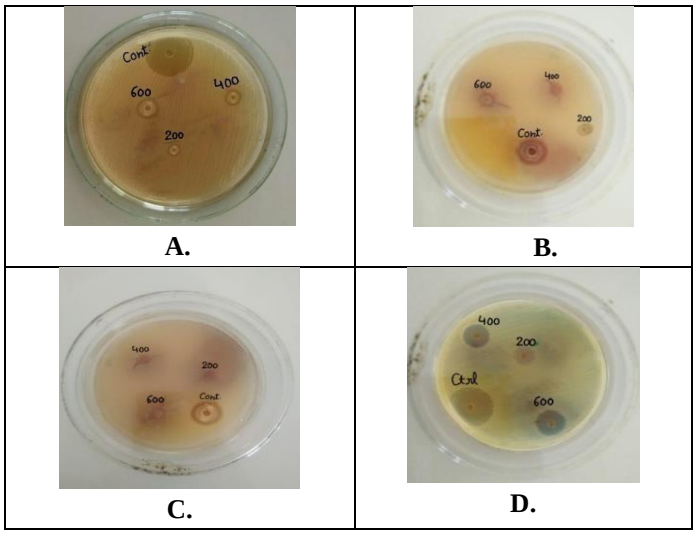

Figure 2. Agar well diffusion assay for *M. indica* seeds extracts(A) Hexane extract (B) Chloroform extract (c) Ethyl acetate extract (d) Methanol extract.

Table 1. Zone of inhibition of extract of *M. indica* &*S. cumini* seeds extract.

Extracts	Concentration (µg/ml)	*Mangifera indica*	*Syzygium cumini*
		Propionibacterium acne	
		Zone of inhibition (mm)	
Hexane	200	3.067±0.34	2.66±0.66
	400	7.2±1.10	6.83±0.58
	600	11.3±0.57	9.46±0.81
Chloroform	200	3.96±0.58	4.26±1.516
	400	5.4±1.45	5.46±0.721
	600	8.56±1.33	7.56±0.74
Ethyl acetate	200	3.560±0.84	2.56±0.34
	400	4.63±0.80	4.26±0.75
	600	3.26±0.30	5.73±1.46
Methanol	200	12±1.527	11.33±1.85
	400	20.33±0.62	19.16±1.81
	600	24.96±0.89	23.33±0.48

*Values expressed as mean±SEM *P<0.05, P<0.01, ***P<0.001 as compared to standard*

Zone of inhibition of plant extracts (mm) against microorganism in triplicate (mean±SEM), zone of inhibition of control (erythromycin at Conc. 600 µg/ml) is 25.43 mm, Zone of inhibition of methanolic extracts (at Conc. 600 µg/ml) of *M. indica*s eeds and *S. cumini* seeds of were 24.96 and 23.33 mm respectively . SEM: Standard error of mean.

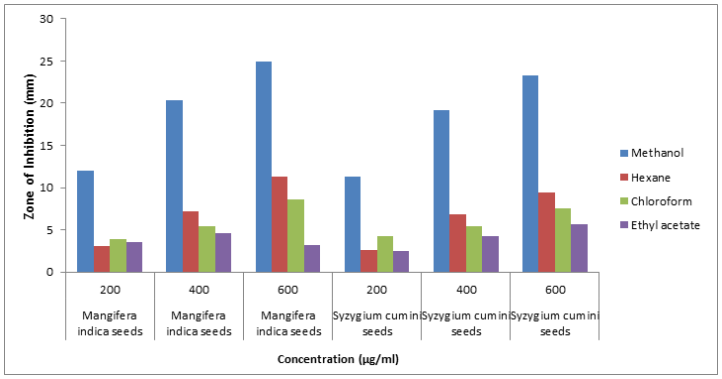

Figure 3. Comparative study of Zone of inhibition of extract of *M. indica*&*S. cumini* seeds extract.

Evaluation parameters of Formulated Antiacne gel of methanolic extract of *M.indica&Syzygiumcumini seeds.*

Table 2 shows physical parameter for prepared gel of a composite extract *M.indica& S. cumini seeds* methanolic extract.

Anti-acne activity

Acne was induced by *P. acnes* injections in rats . The formulated gel was applied for 21 days, The visual appearance of the skin was improved. Further effect was evaluated by histopathological evaluation .Table 3 and Figure 4 shows the mean thickness of excised skin after 21 days.

Table 2. Evaluation parameters.

S.No.	Parameters	Observation
1.	Colour	Brownish yellow
2.	Odor	Characterstic

S.No.	Parameters	Observation
3.	Viscosity	41120 ±0.3 cps at 32^0C
4.	Stickiness	No
5.	pH	7.2

Table 3. Effect of gel, standard and negative on acne.

S.no	Group	Mean Thickness±SEM(excised skin)
1	**Control**	0.1717±0.035
2	**Negative**	1.1183±0.075**
3	**Gel**	0.3900±0.064**
4	**Standard**	0.4117±0.86**

*Values expressed as mean±SEM *P<0.05, P<0.01, ***P<0.001 as compared to standard*

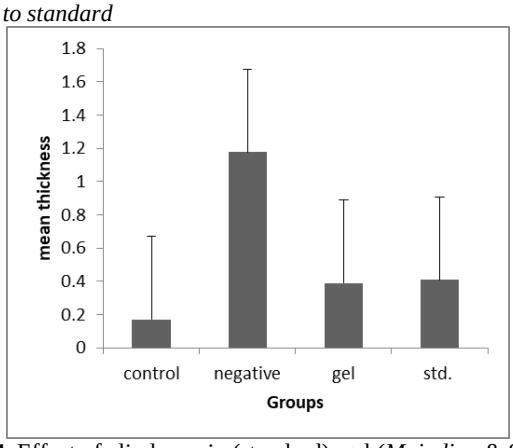

Figure 4. Effect of clindamycin (standard), gel (*M. indica & S. cumini seeds*) on acne.
*Values expressed as mean±SEM *P<0.05, P<0.01, ***P<0.001 as compared to standard.*

Figure 5 shows the effect of gel over skin. Within 48h, rat skin showed papules and acne formation. In treatment group clindamycin gel reduced acne formation in 7 days. After 21 days application, almost 99 % acne disappeared. Further Figure 6 shows histopathological observations for the different treatment and control group.

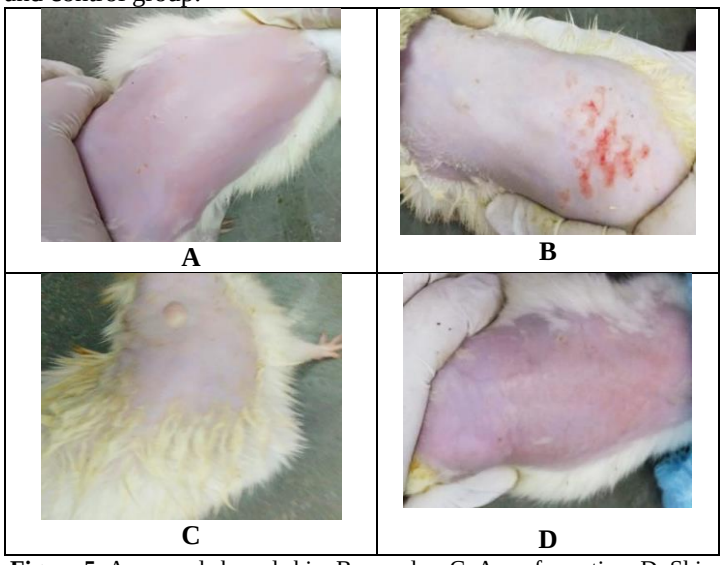

Figure 5. A. normal shaved skin. B. papules. C. Acne formation. D. Skin after test gel (21 days).

Histopathology of rat skin after 21days.

The main components of acne are comedones, inflammatory lesions and scars. In this histopathology report, interscapular region of skin of control group has shown normal skin architecture with absence of inflammation, edema, congestion and hyperkeratosis. The negative group with *P. acne* group has shown, inflammation, Edema and dilated sebaceous gland. The standard group of Clindamycin group has shown slight acanthosis, reduction of leukocyte infiltration and reduced inflammation. The Test gel group of methanolic extract of *M.indica & S. cumini seeds* has shown decreased inflammation and oedema.

The gel of composite methanolic extract of the plants *M.indica & S. cumini* seeds showed maximum reduction of acne, scars, comedons, papules and inflammation and histology shown was close to normal as shown in Figure 6.

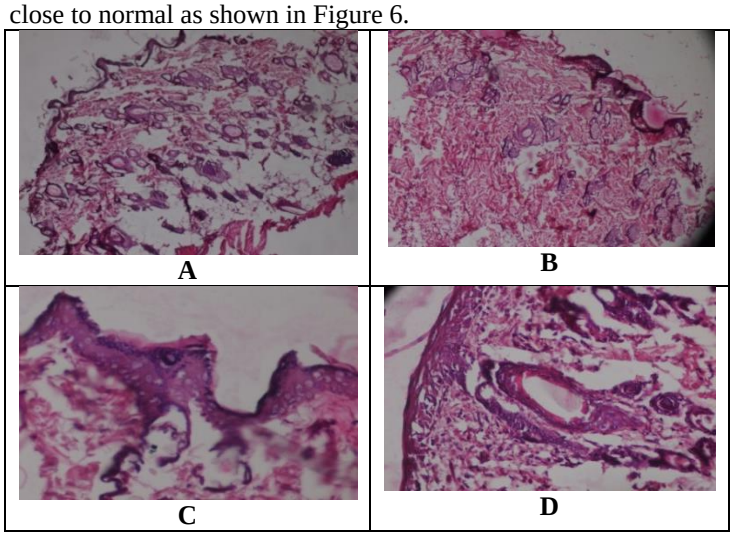

Figure 6. Histopathlogicalstudyof rat skin. A. Control group- normal skin with no acne. B. Negative group- inflammation, Edema and dilated sebaceous gland. C. Standard group- slight acanthosis, reduction of leukocyte infiltration and reduced inflammation. D. test group- decreased inflammation and oedema.

Discussion.

In the present work, Topical formulations containing methanolic extract of the seeds of *Mangifera indica&Syzygium cumini* was prepared for antiacne activity.

The extracts of *M. indica* &*S. cumini* seeds have been tested quantitatively for phytoconstituents. Phytochemical testing displayed that there was the presence of Saponins, phenols, alkaloids, tannins, glycosides, flavonoids, proteins, carbohydrates, anthraquinones, steroids. The extraction was performed with methnol and after extraction, fractionation was done with hexane, chloroform, ethyl acetate.

All *M. indica & S. cumini'*sseeds extracts were assessed by disk diffusion technique for *in-vitro* antibacterial activity and Minimum Inhibitor Concentration (MIC)was determined by method of broth dilution against *Propionibacterium acne.* In disk diffusion method, the experiment was carried out in triplicates (three petriplates for each solvent). Mainly the extracts demonstrated antimicrobial activity at higher concentration against *P. acne*. The control showed 25.43mm ZOI. For *M. indica* seeds extract,maximum ZOI(24.9mm) was shown by methanolic extract, followed by hexane extract(11.13mm), chloroform extract(8.53mm) and ethyl acetate extract(3.56mm). Forextract of *S. cumini* seeds, maximum ZOI(24.33mm) was shown by methanolic extract, followed by hexane extract(11.3mm), chloroform extract(8.56mm) and extract of ethyl acetate(3.56mm). The outcome showed that methanol extract from seeds has the largest inhibition area and the minimum concentration of inhibitors was 600µg / ml.

As with the antimicrobial result, it is suggested that the plants have synergistic effect against acne. Topical gel formulation has been developed by methanolic extract gel. The gel was developed and analyzed for all its parameters like pH, viscosity, physical appearance and compared with commercial gel for *in vivo* activity

against *P.acne* caused acne 21 days in Wistar albino rats. It has been discovered that antiacne activity is significant and prevents the development of acne, papules &comedon in the cross-scapular area of Wistar albino rat.

The impact of antimicrobial *in vitro&in vivo* antiacne against *P.acne* has been verified *(*MTCC, 1951) in Petriplates& in Wistar rats respectively. Physical assessments were performed at periodic intervals over the rat skin & gradual feeding of comedo was observed on treated animals. The gel has shown considerable improvements on comedo or papules or acne reduction on the skin. Compared with standard, the test group found substantial ($p<0.001$*) improvement. Apart from this study, no reports are available of *M. indica & S. cumini* seeds topical polyherbal gel.

4. CONCLUSIONS

In this study, antiacne effect of polyherbal topical formulation of methanolic extract of seeds of *M. indica and S. cumini*was determined in-vivo against *P.acne*in rats. The presence of the phytochemicals present in the gel extract like Saponins, phenols, alkaloids, tannins, glycosides, flavonoids, proteins, carbohydrates, anthraquinones, steroids has shown potential free radical scavenging activity. *Methanolic* extract displayed a potent in vitro antimicrobial action. Minimum Inhibitory Concentration of the extract indicated that these plant seeds could be a good source for antiacne action. The methanolic extract possessed the highest anti microbial activity. The better antiacne potential of the formulation can be attributed to a synergistic effect of the plant extracts.

Hereby it is concluded that the herbal plant gel showed promising antiacne potential as compared to synthetic medicine.

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