



Formulation and In Vitro in Vivo Evaluation of Anti-Acne Gel Containing Peanut Skin Extract

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Abstract: Acne vulgaris is a common skin disorder affecting more than 85% of the population of the world. Management of acne consists of use of topical or systemic antibiotics, retinoids etc. Prolonged use of antibiotics may lead to the development of antibiotic resistance and various side effects. Due to the increasing frequency of intake of antibiotics, expensive and its side effects, there is a need to focus on the scientific exploration of herbal drugs. Peanut or groundnut taxonomically classified as *Arachishypogaea* is a species of legume that belongs to the family of Fabaceae, has antibacterial, antioxidants and anti-inflammatory properties. The objective of the study was to develop a topical poly herbal gel for the treatment of mild acne vulgaris. Six different formulations of the herbal gel from ethanolic extract of peanut skin (EEPS) were prepared by varying the proportions of polymers Carbopol934 and Xanthan gum as a gelling agent and evaluated for their physicochemical properties like pH, spread ability, viscosity and microbial assay, skin irritation and its comparison with marketed product. Peanut skin was extracted using ethanol reflux method. An anti-acne activity of the extract of the peanut skin was performed by Kirby-bauer diffusion method using *S. Epidermidis*, *P. acne*. Antibacterial activity against *P. acne* and *S. epidermidis* was 7.6 mm and 6.6mm respectively. In comparative study of the formulation compared with marketed products, the zone of inhibition of peanut skin extract and marketed product against *P. acne* and *S. epidermidis* was 11.1mm, 10.4mm, 8.3mm and 7.6mm respectively. The visual inspection of the prepared formulation indicated no lumps and had uniform brown color dispersion, free from any fiber and particle, pH was found to be in the range 6.2-6.8, near to the skin pH which indicates that the prepared formulation is compatible with skin and viscosity found to be in the range of 2342 ± 0.8 - 2704 ± 1.2 cps. *In vivo* Animal studies revealed no pathological lesions. It was observed that the developed peanut skin extract formulation has good minimum inhibitory concentration; it may be due to phytoconstituents present in the peanut skin extract. Finally we concluded that the developed herbal gel containing peanut skin extract which is non-toxic, safe, effective and will improves patient compliance.

Keywords: Acne vulgaris, peanut skin extract, topical gel, *S. epidermidis*, *P. acne*.

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I. INTRODUCTION

Acne vulgaris is the most common infection of the skin. Acne vulgaris is a common skin disorder affecting more than 85% of the population of the world, specifically teenagers and adolescents.¹ It is a chronic inflammatory disease affecting the pilosebaceous unit. An increased secretion of sebum and abnormal desquamation of the follicular epithelium leads to obstruction of the pilosebaceous unit and comedo formation. The presence of sebum in the pilosebaceous unit attracts *Propionibacterium acnes*.² Acne can be treated by antibiotics either oral or topical application, hormonal therapies, corticosteroids or surgery. Prolonged use of antibiotics may lead to development of antibiotic resistance and various side effects such as erythema, photosensitivity, allergic dermatitis, excessive skin irritation, urinary problem, joint and muscle pain, headache, depression. Due to the increasing frequency of intake of antibiotics, expensiveness and its side effects, there is a need to focus on the scientific exploration of herbal drugs in the belief that they are safer with fewer side effects than the synthetic ones³. The plant such as peanut skin possess many potential therapeutic activities like antioxidant activity, anti-inflammatory⁴, Hypoglycaemic activity⁵ in the traditional system of medicinal practice and possess rich phytoconstituents Catechins. The plant of peanut skin contains polyphenols, polyunsaturated and monounsaturated fats, phytosterols dietary fibers and rich in catechin. The peanut skin is reported to have significant amounts of flavonoids and phenols which are used for various skin diseases⁶. In the formulation of gel Carbopol 934 and Xanthan gum are used as gelling agents. The study was conceived to formulate herbal gel from ethanolic extract of peanut skin (EEPS) and to evaluate its physicochemical parameters, microbial assay, skin irritation and its comparison with marketed products.

2. MATERIALS AND METHODS

2.1. Collection of Plant

The peanut red skin was purchased from the local market

The peanut red skin was purchased from the local market and authenticated BSI/WRC/IDEN.CER./2018/H3138 Date 27.12.2018 by Botanist Priyanka A. Ingale, at Botanical Survey of India Pune, India.

2.2 Chemicals

Carbopol, methyl paraben, methanol, ethanol were purchased from Thermosil Fine Chem. Pune. Xanthan gum, polyethylene glycol were purchased from Research Lab Fine Chem. Mumbai. Glycerine was purchased from Analytical fine chemical, Mumbai. All reagents used were of analytical grade. The test organism, *P.acne* and *S. epidermidis*, were obtained from monera lab, Pune.

2.3 Preparation of Plant Extract

10 g of roasted peanut red skin was weighted and added in a round bottom flask. To this 100ml 70% ethanol was added. Allowed to reflux for 2 hours at 50°C. The ethanolic extract was filtered and concentrated to a dry mass. A dark brown color residue was obtained. In 2 g Ethanolic extract was dried under the shade to get a dry powder.

2.4 Formulation of Gel

Different concentrations of carbopol 934 and xanthan gum were taken as gelling agents as per formula given in Table I. The gel phase in the formulation was prepared by dispersing Carbopol 934 and Xanthan gum in purified water with constant stirring at a moderate speed using mechanical stirrer at room temperature, then the pH was adjusted to 5.0-7.4 using triethanolamine. Methyl paraben was dissolved in propylene glycol and mixed with the aqueous phase with continuous stirring until it cooled to room temp. The obtained gel mixed 1:1 ratio with gentle stirring to obtain the gel⁷.

Table I. Ingredients for formulation of gels

Sr. No	Ingredients	F1	F2	F3	F4	F5	F6
1	Ethanolic extract of Peanut skin (gm)	3	3	3	3	3	3
2	Carbopol (gm)	0.5	1	1.5	2	2.5	3
3	Xanthan gum (gm)	0.5	1	1.5	2	2.5	3
4	Polyethylene glycol (ml)	2	2	2	2	2	2
5	Methyl paraben (gm)	0.8	0.8	0.8	0.8	0.8	0.8
6	Glycerine (ml)	1	1	1	1	1	1
7	Triethanolamine (ml)	1	1	1	1	1	1
8	Methanol (ml)	2	2	2	2	2	2
9	Water	q.s	q.s	q.s	q.s	q.s	q.s

2.5 Preliminary Photochemical Screening of Ethanolic Extract

The present preliminary phytochemical analysis gives the information about photo constituents in the crude drug. This information is important for the ethno pharmacological screening of the drug. Hence chemical tests were carried out on with the ethanolic extract using standard procedure in order to identify the phytoconstituents⁷.

2.6 Antimicrobial activity

The antimicrobial activity was determined by Kirby-Bauer diffusion method^{13,14}. Sterile SDA (Subouraud dextrose agar) was poured into plates and the depth of the medium was 4mm. After solidification the plates were dried for 30 minutes. The broth culture incubated at 35-37°C for 2-5 hrs. The lid of Petri dishes were closed and kept at room temperature for 5-10 minutes to dry the inoculums, confluent growth is desirable. The

sensitivity disc removed with the help of a forceps and placed carefully in the plates at least 24nm away from the edge. Allowed to stand at room temperature for 30 min. Preparation of well at 7mm diameter in each plate with the help of syringe, with some distance. Sample applied was observed in well. Incubate all plates at 37°C for 24-48 hrs. Measured zone of Inhibition around the each well against *S. Epidermidis* (MTCC NO.435), *P.acnes* (MTCC NO.1951) by vernier calliper. Sample preparation application on well⁸

- 10mg peanut skin extract/0.5ml DMSO
- 20mg peanut skin extract/0.5ml DMSO
- 100mg peanut skin extract/0.5ml DMSO
- 200mg peanut skin extract/0.5ml DMSO

2.7 Physicochemical Evaluation of Formulations:⁹⁻¹⁶

Physical parameters such as color, appearance and consistency were checked visually. After physicochemical evaluation, it was clear that all the batches have brown, transparent, homogenous with good homogeneity, smooth in texture.

2.8 Colour

The color of the formulations was checked against white background. It's brown in colour. Colour increases the aesthetic appearance or to prolong the stability or to produce standard preparations or for identification of a particular formulation. Color psychology says that, the color of the product may also influence the efficacy of therapy.

2.9 Physical appearance

The physical appearance of the formulation was checked visually.

Formula: Percentage yield = (actual yield/theoretical yield) x 100.

2.15 Texture Profile Analysis¹⁷⁻²⁰

Texture profile analysis was performed using a Brookfield CT3 Texture Analyzer in compression mode by using spreadability test accessory. Gel formulation was filled into the female probe, taking care to avoid air pockets into the samples. A conical analytical male probe was forced down into each sample at a defined rate and to a defined depth, At least two replicate analyses of sample were performed at temperature. From the resulting force time plots, the hardness, cohesiveness and adhesiveness were derived. Spreadability was calculated from the energy required to deform the sample or from the hardness of the sample.

2.16 pH

The pH of the various formulations was determined using Digital pH Meter. About 20mg of the formulation were taken

2.10 Odour

The odour was checked by mixing the gel in water and taking the smell.

2.11 Viscosity

The viscosity of gel formulation was determined using small volume Brookfield viscometer. The determinations were carried out at spindle # 7 at 50 rmp and 25°C. The corresponding dial reading on the viscometer was noted. Then the spindle was lowered successively. The dial reading was multiplied by the factor mentioned in catalogue.

2.12 Consistency

Consistency is very important parameters in gel. It was evaluated by just applying small quantity of gel and checking whether there was the presence or absence of stickiness after application of the formulation. The consistency was checked by applying on skin.

2.13 Greasiness

It is very important parameters for gel quality. Prepared gel must have skin feel effect like no greasiness, stiffness and, grittiness, in it. The consistency of the gel formulations and presence of coarse particles were used to evaluate the texture and homogeneity of the formulations. The greasiness was assessed by the application on the skin.

2.14 Percentage Yield of Ethanolic Extract of Peanut Skin

Percent yield is the percent ratio of actual yield to the theoretical yield. It is calculated to be the experimental yield divided by theoretical yield multiplied by 100%.

in a beaker and were subjected to the pH measurement using a digital pH meter within 24 hrs of manufacture.

2.17 In Vivo Studies:¹⁷⁻²⁰

2.18 Skin Irritation Test

The skin Irritation test was carried out to check the possibility of skin irritation on application of peanut skin extract gel using female Wistaer rats.⁶ Animals were procured from National Institute of Biosciences, Pune and acclimatized in the well ventilated animals house of RajarshiShahu College of Pharmacy and Research, Tathawade, Pune. The standard laboratory conditions were maintained by providing relative humidity of 45 - 47 % and temperature of 23± 2° C following the 12:12 hour light dark cycle. Animals were feeded standard palatable diet and water ad libitum during the study. The protocol was approved by

Institutional Animal Ethics Committee (Reg.no. 1249/PO/Re/S/09/CPCSEA) and IAEC protocol no. RSCPR/IAEC/2019/03 as per the guidelines of CPCSEA. Female Wistaer rats having average weight 200-250 g were used and divided into 3 groups containing 6 rats in each group. Group I is a normal control group and had not received any formulation. Group II, is standard group received marketed formulation and Group III is treatment group received peanut skin extract gel. To determine the effect of gel on skin, albino Wistaer female rats were used. Entire undamaged skin was used and the hairs were shaved 3 days before the experiment. In test group III, topical herbal gel was applied and in standard group, the marketed gel was applied on the back of the animal. The gel was applied daily

up to 7 days and then the treated skin was visually examined. Score for skin irritation was assigned as¹¹

- 0 (no irritation) no involvement
- 1 (no erythema but definite dryness) 10%
- 2 (moderate erythema) 10-29%
- 3 (moderate to severe erythema with slight edema) 30-49%
- 4 (moderate to severe erythema with severe edema extending beyond the marked area) 50-69%

2.18 Histopathological examinations

In order to conduct histological investigations gel was applied topically to Wistaer rats as shown in figure 1 and samples were collected on 7th day. The fixation of the samples was done using 10% formalin and histopathological examination was done. 24



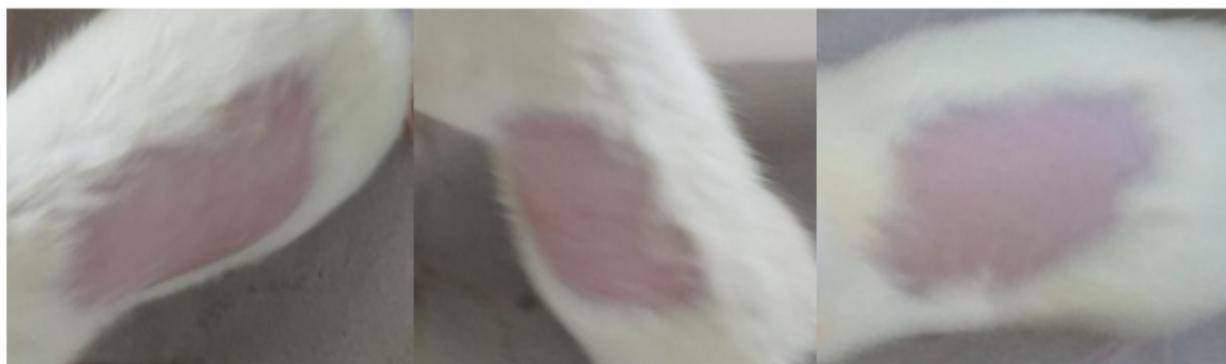
1st day

Group I Normal control



4th day

Group II: Standard Marketed (Marketed gel 1%)



7th day

Group III: Treatment (Formulated gel)

Fig 1. Topical applications of gel for animal study

2.19 Comparative Study of the Formulation

In this study we measured the zone of inhibition of peanut skin extract, placebo gel and marketed products against *S. Epidermidis* and *P. acne* by vernier calliper scale.²¹

3. RESULTS AND DISCUSSION

Table 2. Percentage Yield of Extract of the Peanut Skin.

S. No.	Solvent	Extraction process	% yield
I	Ethanol	Reflux method	17.1%

Percent yield is the percent ratio of actual yield to the theoretical yield. It is calculated to be the experimental yield divided by theoretical yield multiplied by 100%. On the basis of table 2 it is concluded that reflux method gives 17.1 % yield of extract which is sufficient for further study.

Table 3. Preliminary Phytochemical screening of Ethanolic Extract of Peanut Skin Phytochemical screening

Chemical tests	Results
Carbohydrates	+
Polyphenol	+
Glycoside	+
Phytosteroids	+
Tannins	+
Flavonoides	+
Alkaloids	+

+ Indicate above mentioned Phytochemicle are present in Extract

The above secondary metabolites were found to present in the Ethanolic Extract of Peanut Skin. The metabolites were recorded in table 3. Flavonoid is well known antibacterial agent that had been studied. Flavonoid had been reportedly showed antimicrobial activities against *P. acnes*, and *S. Epidermidis*.

3.1. Physicochemical Evaluations of Gel Formulations

The visual inspection of the prepared formulation indicated

no lumps and had uniform brown color dispersion, free from any fiber and particle, smooth in appearance, pH was found to be in the range 6.2-6.8, it is near to the skin pH which indicates that the prepared formulation is compatible with skin and viscosity found to be in the range of 2342 ± 0.8 - 2704 ± 1.2 cps¹⁹. Results are shown in Table no.4. Results showed that the gels had a cosmetically appealing appearance and smooth texture, and they were all homogenous with no signs of phase separation and greasiness.

Table 4. Physicochemical Evaluation of Gel Formulation

Formulation	pH	Viscosity (cps)	Homogeneity	Appearance	Color
F1	6.7	2192 ± 0.4	Homogeneous	Smooth	Brown
F2	6.5	2704 ± 1.2	Homogeneous	Smooth	Brown
F3	6.2	2342 ± 0.8	Homogeneous	Smooth	Brown
F4	6.8	2298 ± 1.4	Homogeneous	Smooth	Brown
F5	6.3	2294 ± 0.5	Homogeneous	Smooth	Brown
F6	6.4	2455 ± 0.2	Homogeneous	Smooth	Brown

3.2. The Antimicrobial Activity

The antibacterial activity of developed formulations was evaluated by measuring the diameter of zones of inhibition (in mm). Study results showed antimicrobial activity against acne causing bacteria *P. acne* and *S. Epidermidis*²⁰. The antibacterial

activity study results are shown in Table 5 and Figure 2. The zones of inhibition are given in table 5 & 6. Table 6 showed inhibitory effect on the test bacterium which is more to marketed preparation. This suggests that the other active ingredients of the formulations containing solvents such as methanol may have contributory antibacterial activity.

Table 5. Antimicrobial Activity of Peanut Skin Extract.

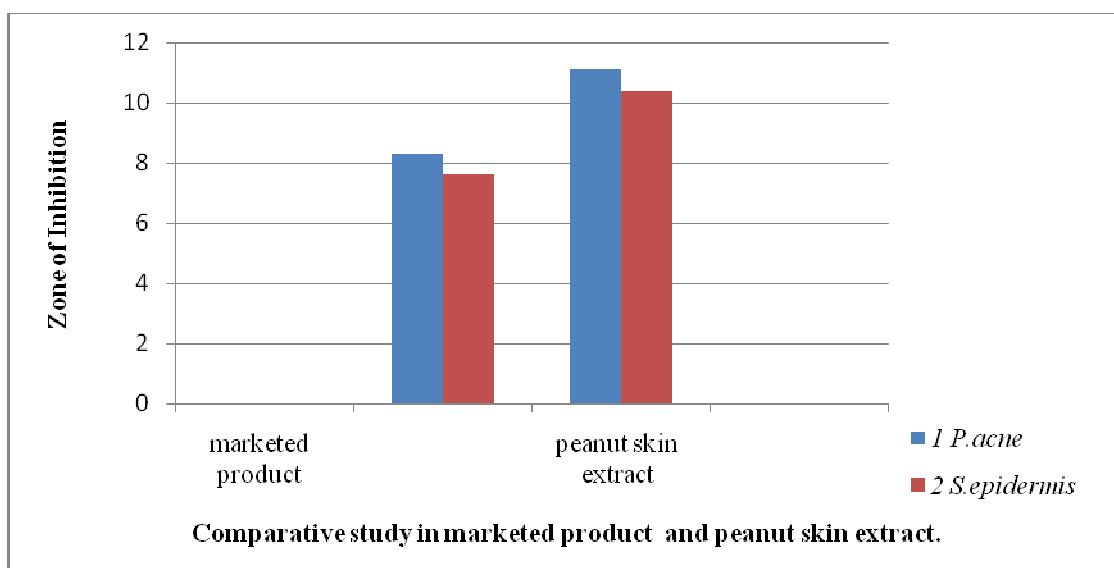
Test sample	Zone of inhibition		MIC	
	<i>P. acne</i> (MTCC NO.1951)	<i>S. epidermidis</i> (MTCC NO.435)	<i>P. acne</i>	<i>S. epidermidis</i>
Peanut Skin extract	7.6± 0.25 mm	6.6±0.41mm	300mg	300mg

**Fig 2. Antibacterial activity of Peanut Skin Extract against *P.acne* and *S. epidermidis*****Table 6. Anti-acne Study of the Formulation**

Formulation	Zone of inhibition	
	<i>P.acne</i>	<i>S. epidermidis</i>
Peanut skin extract	11.1±0.16 mm	10.4±0.43 mm
Marketed formulation	8.3±0.37 mm	7.6±0.26 mm

In comparative study zone of inhibition of the formulation was compared with marketed product against *P.acne* & *S. epidermidis* as shown in figure 3. The zone of inhibition of peanut skin extract and marketed product against *P.acne* and *S. Epidermidis* was 11.1mm, 10.4mm, 8.3mm and 7.6mm

respectively ²¹. On the other hand, the activity of selected formulations against *P.acne* & *S. epidermidis* further showed remarkable inhibitory effect as compared to marketed product.

**Fig 3. Antibacterial activity of peanut skin extract gel & marketed product against *P.acne* and *S. epidermidis***

3.3. Texture profile analysis

As per the Figure 4 Texture profile analysis of gel showed the Spreadability affects how area on which gel spread when applied. Retention time of gel depends upon its viscosity and spreadability. Texture profile analysis of the

formulated gel showed the hardness of 154 g which was the maximum force value in the graph. The area under the positive curve is the energy required to deform the sample. The hardness work done and firmness show the spreadability of the sample. Higher value of firmness and hardness work indicated less spreadable sample conversely the less value

indicated more spreadable sample. The maximum negative force on the graph indicated sample adhesive force; the more the negative value the more 'sticky' the sample. The area under the negative part of the graph is known as

adhesiveness which is the energy required for breaking probe sample contact. These results expressed the retention time of the gel on the site of application²².

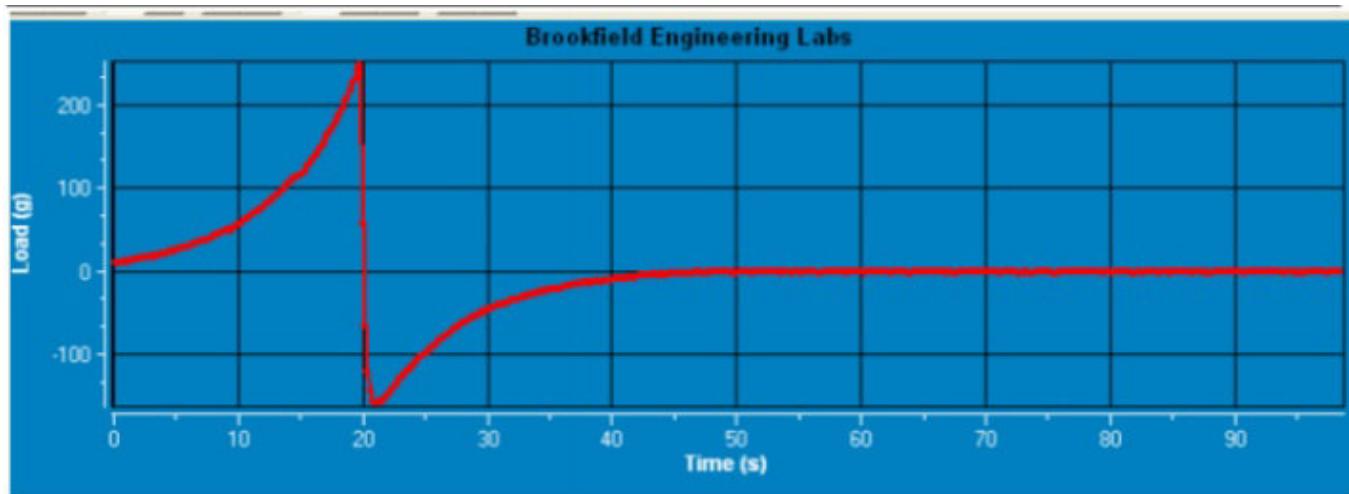


Fig 4. Texture profile analysis of gel

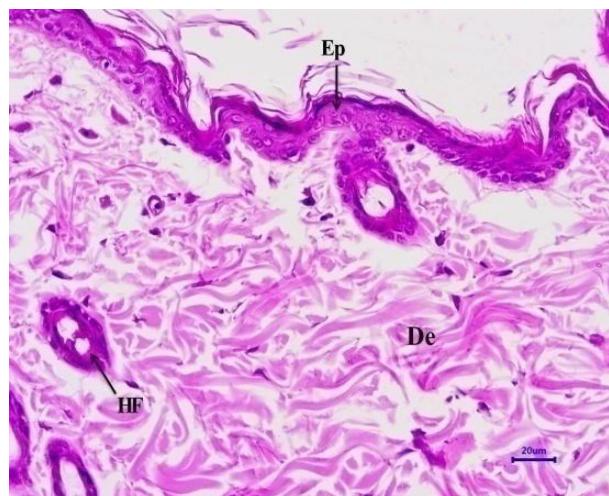
3.4. Skin Irritation Test

The topical formulation should be free of skin irritation and erythematous reaction, existing marketed formulation has been linked to markable skin irritation, which restricts the applicability and acceptability. In the present study no skin irritation (redness, swelling, or edema) observed in the normal control group. Group II peanut extract gel showed slight redness but no irritation and group III Marketed

product should also have no redness and edema. Thus the formulated gel can be considered non irritating and safe for topical used.²³

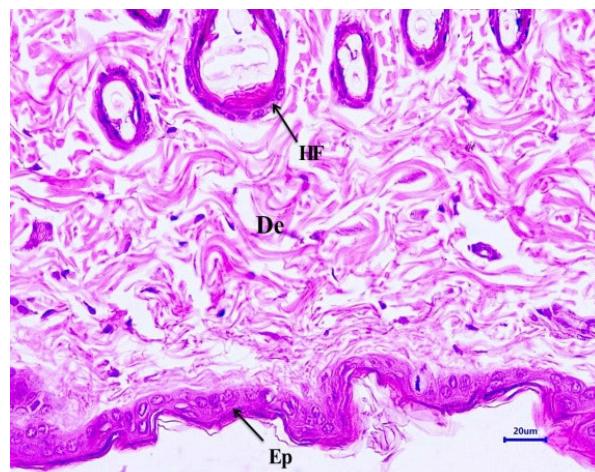
3.5. Histopathology

Histological examination of normal specimen of control was studied. Normal control showed intact epidermis dermis and presence of hair follicle did not show any pathological lesion.



Showing normal histology, Epidermis (Ep), Dermis(De), Hair Follicle(HF) {H&E, 400X}

Fig 5. Normal

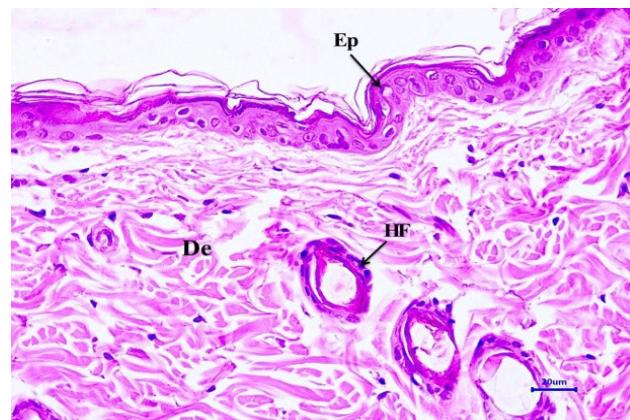


Showing normal histology, Epidermis(Ep), Dermis(De), Hair Follicle(HF) {H&E, 400X}

Fig 6.Peanut extract

Microscopic examination of skin of rats treated with Peanut extract did not show any lesion of pathological lesion²⁴. Results obtained from the histological studies showed that the prepared

Peanut extract formulation was effective in the treatment of acne. Also Microscopic examination of skin of rats treated with Peanut extract did not show any lesion of pathological lesion.



Showing normal histology, Epidermis(Ep), Dermis(De), Hair Follicle(HF) {H&E, 400X}

Fig 7.Market product

Microscopic examination of skin of rats treated with marketed products did not show any lesion of pathological lesion. Comparison of the control sample treated Peanut extract and marketed products did not show any lesion of pathological lesion. From this comparative study & as per figures 5,6 & 7, it can be concluded that developed Peanut extract is safe and effective for treatment of Acne vulgaris. The present study was undertaken with an aim to formulate and evaluate the topical poly herbal gel for the treatment of mild acne vulgaris. Six different formulations of the herbal gel from ethanolic extract of peanut skin²⁵. An anti-acne activity of the extract of the peanut skin was performed by Kirby-bauer diffusion method using *S. Epidermidis*, *P. acne*. Antibacterial activity against *P. acne* and *S. epidermidis* was 7.6 mm 6.6mm respectively. In comparative study of the formulation compared with marketed products the zone of inhibition of peanut peanut skin extract and marketed product against *P. acne* and *S. epidermidis* 11.1mm, 10.4mm, 8.3mm and 7.6mm respectively. This suggests that the other

active ingredients of the formulations containing solvents such as methanol may have contributory antibacterialeffect²⁶. The visual inspection of the prepared formulation indicated no lumps and it shows uniform brown color dispersion, free from any fiber and particle, pH was found to be in the range 6.2-6.8, it is near to the skin pH which²⁷ indicates that the prepared formulation is compatible with skin and viscosity found to be in the range of 2342 ± 0.8 - 2704 ± 1.2 cps. In vivo Animal studies revealed no pathological lesions²⁸. The topical application of the gel at the affected site would offer the potential advantages of the delivery of the drug directly to the acne site²⁸⁻²⁹.

3. CONCLUSION

Recent treatment of acne consists of use of topical or systemic antibiotics, retinoids etc. The major problem associated with use of antibiotic is resistance and its uncommon side effects. Natural remedies are more

acceptable in the belief that they are safer with fewer side effects than the synthetic ones. In skin diseases medicaments are mainly applied topically. Herbal medicine is very effective in curing various dermatological diseases and there are many herbal drugs which have been mentioned to be useful in the treatment of acne. The Peanut skin extract was found to have potency against acne inducing bacteria. The study was conceived to formulate herbal gel from ethanolic extract of peanut skin. In the antimicrobial studies, a comparison made with the standard marketed formulation Patanjali aloevera gel (8.3mm)(7.6mm), the Ethanolic extract of peanut formulation had shown the maximum effect for *P. acne* and *S. epidermidis* (11.1mm)(10.4mm) respectively. The phytoconstituents catachine present in the peanut skin might be responsible for the antimicrobial activity. Finally we concluded that the developed herbal gel containing peanut skin extract which is non-toxic, safe, effective and will improves patient compliance. In future formulated Hebal gel can be studied for various activities.

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4. ACKNOWLEDGEMENT

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5. AUTHORS CONTRIBUTION STATEMENT

Nilima A. Chaudhari conceived the presented idea, provided intellectual content, She Performed a part of research work. Ashwini P. Katke did the literature search & performed the research work. Also she evaluated the results. All others authors have participated equally in the execution of laboratory study & reviewed the manuscript.

6. CONFLICT OF INTEREST

Conflict of interest declared none.

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