



Evaluation of Anti-Acne Property of Poly Herbal Formulation

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ABSTRACT

Acne vulgaris is an extremely common skin disorder that affects virtually all individuals at least once during life. Herbal medications are considered safer than allopathic medicines as allopathic medicines are associated with side effects such as contact allergy, local irritation, scaling, photosensitivity, itching, pruritus, redness, skin peeling etc. In this study the anti-acne property of various herbal extract were reconfirmed. The cream formulation was developed, which contains the effective concentration of poly herbal extract. The anti-acne property of developed formulation was evaluated by in vitro method of anti-bacterial activity (broth dilution method and sub culturing method). The characteristics of cream in terms of spreadability, greasiness, tackiness, film forming, softening, comfortable and pleasant were analyzed by skin feel test. The results proved that the chosen formulation also having the effective anti-acne property. So we can suggest the further investigation and in vivo studies will help in developing this formulation as marketed product.

KEY WORDS: Anti-Acne, Polyherbal formulation, evaluation of anti-acne property

INTRODUCTION:

Skin is perhaps the most vulnerable part of our body. Throughout history, humans have been obsessed with caring for their skin. The concept of caring for one's beauty has been around for ages, good skin has always been a key component of overall beauty and good health. It is a well known fact that day to day exposure of human skin lead to number of problems such as acne, pimples, pigmentation and sunburn marks.^[1]

Acne vulgaris is a disease of pilosebaceous unit characterized by the formation of open and closed comedones, papules, pustules, nodules and cysts. It is the most common disorder treated by dermatologists. Lesions are most common on the face, but the neck, chest, upper back, and shoulders may also be affected.

The term acne is derived from Greek word "acme" which means "prime of life". Although generally considered to be a benign, self limiting condition, and despite its apparent cosmetic nature, its effects can go far deeper than the surface of the skin, and can place a heavy emotional and psychological burden on patients that may be far worse than the physical impact.^[2]

Acne is a disease of the pilosebaceous units in the skin. A changed keratinisation pattern in the hair follicle leads to blockage of sebum secretion. It is probable that hyper responsiveness to the stimulation of sebocytes and follicular keratinocytes by androgens leads to the hyperplasia of sebaceous glands and seborrhea that characterise acne. The enlarged follicular lumen attributable to inspissated keratin and lipid debris forms a

closed comedone (whitehead). When the follicle has a portal of entry at the skin, the semisolid mass protrudes forming a plug, producing an open comedone (blackhead).^[3]

Since the most frequent bacteria isolated from acne patients were *Staphylococcus aureus*, it is possible that acne vulgaris is mainly caused by *Staphylococcus aureus* rather than *Propionibacterium acnes*. This is in contrast to some reports which implicated both *Staphylococcus epidermidis* and *Propionibacterium acnes* as bacteria causing acne vulgaris. It may be concluded that geographical regions affect the bacteria involved in acne vulgaris.^[4]

However, the presence of microorganisms is not a strict prerequisite for comedo formation. Further, *P. acnes* belongs to the resident cutaneous flora.

P. acnes colonizes the follicular duct and proliferates, breaking down the sebum to triglycerides, irritants that probably contribute to the development of inflammation. When the follicular epithelium is invaded by lymphocytes it ruptures, releasing sebum, microorganisms, and keratin into the dermis. Neutrophils, lymphocytes, and foreign body giant cells accumulate and produce the erythematous papules, pustules, and nodular swelling characteristic of inflammatory acne.^[5]

The use of natural remedies, particularly herbal medicine, dates back thousands of years. In recent years, natural approaches to combating acne and its disfiguring effects have gained popularity. Several botanicals with a

history of use in traditional cultures have entered the growing 'cosmeceuticals' market.

With fewer adverse side effects and the added advantage of multi-functionality, botanicals are increasingly being used in mainstream cosmetic products, including acne fighting compositions. Quinones, flavanoids, polyphenols, tannins, terpinoids, alkaloids and essential oils all exhibit antimicrobial activity.^[6, 7, 8]

In this study there are four herbal materials which are used to investigate the anti-acne property. They are: Turmeric, Thyme, Sandalwood and Safflower. The aim of this study was to find the anti-acne property of a poly herbal formulation. The Objectives were

- Preparation of a poly herbal extract.
- Evaluation of the poly herbal extract.
- Preparation of a poly herbal cream formulation.
- Evaluation of the poly herbal cream formulation

MATERIALS AND METHODS:

PLANT MATERIAL:

Local Omani traditional herbs were used for t study.

Turmeric powder (rhizome), Thyme (leaves), Sandalwo powder (wood) and Safflower (petals) were purchas from a local herbal drug store in Seeb, Muscat. Thyme a safflower were made into fine powder to be used.

SOLVENTS:

Methanol and Propylene glycol

CHEMICALS:

Hard paraffin, Liquid paraffin ,Emulsifying wax, Glycerin and Propyl paraben

APPARATUS:

Electronic weighing apparatus, Autoclave (DAIHAN Scientific), Incubator (GALLENKAMP), Hot plate, Camera.

METHODS:

EXTRACTION PROCEDURE:

Different weights of the plant material were used to obtain different concentrations of the extracts. Powdered herbs were extracted with methanol using maceration technique, weights in Table. 1 were taken and transferred into beakers. HRB 1, HRB 2, HRB 3, HRB 4 and HRB 5 represent different concentrations. Each concentration contains the 4 herbs in equal amounts. In HRB1 0.5 g of each herb was weighed and transferred into a 100 ml beaker and 20 ml of methanol was added. (Table No.1: the exact amount of each herb was noted)

1. The same step was followed for the rest of concentrations with corresponding weights, HRB 2 with 40 ml, HRB 3 with 60 ml, HRB 4 with 100 ml and HRB 5 with 120 ml. The solutions were kept for 6 days and shaken frequently. After 6 days the extract solutions were filtered through filter paper using vacuum and transferred into pre-weighed beakers. The methanol was allowed to evaporate using a slightly warm water bath. The extracts were weighed and kept to use later in the study.^[8]

Plant Material	HRB 1 (g)	HRB 2 (g)	HRB 3 (g)	HRB 4 (g)
Turmeric	0.5091	1.0790	1.5110	2.0420
Thyme	0.5067	1.0050	1.5321	2.0051
Sandalwood	0.5034	1.0012	1.5132	2.0110
Safflower	0.5045	1.0063	1.5280	2.0112
Total weight (g)	2.0237	4.0915	6.0843	8.0693

Table No.1: Different amount of plant material

HRB 1 (g)	HRB 2 (g)	HRB 3 (g)	HRB 4 (g)	HRB 5 (g)
0.3002	0.5188	0.8788	1.2015	1.4096

Table No. 2: Amount of extracts.

Note:

SAMPLE PREPARATION:

Solutions of extracts were prepared using the different amount each dissolved in 10 ml of propylene glycol to be used in the antibacterial evaluation.

HRB 1 (g)	HRB 2 (g)	HRB 3 (g)	HRB 4 (g)	HRB 5 (g)
30	51.88	87.88	120.15	140.96

Table No. 3: Concentrations of extracts (mg/ml) for anti-bacterial activity

EVALUATION OF ANTI-ACNE PROPERTY (ANTIBACTERIAL ACTIVITY) OF EXTRACTS:

MICROORGANISM AND MEDIA:

The test organism used in this study was: *Staphylococcus aureus*

STERILIZATION PROCEDURE:

All equipments required for this test were autoclaved at 120°C.

EVALUATION PROCEDURE:

The antibacterial activity was determined by broth dilution assay. In this method, 7 sterile test tubes with 9 ml sterile nutrient broth were taken. 1 ml of different concentrations of extract solutions was added and 0.1 ml

inoculums was also added to 5 test tubes. A negative control with the nutrient broth and extract solution was prepared; a positive control was also prepared containing nutrient broth with 0.1 ml inoculums to indicate the growth promotion capacity of the media. A drop of sterile oil was added to the test tubes to maintain an anaerobic condition. Test samples were incubated at 37°C for 24 hours. For confirmation of the results, sub culturing of the samples on to sterile nutrient agar plates was done. The evaluation test was done to find the effective concentration which inhibits growth from the concentrations in Table No: 3 to be later used in the formulation. [9]

PREPARATION OF FORMULATION:

A cream formulation of 10 g was prepared.

Ingredients	Amount for 10 g
Liquid paraffin	4 ml
Hard paraffin	0.9 g
Emulsifying wax	0.7 g
Propyl paraben	0.02 g
Glycerin	0.9 ml
Water	3.5 ml
Poly-herbal extract	1.2015 g

Table No. 4: Ingredients and quantity for the formulation

PROCEDURE:

The procedure for cream formulation was followed as per the reference. [10]

According to the procedure, 2.25 g of hard paraffin, 10 ml of liquid paraffin and 1.75 g of emulsifying wax were weighed, transferred into a 100 ml beaker and they were allowed to melt at 60°C in a water bath constituting the oil phase. 0.05 g of propyl paraben was dissolved in 8.7 ml of water, and then 2.25 ml of glycerin was added to them and allowed to heat at 60°C in a water bath constituting the aqueous phase. When the phases reached the same temperature, the aqueous phase was added to the oil phase gradually and stirred with a glass rod until cooled, firm and homogenous. Finally 1.2015 g of

the poly-herbal extract was added to the cream and mixed. Concentration of the cream was 12% w/w.

EVALUATION OF THE FORMULATION:

EVALUATION OF THE ANTI-ACNE PROPERTY (ANTIBACTERIAL ACTIVITY):

The antibacterial activity was determined by broth dilution assay. In this method, 2 sterile test tubes with 9 ml sterile nutrient broth were taken. 1 gram of the cream was added to each test tube. 0.1 ml inoculums was added to one test tube, the other was left plain and marked as the negative control. A drop of sterile oil was added to each test tube to maintain an anaerobic condition. The test

tubes were incubated at 37°C for 24 hours. After incubation subculturing on sterile nutrient agar plates was done for confirmation of the results.

SKIN FEEL TEST:

Skins feel test was conducted on 10 volunteers (8 females and 2 males, AGE: 18-22 years).

The volunteers were questioned on the skin feel characteristics of the cream after application on the back of the hand. The number of "Yes" answers was represented in a graph.

The following characteristics of the cream were evaluated^[11].

- **Spreadability:** Spreadability is the ease of moving the product in circular movements over the skin, upon application. Volunteers were asked whether the creams had a good spreadability
- **Greasiness:** Volunteers were asked whether they perceived a greasy feel when rubbing the creams on the hands.
- **Tackiness:** Tackiness defines the feeling of a force required to remove fingers from the skin surface. Volunteers were asked whether the cream tested felt tacky right after application.
- **Film coating:** This is the feeling that a cream has formed a film on the skin, after application. Volunteers

were asked whether the cream formed a film on the skin after application.

- **Softening:** Volunteers were asked whether the cream left a soft feeling on the skin upon application.
- **Comfortable:** Volunteers were asked to overall evaluate whether the cream was comfortable.
- **Pleasant:** Volunteers were asked if the cream was pleasant.

SAMPLE OF THE QUESTIONNAIRE:-

Questionnaire for evaluation of the poly-herbal cream skin feel test:

Instructions: Please put a tick in the box next to the answer of your choice.

1. Sex Male Female
- The cream has good spreadability? Yes No
- Is there a greasy feel when you rub the cream on the hand?
 Yes No
- Did the cream feel tacky after application? Yes No
- Did the cream form a film on the skin after application? Yes No
- Did it leave a soft feeling on the skin? Yes No
- Overall is the cream comfortable? Yes No
- Is it pleasant? Yes No

RESULT:



(a) All test tubes after incubation

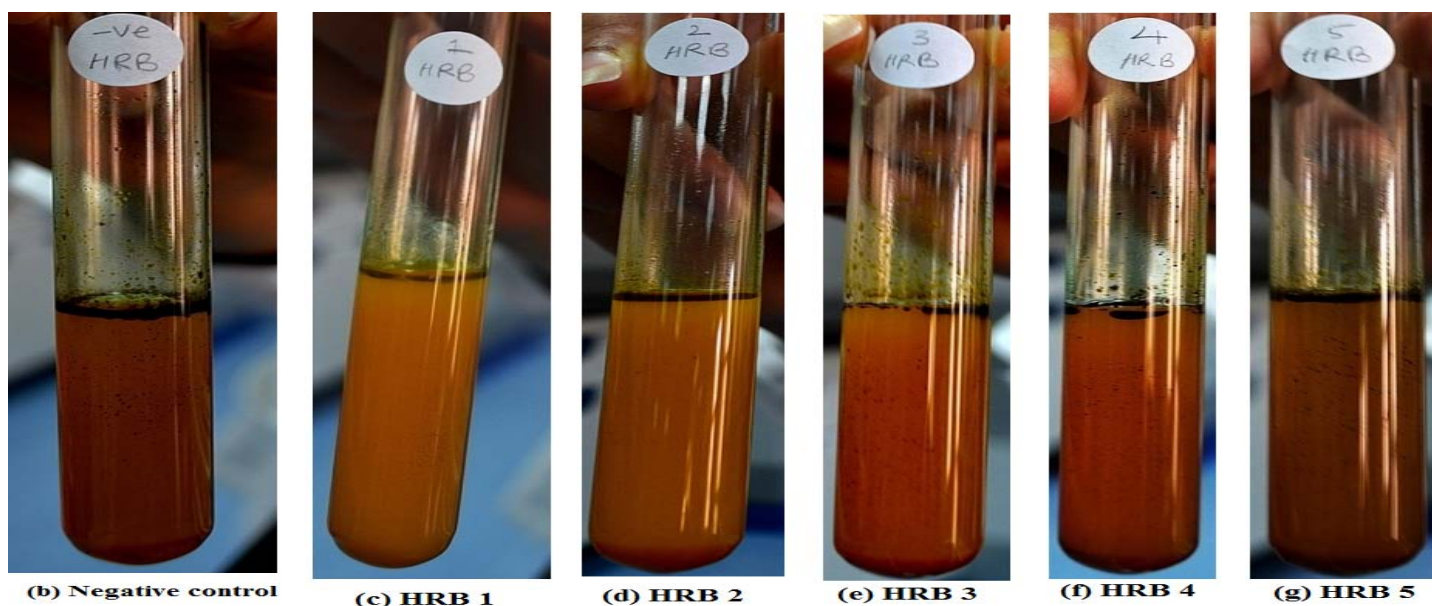


Figure No.9: Results of the evaluation of anti-acne property (antibacterial) of poly herbal extracts.

Sample	HRB 4	HRB 5
Conc. (mg/ml)	120.15	140.96

Table No.5: Effective anti-acne (antibacterial) concentrations of extracts

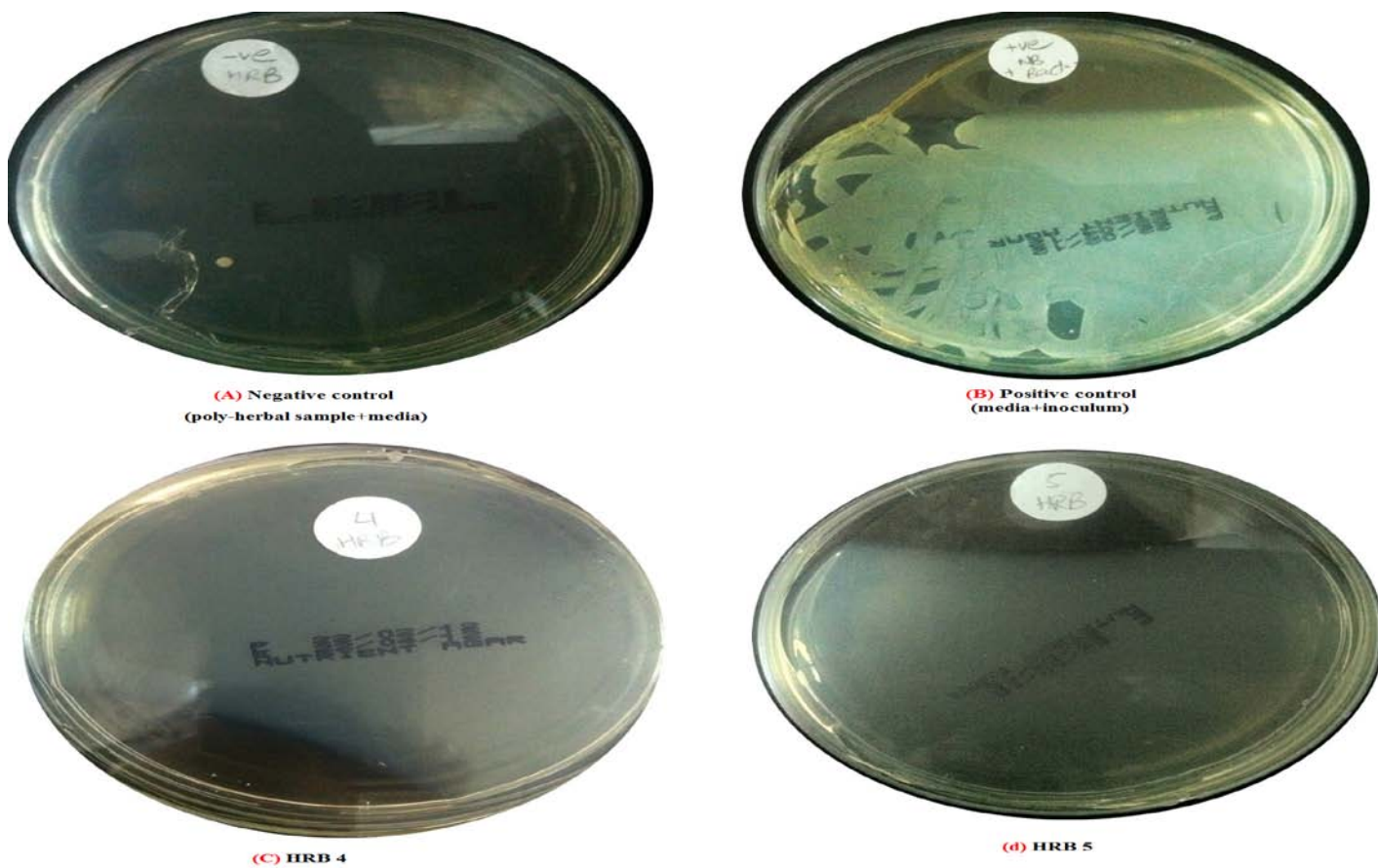


Figure No.10: Results for subculturing the poly herbal extracts (Confirmation test)



(A) Negative control (poly-herbal cream+media)

(B) poly-herbal cream+inoculum

Figure No.11: Results for subculturing the poly herbal Cream (confirmation test)

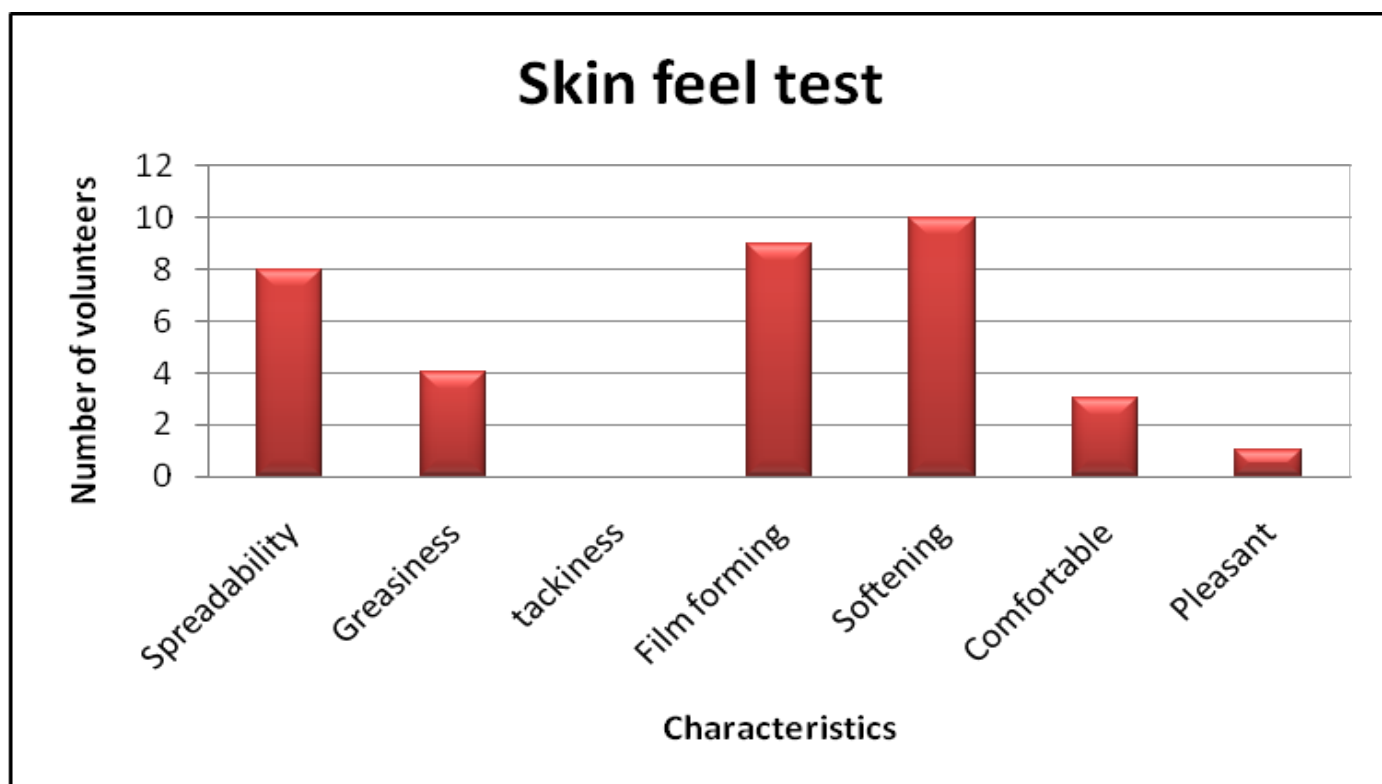


Figure No.12: Bar graph representing results of the skin feel test

DISCUSSION:

In the present research work deals with the evaluation of anti-acne property of alcoholic extract of poly-herbals and cream formulation containing poly herbal alcoholic extract.

Turmeric, thyme, sandalwood and safflower extracts have been used to find out their anti-acne property all together in the form of a poly-herbal formulation.

In Figure.9: (Results of the evaluation of anti-acne property (antibacterial) of poly herbal extracts) the color of samples was compared to the negative control to check which concentration exhibited antibacterial activity. The color of HRB 4 and HRB 5 samples was similar the negative control, while HRB 1, HRB 2, and HRB 3 showed different colors with turbidity, indicating that there is growth of bacteria (*S.aureus*) when compared to the positive control. In Table.5 :(Effective anti-acne (antibacterial) concentrations of extracts) the table shows the concentrations which exhibited antibacterial activity; HRB4 with 120.15 mg/ml and HRB 5 with 140.96 mg/ml

In Figure.10 :(for subculturing the poly herbal extracts (Confirmation test)) for confirmation of the results; subculturing on nutrient agar plates at 37°C for 24 hours was conducted. The figure shows no growth on both plates of HRB 4 and HRB5 therefore, we can say that HRB 4 and HRB 5 exhibited antibacterial activity against *S.aureus* at concentrations 120.15 and 140.96 (mg/ml) respectively. HRB 4 weight of extract was taken to be used in the formulation as it was the least concentration that is effective.

A cream formulation of 10 grams was prepared with the ingredients in Table.4 containing the same weight of extract from which the sample concentration was effective which is 1.2015 g accounting for 12% of the weight of cream.

Evaluation of the cream was done in terms of antibacterial activity and skin feel test.

In Figure.11 (Results for subculturing the poly herbal Cream (confirmation test)) the figure shows the results for the antibacterial activity test after confirmation, indicating that the cream formulation is effective as no growth was observed in the plate.

In Figure.12 (Bar graph representing results of the skin feel test) results of the skin feel test which was conducted are shown.

As seen the spreadability of the cream was good as 8 people described that it was easily and smoothly applied and moved on the skin. Regarding the greasiness 4 people said the cream felt a bit greasy.

For the tackiness, the cream was not tacky at all as all volunteers expressed that fingers were easily removed without requiring force.

9 volunteers said the cream formed a thin film on the skin, and regarding softening apparently the cream left a soft feeling as it was applied on skin as all volunteers said. Disadvantages were that only 3 volunteers felt that the cream was overall comfortable and this was due to the light yellowish green color the cream left on the skin, another thing that only one volunteer thought that the cream was pleasant as the rest claimed that the smell of the cream was a bit strong and kind of unpleasant.

CONCLUSION:

Acne vulgaris is an extremely common skin disorder that affects virtually all individuals at least once during life. Acne can have important negative psychological consequences for the affected individual, including diminished self-esteem, social withdrawal due to embarrassment and depression. Herbal medications are considered safer than allopathic medicines as allopathic medicines are associated with side effects such as contact allergy, local irritation, scaling, photosensitivity, itching, pruritus, redness, skin peeling etc.

Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones, so herbal anti-acne cream which is non toxic, safe, effective and improves patient compliance by the utilization of herbal extracts would be highly acceptable.

In this study the anti-acne property of each herbal extract was reconfirmed.

The cream formulation was developed, which contains the effective concentration of poly herbal extract. The anti-acne property of developed formulation was evaluated by in vitro method of anti-bacterial activity (broth dilution method and sub culturing method)

The characteristics of cream in terms of spreadability, greasiness, tackiness, film forming, softening, comfortable and pleasant were analyzed by skin feel test. The results proved that the chosen formulation also having the effective anti-acne property. So we can suggest the further investigation and in vivo studies will help in developing this formulation as marketed product.

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