

Development of Poly Herbal Cream Formulations Containing Wild Growing Omani Medicinal Plants

ABSTRACT

Acne is derived from the Greek word “acme” which means “prime of life”. It is mostly considered to be benign and self-limiting condition but if it is ignored than it can progress to lifetime presence and scarring of the skin. Almost 85% of adolescence and preadolescence are affected by acne and from here males are at higher ration that female due to testosterone level that causes increase size in sebaceous gland which in turn causes more production of sebum (oil production). Women are affected more during adulthood due to over use of oil based cosmetics and premenstrual hormones. Acne starts to decline sharply after the age of late 40s. There are many different factors that contribute to acne formation and it has many different forms. Herbal medication are considered safer than allopathic medicines because allopathic medicines are associated with various side effects such as like contact allergy, local irritation, scaling, photosensitivity, itching, redness, skin peeling, necrosis of the skin etc. In this study there are 3 herbal plants were used to prepare cream formulation that were used to investigate the anti-acne property. The aim of this research work was to develop formulations and to perform its evaluation as an herbal anti-acne cream. The objective of this study was to focus on the ant-acne or anti-bacterial effect of the formulated polyherbal cream containing three plants, thyme, aloe Vera and basil. The results showed that the formulation 10 showed potential anti-acne property had that twice more concentration of aloe Vera than basil and thyme.

KEY WORDS: Anti-Acne, cream formulation, evaluation of anti-acne property, medicinal plants in Oman.

INTRODUCTION:

There are total 5 types of acne which are acne vulgaris, acne rosacea, acne conglobate, acne fulminant, and pyrodermafaciale. Among these acne vulgaris is the most common affecting the population^{1, 2}. The scarring is a normal body phenomenon to compromise the cell damage that has taken place. It is usually the result of collagen production to heal the skin damage. Patient with moderate to severe type acne have severe scarring where else the mild acne have mostly color change³.

In case of acne vulgaris, how much active ingredient is important that much the vehicle is also important as one of the reason for acne is increased sebum production and also the after effect of acne may causes scars on the

skin that may cause dryness⁴. Dryness leads to more skin itch and irritation which is solved by the greasy feeling of the cream as it not only causes skin moisture but also prevent water loss. Creams works best for dry skin which is not accomplished by gels.^{5,6}

In terms of epidemiology, Sebum is an oily substance that composes of mixture of different types of lipids and phospholipids along with triglycerides and sterols. Its production rate is more in males than in females during the adolescence and declines in production as they age due to decrease in secondary hormones such as testosterone in males and estrogen in females. It is this environment that helps in growth of *Propionibacterium acnes*, the major type of bacteria that is known to be the cause of acne along with other minor ones.⁷

Even though the main pathogen for acne is *Propionibacterium acnes*, there are other that showed to cause acne in some studies eg: *Staphylococcus epidermis* and *Staphylococcus aureus*^{8,9}. Some studies also showed that *Staphylococcus aureus* is the main cause instead of *Propionibacterium acnes* as it was more isolated from acne patient in an in-vitro studies which contradicts the known information on *Propionibacterium acnes*¹⁰.

The present research work was designed with the aim to develop formulation and to perform its evaluation as an herbal anti-acne cream. The plant materials used for the formulations were extract of leaves of Basil, Thyme and gel of Aloe-Vera.

The developed formulations were evaluated for their physical as well as microbiological characteristic activity against *Staphylococcus Aureus*.

MATERIALS & METHODS

Plants

Aloe Vera, Thyme, Basil. They were purchased from a local herbal store in Seeb.

Solvents: Methanol, Distilled water

Table 1: Amount of herbs and extracts

A: Aloe vera, B : Basil, T: Thyme

Formulation	Form of herbs	Ratio (A:B:T)	Amount (A:B:T)
F1	Dry powder (B+T) gel of A	16:1:1	1ml : 0.062g : 0.062g
F2	Dry powder (B+T) gel of A	32:1:1	2ml : 0.062g : 0.062g
F3	Dry powder (B+T) gel of A	16:1.6:1	1ml : 0.1g : 0.062g
F4	Dry powder (B+T) gel of A	16:1:1.6	1ml : 0.062g : 0.1g
F5	Oil extract(B+T) gel of A	1:2:1	0.5ml : 1ml : 0.5ml
F6	Oil extract(B+T) gel of A	1:1:2	0.5ml : 0.5ml : 1ml
F7	Oil extract(B+T) gel of A	2:1:1	1ml : 0.5ml : 0.5ml
F8	Methanolic extract (B+T) gel of A	1:2:1	1ml : 2.003g : 1.004g
F9	Methanolic extract (B+T) gel of A	1:1:2	1ml : 1.014g : 2.020g
F10	Methanolic extract (B+T) gel of A	2:1:1	2ml : 1.032g : 1.005g

PROCEDURE:

Table 1 represented the composition of pure herbs, their oil extracts and mathanolic extract.

- 1) The dried leaves of thyme and basil were pulverized to get course powder and different ratio of these two powders were weighed for three preparing batches. F8, F9, and F10 represents three different batches contains various proportion as per the Table No: 1.
- 2) Aloe vera gel was collected in a 10ml measuring cylinder by cutting through the leave and squeezing it.

- 3) Three separate 100ml beaker was taken with different weight of each sample and to these beakers of polyherbal mixture, 20ml of methanol was added.
- 4) The beakers were heated on a hot plate at 60-80 degree Celsius until bubbles started to appear and methanol started to evaporate.
- 5) The mixture was filtered out and the filtrate was dried under room temperature for several hours until all the methanol evaporated and left behind the semisolid methanolic extracts of the samples.
- 6) The extracts were weight and kept for further use

PREPARATION OF POLYHERBAL CREAM.

Excipients used in the cream formulation: Steric acid, Glycerin, Methanolic extracts of polyherbs, Potassium hydroxide, Methyl paraben, Propyl paraben, Distilled water

Procedure for Preparation of polyherbal cream¹¹:

- 1) Accurately weighed amount of stearic acid was taken in a 100 ml beaker and kept on water bath at 80°C.
- 2) All the oils were dissolved in melted stearic acid which was introduced with the help of dropper.
- 3) Potassium hydroxide, methyl paraben and propyl paraben were weighed and dissolved in water in a separate 100ml beaker which was also kept at same temperature.
- 4) The aqueous solution was added slowly in to the stearic acid with stirring and allowed the mixture to cool.
- 5) To this cream the previously weighed amount of total methanolic extract (table 2) was added slowly with homogenous stirring.
- 6) The prepared cream was filled in an aluminum wrapped glass container and the openings were sealed well.

Table No 2 represents the total amount of extracts in batches of F8, F9 and F10.

Plants	Extract sample 1/F8	Extract sample 2/F9	Extracted sample 3/F10
Aloe Vera	1ml	1ml	2ml
Basil	2.003g	1.014g	1.032g
Thyme	1.004g	2.020g	1.005g
Total sample weight extracted	0.2601g	0.3103g	0.1802g

Table No 3: Composition of formulation of polyherbal cream

Sr. No	Ingredient	Quantity for 2g		
		F8	F9	F10
1	Amount of total methanolicextract	0.2601g	0.3103g	0.1802g

2	Steric acid	0.376g	0.376g	0.376g
3	Glycerin	0.0768g	0.0768g	0.0768g
4	Potassium hydroxide	0.024g	0.024g	0.024g
5	Methyl praben	0.004mg	0.004mg	0.004mg
6	Propyl paraben	0.0008mg	0.0008mg	0.0008mg
7	Distilled water	1.32g	1.32g	1.32g

EVALUATION OF ANTI-ACNE PROPERTY (ANTIBACTERIAL ACTIVITY)

1) Evaluation of anti-acne property of dry herbs:

Microorganism and Media:

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

Sterilization Procedure:

All equipment required for this test was autoclaved at 120°C and the loop was sterilized by hot flame.

Microbial Evaluation Procedure:

The antibacterial activities of different methanolic extract were determined by modified agar well diffusion method. The base medium used for the screening of antibacterial activity was nutrient agar (NA), and liquid nutrient broth (NB) was used to ensure the growth of the bacteria.

The well diffusion technique was performed for each extract sample as following:

- A cotton bud was soaked in the liquid nutrient broth (NB) which contains the test organism to pick up some colonies of the *Staphylococcus aureus* (test organism).
- A streaking motion was used to spread the bacteria properly on the nutrient agar (NA) plate.

- A sterile loop and 8 mm borer were used to cut wells in the nutrient agar (NA) plate.

3 mg of thyme and basil were taken and made into paste form separately with the help of distilled water. These pastes were inserted into the wells of nutrient agar.

As aloe vera was in gel form, it had to be mixed with warm beeswax to increase the viscosity along with glycerin and mixture was placed into the well.

- The plate was incubated at 37°C for 24 hours.
- The anti-acne activity was evaluated by measuring the diameter of zone of inhibition in mm. (Table No 4)

2) EVALUATION OF ANTI-ACNE PROPERTY OF METHANOLIC EXTRACTS (F8, F9 AND F10):

Microorganism and Media:

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

Sterilization Procedure:

All equipment required for this test was autoclaved at 120°C and the loop was sterilized by hot flame.

Microbial Evaluation Procedure:

The antibacterial activities of different methanolic extract were determined by modified agar well diffusion method. The base medium used for the screening of antibacterial activity was nutrient agar (NA), and liquid nutrient broth (NB) was used to ensure the growth of the bacteria.

The well diffusion technique was performed for each extract sample as following:

- A cotton bud was soaked in the liquid nutrient broth (NB) which contains the test organism to pick up some colonies of the *Staphylococcus aureus* (test organism).
- A streaking motion was used to spread the bacteria properly on the nutrient agar (NA) plate.
- A sterile loop and 8 mm borer were used to cut wells in the nutrient agar (NA) plate.
- A small circle with 1 cm diameter was drawn in the filter paper and then it was cut.

- The piece of filter paper was soaked in the methanolic extract beaker and put in the nutrient agar (NA) plate. The plate was incubated at 37°C for 24 hours.
- The anti-acne activity was evaluated by measuring the diameter of zone of inhibition in mm. (Table No 5)

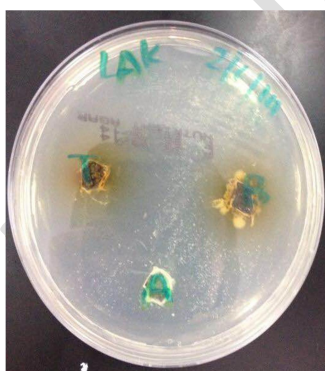
RESULTS:

1) Results of evaluation of anti-acne property of dry herbs

Table 4: Zone of inhibition for raw herbs

Herbs	Zone of inhibition in cm
Aloe vera	2.5
Basil	3
Thyme	4

Figure 1: subculture of raw herb paste



2) Results of evaluation of anti-acne property of methanolic extracts

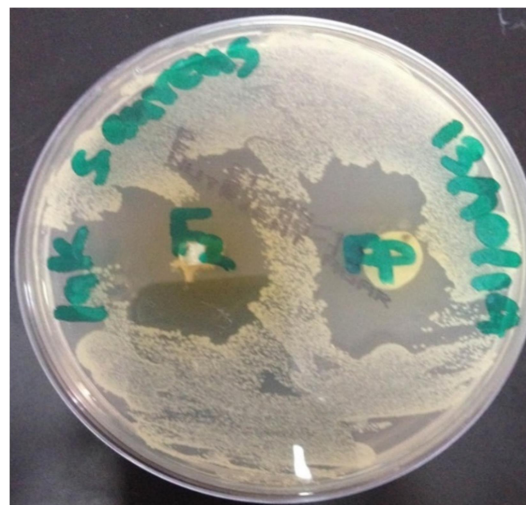


Figure 2: subculture of polyherbalmethanolic extract and its cream formulation

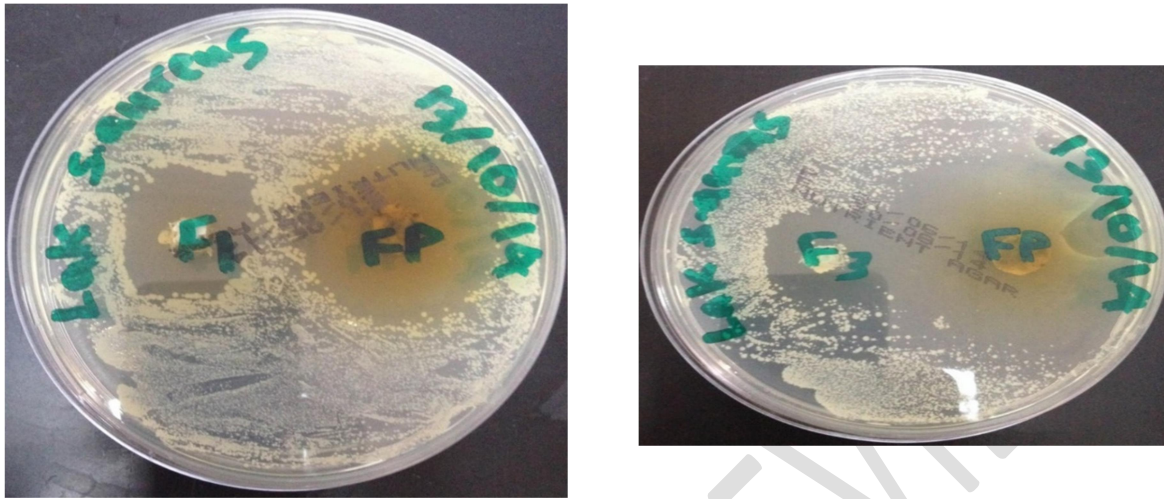


Table 5: Zone of inhibition for mixture of polyherbalmethanolic extract

Beaker of poly-herbal extract	Zone of inhibition in cm
1	3.1
2	2
3	4.2

3) Results of evaluation of anti-acne property for all cream formulations.

Formulation / Batch (Code)	PH	Appearance Homogeneity /Colour)	spreadability	Greasiness & Tackiness	Consistency	Film coating	Softening	Zone Of Inhibition (mm)
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F4			0					Poor
F5			0.1					Poor
F6			0					Poor
F7			0.15					Poor
F8			2.1					Good
F9			2.3					Good
F10			2.5					Good

Indication: poor < 1cm, moderate 1-2 cm, good >2cm

Table No: 6: summary of zone of inhibition of all the cream formulations

Marketed Clindamycin in 1% gel	7.0 5	Colorless	Perfect	Not	Gel	No t	Soft	32 mm
Blank cream	7.4 4	White	Goo d	Not	Semi- solid	No t	Very soft	No zone
Extract of F8	–	Dark green	–	–	Semi- solid	–	–	31 mm
Extract of F9	–	Dark green	–	–	Semi- solid	–	–	20 mm
Extract of F10	–	Dark green	–	–	Semi- solid	–	–	42 mm
Formulation (8)	6.7 2	Pale green	Goo d	Not	Semi- solid	No t	Soft	18 mm
Formulation (9)	6.8	Pale green	Goo d	Not	Semi- solid	No t	Very soft	23 mm
Formulation (10)	7.4	Pale green	Goo d	Not	Semi- solid	No t	Very soft	6 mm

DISCUSSION

In the treatment of acne herbal medications are considered safer than allopathic medicines due to the fact that allopathic medicines are associated with side effects such as contact allergy, local irritation, scaling, photosensitivity, itching, pruritus, redness, skin peeling, necrosis of the skin etc. Acne vulgaris is an extremely common skin disorders that affects virtually all individuals at least once during life.

The present research work was designed with the aim to develop formulation and to perform its evaluation as an herbal anti-acne cream. The plant materials used for the formulations were extract of leaves of Basil, Thyme and gel of Aloe-Vera.

The developed formulations were evaluated for their physical as well as microbiological characteristic activity against *Staphylococcus Aureus*.

The characteristics of cream in terms of appearance, pH, viscosity and spread-ability were analyzed by reported method.

The results proved that the prepared formulations are also having the acceptable property. The zones of inhibitions for the anti-acne activity were compared with the herbal marketed preparation for acne vulgaris.

All the formulations (F8, F9, and F10) have shown effective zones of inhibitions but formulation 10 has shown comparable zones of inhibitions to that of the marketed preparation. This suggests that the active ingredients of the formulations may have contributory antibacterial activity.

In table 1, represents all the forms of herbs in different ratios and its oil extract as well as methanolic extract.

The table was designed to optimize the maximum anti-acne property for each herb, which was taken as maximum quantity in 3 different batches. F8 contained more basil than the other two herbs where else F9 contained more thyme and F10 contained more aloe vera than the rest.

In table 2, the sample weight of herbs being used in methanolic extraction was shown along with total amount of extract result. Three separate beakers were used that each contained mixture of different ratios of the three herbs along with specific amount of methanol (20ml). The exact amount of herbs showed in table 1.

Table 5, represents the zone of inhibition of poly-herbal methanolic extract (table 2). This was done to test the poly-herb's (combination of all three plants in different proportions) antibacterial property and potential effect.

According to the results beaker 3(F10) had the most zone of inhibition having 4.2cm that contained high concentration of aloe-vera and beaker 2(F9) showed the least zone of inhibition of 2cm that contained high amount of thyme.

Even though the raw form of alone aloe-vera had the least zone of inhibition but when it was combined with basil and thyme for methanolic extraction, the extracted combination had the most zone of inhibition.

Expected result from beaker 2(F9) was high because alone raw form of thyme showed the highest zone of inhibition, and beaker 2 contained double of thyme's concentration in the combination but the result was opposite having the least zone of inhibition. The anti-bacterial property of thyme was decreased when it was combined with aloe vera and basil.

Figure 4, showed the subcultural result of polyherbalmethanolic extract and its cream formulation. Fig 4i and 4iii, both have their methanolic extract zone of inhibition (fp=filter paper) more than its cream (F1 and F3). Fig 4ii, zone of inhibition for methanolic extract and its cream was almost the same.

Table 3, shows the blank cream composition that was used for most of the blank cream formulation except for cream formulation with oil. In this decreased the amount of steric acid was used to balance with the aqueous composition of the cream.

Table 4, The zone of inhibition of raw herb's anti-acne property in their paste form was presented. This was done to confirm the antibacterial property of each plant before they are formulated into cream. According to the results, thyme had the most antibacterial property as its zone of inhibition that is the highest being 4cm and least is aloe vera being 2.5cm.

Figure 3, showed the subculture (anti-bacterial evaluation in table 4) results of anti-acne property of raw herb's paste form

In table 6, it showed that the zone of inhibition of all the cream that had been formulated with different type of plants forms (oil, dry powder, methanolic extract). Also indicates the indication of its bacterial property based on its zone of inhibition. Most of it having zero antibacterial property except the last three formulation.

Three separate blank creams were prepared and to this accurately weighed amount of the plant were added and mixed vigorously. These creams were evaluated for anti-microbial property but showed no zone of inhibition in formation 1 to formulation 4.

Oil extracts of these plants that were purchased from local store, few drops of these oils were added to the final cream and tested for anti-microbial properties. It showed minimum zone of inhibition.

The weight of steric acid was decreased as the oily composition was more than the aqueous composition but this also showed minimum effect. Formulation 5 to 7

The final cream formulation prepared with methanolic extracts. These showed potential anti-microbial properties as the phytochemical components in the plants were water soluble (confirmation after the above failed cream and also literature survey) for which the phytochemical diffused through the cream in to the agar plate (anti-microbial test). Formulation form 8 to 10.

Table 7, represents the complete physical and microbial evaluation of marketed cream, clindamycin being the standard medicated cream and methanolic extracts and their cream formulation (F8, F9 and F10)

With the help of pH meter it was observed that clindamycin had pH of almost neutral 7, the closest to this pH was first cream formulation 9 which is pH 6.8 and then second cream formulation 10 that has pH 7.4.

Formulation 10 has the highest zone of inhibition of 26 mm that F8 and F9 making its anti-acne property closest to the standard cream, clindamycin that has 31mm of zone of inhibition.

The overall physical evaluation of the extracts and creams were good and acceptable.

Considering all the results from the test and feedback from volunteers, it was noted that cream formulation 10 was best accepted and has good potential.

CONCLUSION

Herbal plants have been traditionally used over centuries before even synthetic drug came to this era. It is believed to have more pharmacological action than toxicity effect. Many drugs of today's date have been evolved from the plants chemical component. Scientists are on the research of developing more drugs from herbals due to the bacterial resistance towards the antibiotics.

Herbal plants are taken either as direct dry powder or making infusion out of it. It was and still being used for external application as a paste and also as internal. Anti-inflammatory, anti-bacterial, whitening agent, scars are the few uses of herbal medicine that have been reported along with potential anti-acne effect.

Acne has always been one of the most troublesome diseases in teens mostly which affects both males and females. Not only acne leaves physical scars but also mentally disturbs the patient and decreases their self-esteem and confidence.

This study focused on the ant-acne or anti-bacterial effect of the three plants, thyme, aloe Vera and basil. The phytochemical components of these plants were investigated based on the literature survey and formulated polyherbal cream as per the modified procedure.

After completion of evaluation studies, we found that the formulation 10 showed potential anti-acne property had that twice more concentration of aloe vera than basil and thyme.

This shows that aloe vera has more action and its action was potentiated by the combination of the rest.

Not only in-vitro evaluation confirmed the anti-acne property but it was also accepted physically by the volunteers through physical test like odor, wash-ability, spread ability.

In conclusion formulation 10 (Methanolic extract (B+T) gel of A) can be used as anti-acne and make as marketed drug after further investigation of it and also in-vivo evaluation.

NOTE:

The study highlights the efficacy of "Herbal" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

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