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Development and evaluation of herbal cream for the treatment of acne

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Abstract
Acne is a disease characterized by inflammatory and non inflammatory lesions. The pathogenesis includes various factors like hormonal, bacterial and immunological, which causes acne lesions. *Propionibacterium acnes* and *Staphylococcus epidermidis* have been recognized as pus-forming bacteria triggering an inflammation in acne. The present research work deals with formulation and evaluation of herbal cream against this etiologic agent of acne vulgaris. The *boswellia* oil was seperated and formulated into a topica ceram. In vitro antibacterial activity was performed against *P. acnes, S. epidermidis* and *S. aureus*, using agar well diffusion method. The measured zones of inhibitions of the prepared formulations were compared with standard antibiotic (Clindamycin) and standard marketed topical herbal formulation. The prepared creamss were evaluated for pH, viscosity, spreadability, stability, drug content and *in vitro* diffusion. The results from the agar well diffusion showed that *boswellia* oil herbal formulations would inhibit the growth of *P. acnes, S. epidermidis* and creams showed significance antimicrobial activity against these bacteria as compared to standard.

Keywords: Acne vulgaris, P. acne, *boswellia* oil

Introduction
It is a disease characterized by inflammatory and non inflammatory lesions.
The pathogenesis includes various factors like hormonal, bacterial and immunological, which causes acne lesions. The term acne is obtained from Greek language “acme” which means prime of life. Acne is not a serious type of disease but it impacts the life quality of the patient. This is benign condition can cause severe psychological problems. It can show its symptoms and come out in any stage of life but mostly it affects at age 12-24, the estimated population is around 85% [1]. There are number of drugs available for the treatment of lesions, and its scar, but having some side effects. To reduce the side effects & to enhance the efficacy some fixed dose combinations are used. Medicinal herbs as a potential source of therapeutic aids have attained a significant role in health system all over the world for both humans and animals, not only in the diseased condition but also as potential material for maintaining good health. Since time immemorial, medicinal plants, natures’s hidden and to a large extent unexplored treasure, have been used virtually in all human cultures around the world (over 75 % of the population) as a source of safe and effective medicines [2].
Boswellia serrata is the oleogum resin obtained by incision or produced by spontaneuos exudation from the stem and branches of Boswellia serrata Roxb. (Fam. Burseraceae). Salai guggal is an oleo-gum-resin obtained from Boswellia serrata. It is also known as frankincense in English and Olibanum in arabian. This tree abundantly growing in dry hilly tracts of the India which has been used for variety of the therapeutic purpose such as cancer, inflammation, arthritis, asthma, psoriasis, colitis, crohn’s disease and hyperlipidemia [3-4].
This oil is soluble in colophony & dammer, but more volatile in nature. Gum is mainly composed of arabinose with small amounts of xylose and galactose. Gum als conatins oxidizing and diastatic enzyes. The highly brittle resin is soluble in various organic solvents. It softens between 65-72°C and melts between 73-78°C. Resin is mainly employed in preparation of varnishes. Indian olibanum contains β-boswellic acid in resin portion, Volatile oil contains P-cymene, α-limonene, terpinolene, α-thujone, α-thujone, α-phellandrene, α-terpiol, bornyl acetate, and methyl chacicol. A diterpene alcohol viz. serratol has been reported from gum resin. The fixed oil is usually pale yellow in colour and has an agreeable odour. Essential oil is obtained in yield of up to 16% oleo-gum-resin by steam distillation [5].

Materials and Methods
Collection and authentification
The boswellia gum was collected from local market of Bhopal, Madhya Pradesh and authentification was done at Agarkar Research institute Pune.

Extraction of boswellia oil from gum

The shade dried boswellia gums were finely powdered and boswellia oil was extracted by using Clevenger apparatus, through hydro distillation method with a flow rate of 4 ml/min for 4 hrs. Percentage yield of boswellia gum oil was found as 2.8% [7-10].

Phytochemical screening

Phytochemical screening of boswellia serrata oil was done and results were shown in table no.1.

Development of herbal anti acne cream

Three formulations of herbal anti acne cream were prepared containing varied quantity volatile oils (F4A, F4B, F4C) as in Table 7. 4 and the formulations were checked for antimicrobial activity against *Propionibacterium acnes* and *Staphylococcus epidermidis.*

Table 1: Concentration of various ingredients in different formulations

Ingredients	Formulation code		
	F4A	F4B	F4C
Bees wax	16.67%	16.67%	16.67%
Mineral oil	50%	50%	50%
Borax	0.83%	0.83%	0.83%
Water	32.5	32.5	32.5
Methyl paraben	0.5%	0.5%	0.5%
Rose oil	0.5%	0.5%	0.5%
Boswellia oil	1%	2%	4%

Procedure

- Melt the bees wax and mineral oil (almond oil) together and bring to a temp to 70^0c.
- Dissolve borax in water and bring the temp of this solution to 70^0c.
- Add water phase to oil phase with rapid stirring.
- After the addition of water, agitated slowly while cooling.
- Add the perfume and preservative at the temp at 50^0c.
- Fill into the jars when cream has cooled to 42^0c.

Evaluation of cream

1. Appearance

- All developed cream formulations were inspected for their physical appearance, which consist of Colour- The colour of all the formulations was checked against white background.
- Consistency- The consistency was checked by applying on the skin.
- Greasiness- The greasiness was assessed by the application on to the skin.
- Odour- The odour of the cream was checked by mixing the cream in water and taking the smell [11].

2. pH determination

The pH of the cream was determined using digital pH meter. The readings were taken for average of 3 times. The pH values of all developed formulations were in the range of 6-7 which is considered acceptable to avoid the irritation upon application to the skin. pH found which is well within the limits of skin pH i.e 5.6-7.5. Hence it was concluded that all the formulation could not produce any local irritation to the skin. Result are shown in table no.6 [12]

3. Homogeneity

All developed gels were tested for homogeneity by visual inspection after the creams have been set in the container. They were tested for their appearance and presence of any aggregates [13].

4. Viscosity

Viscosity of the cream was measured by using Brookfield viscometer. DVII model with a T-bar spindle in combination with a helipath stand. Spindle T 95 was used for the measurement of viscosity of all the gels. The viscosity was measured using 50 gm of cream filled in a 100ml beaker. The T-bar spindle (T 95) was lowered perpendicular in the center taking care that spindle does not touch the bottom of the jar.

Fig 1: Brookfield viscometer

5. Measurement of viscosity

The T-bar spindle (T95) was used for determining the viscosity of the creams. The factor like temp, pressure and sample size etc. which affect the viscosity was maintained constant during the process. The helipath T-bar spindle was moved up and down giving viscosities at number of points along the path. The torque reading was always greater than 10%. The average of three readings taken in one minute was noted as the viscosity of creams [13].

6. Spreadability

- Spreadability of formulations was determined by an apparatus suggested by Multimer *et al*. which was fabricated in laboratory and used for study. The apparatus consist of a wooden block, with a fixed glass slide and movable glass slide with one end tied to weight pan rolled on the pulley, which was in horizontal level with fixed slide.

Procedure

An excess of cream sample 2.5 g was placed between two glass slides and a 1000g weight was placed on slides for 5 minutes to compress the sample to a uniform thickness. Weight (60g) was added to the pan. The time (seconds) required to separate the two slides was taken as a measure of spreadability.

It was calculated using the formula,

$S = m.l / t$

Where, S -Spreadability in g.cm / sec; m - Weight tied to upper slide; l - Length of glass slide; t - Time in seconds Length of glass slide was 11.3 cm and weight tied to upper slide was (60g) throughout the experiment [14].

7. *In vitro*- diffusion study

In-vitro diffusion studies the in-vitro diffusion studies for all formulations (F1-F8) were carried out using the Franz-diffusion cell. The diffusion cell apparatus was fabricated as an open ended cylindrical tube. A weighed quantity of formulation equivalent to 1gm of the drug was placed onto the dialysis membrane-70 (Hi- Media) and was immersed slightly in 100ml of receptor medium (phosphate buffer pH 6.8+ethanol in ratio 40:60) which was continuously stirred and the temperature was maintained at 37±1°C. Aliquots of 1ml were withdrawn from each of the system at time intervals of 5, 10, 15, 30, 60, 120, 240, and 360 minutes were analyzed for drug content using ultraviolet spectrophotometer. Readings were recorded in table 7 [15].

8. Stability studies as per ICH guidelines

The stability studies were carried out in all formulations at different temperature conditions (4°, 25° and 37°C) for 3 months. All the evaluation parameters i.e. pH, viscosity, spreadability, drug contents, consistency and phase separation studied at different time intervals i.e. 15th, 30th, 60thand 90th days. By using stability chamber model no.CHM-6-PLUS. Results were shown in table no.4, 5, 6 [16-17].

9. Antiacne activity of Herbal formulations of *Boswellia serrata* Oil.

Microorganism

Clinical isolates of Staphylococcus epidermidis MTCC NO 435, propionibacterium acnes MTCC NO 1951 authentic bacterial strains were used in the studies which are procured from Institute of Microbial Technology (MTCC), SECTOR 39 A, Chandigarh.

Herbal formulations of *Boswellia serrata* oil were tested against P. acnes and S. epidermidis by agar well diffusion method. The formulations were prepared by using DMSO as a solvent. P. acnes was incubated in ASLA agar medium under anaerobic conditions. Agar surface of each plate was streaked by using sterile cotton swab with the reference bacterial strain. Agar plate was punched with a sterile cork borer of 4 mm size and 100 µL of herbal formulations of various concentrations (50 mg/mL, 100 mg/mL, 200 mg/mL) were poured with micropipette in each bore. The plates were allowed to stand for 30 min. Then the plates were incubated at 37°C for 48 hrs under anaerobic conditions.

Similarly freshly grown culture of s. epidermidis was diluted with 15-20 mL of Mueller-Hinton agar on glass petri plates of same sizes and allowed to solidify. Agar surface of each plate was streaked by using sterile cotton swab with the reference bacterial strain. Agar plate was punched with a sterile cork borer of 4 mm size and 100 µL formulations of various concentrations (50 mg/mL, 100 mg/mL, 200 mg/mL) were poured with micropipette in each bore. The plates were allowed to stand for 30 min. Then the plates were incubated at 37°C for 24 hrs under aerobic conditions. The control Tetracycline disc (30µg/mL) and dalacin™ (1% clindamycin phosphate solution) were also run simultaneously.

After incubation period the zone of inhibition in mm was measured to determine the anti-acne activity of formulations. Results were shown in table no.9 [18-21].

Result and Discussion

Table 2: Phytochemical screening of *boswellia serrata* gum oil.

Test	Boswellia oil
Carbohydrate	-
Proteins	-
Amino acid	+
Fat & oil	+
Steroids	+
Volatile oil	+
Alkaloids	+
Glycosides	-
Phenolic compound	+
vitamin	-
terpene	+
Flavonoids	+
Tannins	+

Table 3: Evaluation of cream

Formulation	F4-A	F4-B	F4-C
Colour	cream	cream	cream
Odour	bitter	bitter	bitter
Appearance	glossy	glossy	glossy
Consistency	excellent	excellent	excellent
pH[a]	7.0±0.05	7.0±0.05	7.0±0.05
Viscosity (cps)	38.4±0.4	38.4±0.4	38.4±0.4
Spreadability (g/sec)	46.0±0.5	46.0±0.5	46.0±0.5
Extrudability (g)	543.4±0.4	543.8±0.4	543.9±0.4

a=mean±standard deviation,
All experiments were performed in triplicate

Table 4: Stability Study of prepared formulations for one month (30days)

Sr. No.	Formulations	F4-A] B.Serrata oil(API-1%)	F4-B] B.Serrata oil(API-2%)	F4-C] B.Serrata oil(API-4%)
1.	Colour	cream	cream	cream
2.	Odour	bitter	bitter	bitter
3.	Appearance	glossy translucent	glossy translucent	Glossy translucent
4.	Consistency	excellent	excellent	excellent
5.	pH	7.3±0.02	7.2±0.05	7.4±0.05
6.	Viscosity (cps)	38.3±0.2	38.2±0.1	38.6±0.5
7.	Spreadability (g/sec)	46.3±0.2	46.2±0.1	46.1±0.3
8.	Extrudability (g)	543.2±0.2	543.2±0.1	543.4±0.3
9.	Drug content	92.36	91.5	93.2

Table 5: Stability Study of prepared formulations for two months (60days)

Sr. No.	Formulations	F4-A] *B.Serrata oil*(API-1%)	F4-B] *B.Serrata oil*(API-2%)	F4-C] *B.Serrata oil*(API-4%)
1.	Colour	cream	cream	cream
2.	Odour	bitter	bitter	bitter
3.	Appearance	glossy translucent	glossy translucent	glossy translucent

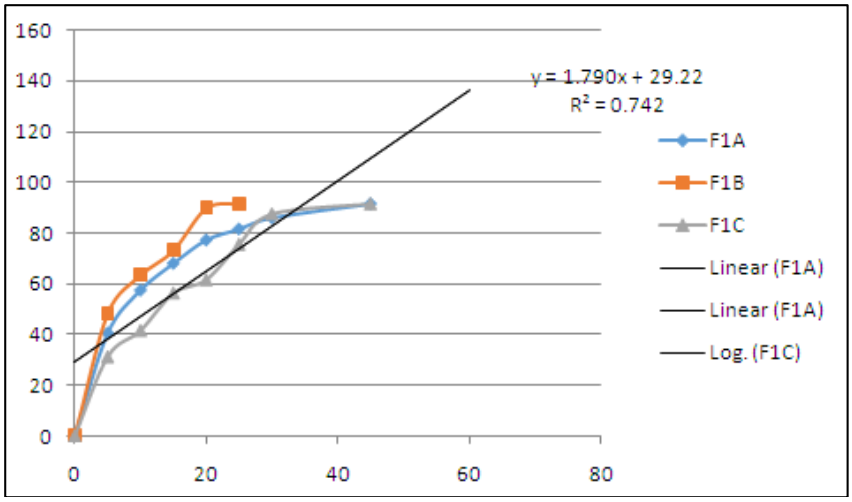
4.	Consistency	excellent	excellent	excellent
5.	pH[a]	7.2±0.01	7.0±0.03	7.5±0.02
6.	Viscosity (cps)	38.3±0.2	38.1±0.5	38.6±0.4
7.	Spreadability	46.1±0.3	46.2±0.2	46.3±0.5
8.	Extrudability (g)	543.2±0.2	543.3±0.3	543.9±0.4
9.	Drug content	91.3	90.0	91.2

Table 6: Stability Study of prepared formulations for three months (90days)

Sr. No.	Formulations	F4-A] B.Serrata oil(API-1%)	F4-B] B.Serrata oil(API-2%)	F4-C] B.Serrata oil(API-4%)
1	Colour	cream	cream	cream
2	Odour	bitter	bitter	bitter
3	Appearance	glossy translucent	glossy translucent	glossy translucent
4	Consistency	excellent	excellent	excellent
5	pH[a]	7.3±0.02	7.2±0.05	7.1±0.08
6	Viscosity (cps)	38.3±0.2	38.2±0.3	38.1±0.2
7	Spreadability (g/sec)	46.8±0.3	46.5±0.2	46.7±0.3
8	Extrudability (g)	543.3±0.2	543.1±0.7	543.6±0.3
9	Drug content	91.01	90.21	91.30

Table 7: In vitro- diffusion study of Boswellia Serrata oil

Time	F4A	F4B	F4C
0	0	0	0
5	40.1	41.99	46.67
10	60.16	53.4	56.53
15	61.13	61.983	69.3
20	71.21	78.63	78.2
25	84.4	83.29	91.87
30	90.37	86.56	92.35
45	91.44	91.81	93.12



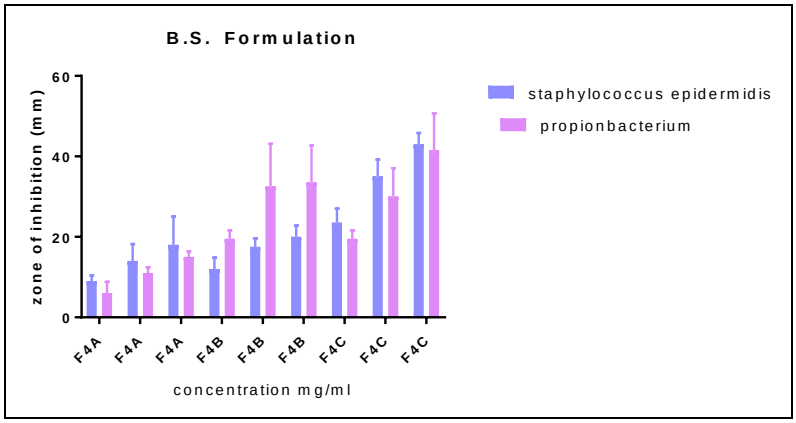
Graph 1: In-vitro drug release for *Boswellia serrata* oil

Table 8: Drug content *in vitro* release

Formulation	Drug content (%m/m)	% cumulative release
F1A	94.68	91
F1B	93.31	92
F1C	92.17	93
F2A	94.0	92
F2B	94.6	93
F2C	95.1	94
F3A	93.21	90
F3B	92.10	91
F3C	93.18	91
F4A	93.2	90
F4B	91.5	88
F4C	92.36	90

Table 9: Anti acne evaluation of all the formulations of B.S. oil

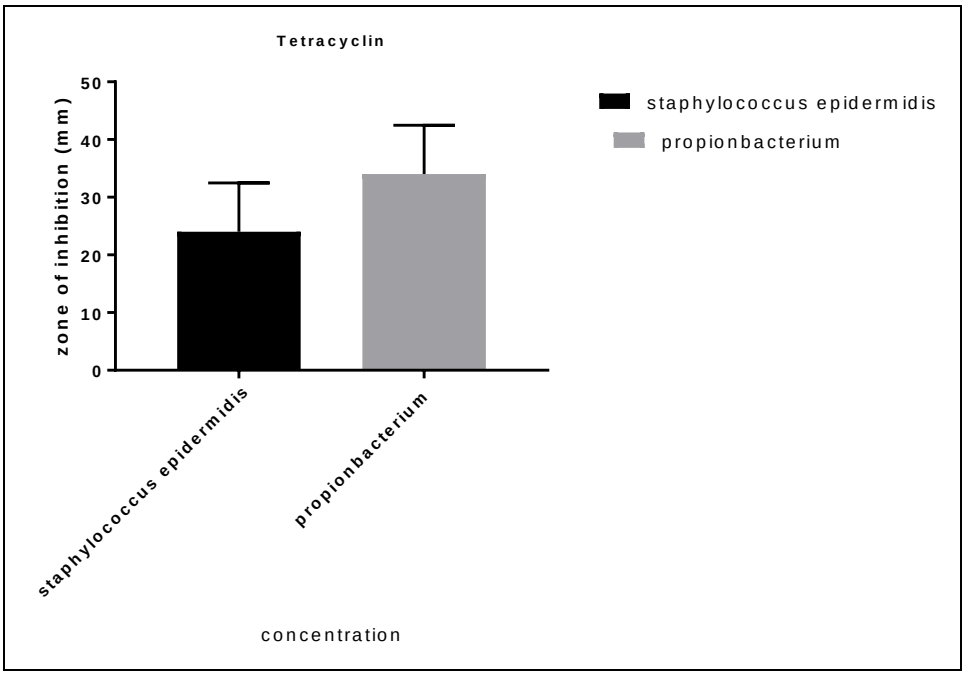
Sr. No.	concentration	*Staphylococcus epidermidis*	Propionbacterium acne
F4A	50	9.000±1.414	6.000±2.828
F4A	100	14.000±4.243	11.000±1.414
F4A	200	18.000±7.071	15.000±1.414
F4B	50	12.000±2.828	19.500±2.121
F4B	100	17.500±2.121	32.500±10.606
F4B	200	20.000±2.828	33.500±9.192
F4C	50	23.500±3.536	19.500±2.121
F4C	100	35.000±4.243	30.000±7.071
F4C	200	43.000±2.828	41.500±9.192

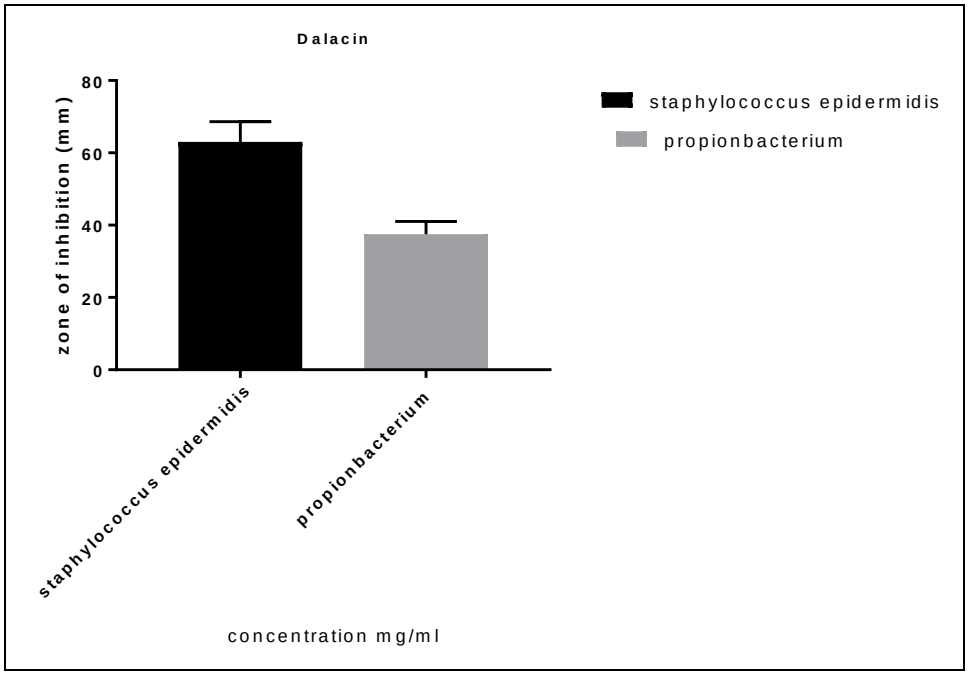


Graph 2: Anti acne evaluation of all the formulations.

Table 10: Concentration, staphylococcus epidermidis and propionbacterium

Sr. No.	Concentration	Staphylococcus epidermidis	Propionibacterium
TETRACYCLIN	30.000	24.000±8.485	34.000±8.485
DALACIN	10.000	63.000±5.656	37.500±3.536





Discussion

The present investigate reveals' that *a Boswellia serrata* oil formulation (F4A, F4B, F4C) shows greater antimicrobial activity against acne causing bacteria. F4A had shown the zone of inhibition of 6.000±2.828, 11.000±1.414, 15.000±1.414, at concentration(50 mg/ml,100 mg/ml, 200 mg/ml) respectively against *P. acne* but less inhibition 9.000±1.414, 14.000±4.243, 18.000±7.071, at concentration (50 mg/ml 100 mg/ml, 200 mg/ml) respectively against *S. epidermidis.* F4B had shown the zone of inhibition of 19.500±2.121, 32.500±10.606 and 33.500±9.192 at concentration(50 mg/ml, 100 mg/ml, 200 mg/ml) respectively against *P. acne* but less inhibition12.000±2.828, 17.500±2.121 and 20.000±2.828 at concentration(50 mg/ml, 100 mg/ml, 200 mg/ml) respectively against *S. epidermidis.* F4C had shown zone of inhibition of 23.500±3.536, 35.000±4.243, 43.000±2.828 for *S. epidermidis* at a concentration of (50 mg/ml, 100 mg/ml, 200 mg/ml) respectively and 19.500±2.121, 33.000±7.071, 41.500±9.192 for *P. acne* at a concentration of (50 mg/ml, 100 mg/ml, 200 mg/ml) respectively as represented in table 8 and figure 1 respectively.

Conclusion

Many plants seem to have inhibitory effects on the growth of bacteria, fungi and viruses in vitro. However, there are a few clinical evidences about the effectiveness and safety of these plants in the treatment of acne and other skin infections. Consumption of alternative and complementary medicine, including medicinal plants, is increasing and is common amongst patients affected by acne and infectious skin diseases. Medicinal plants have a long history of use and have been shown to possess low side effects. This plant is a reliable source for preparation of new drugs. As the herbal formulations of *Boswellia oil* had shown better antiacne activity. F4C shown greater antiacne activity as compare to F4A, F4B at various concentration ranges.

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