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Formulation and evaluation of antiacne cream by using Clove oil

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ABSTRACT

Acne is a common skin problem associated with the microbial infection and needs antimicrobial agents for the treatment. Herbal products containing essential oils as antimicrobial agents are undoubtedly a growing trend. Clove oil is reported to have antimicrobial activity against acne causing microorganisms such as *Propionibacterium acnes*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Candida albicans.* Hence the present study was undertaken with the aim to formulate and develop Antiacne cream by using Clove oil. The essential oil of clove was extracted by steam distillation method and cream formulations were developed with various concentrations of Clove oil; all the formulations were evaluated as per Bureau of Indian Standards guidelines (BIS) guidelines and for antimicrobial activity against the microorganisms responsible for acne by agar well diffusion technique. Also all the Antiacne cream formulations were subjected to stability studies and subjective evaluation of panel of human volunteers. The results showed that the Antiacne cream (C-6) containing clove oil is effective against microorganisms responsible for acne. Clove oil is effective Antiacne agent hence can prove to be beneficial for incorporating in Antiacne preparations.

Selection and peer-review under responsibility of the scientific committee of the International Conference on Advanced Functional Materials (Innovations in Chemical, Physical and Biological Sciences).

1. Introduction

Acne is a chronic inflammatory disease of the pilosebaceous unit. It is characterized by the formation of comedones, papules, pustules, inflamed nodules, superficial pus, filed cysts and in extreme cases canalizing and deep scaring [1]. Acne develops on those areas where sebaceous glands are most numerous: the face, scalp, neck, chest, back, upper arms and shoulders [2]. The bacteria *Propionibacterium acnes*, *Staphylococcus epidermidis* [3], *Staphylococcus aureus* [4], the fungus *Candida albcans* are almost commonly present in the pustular contents of the acne [5]. Acne is common skin problem associated with microbial infections. For its treatment antimicrobial agents are required.

Various antimicrobial agents are used in cosmetic preparations from natural and synthetic sources. Normally synthetic materials are used because of low cost and strong antimicrobial property but synthetic material may give adverse effect to human and environment [6]; also faith of consumer on herbal products is growing fast, hence there is a need to find out effective natural antimicrobial agents.

Essential oils have a wide application in folk medicines, food flavouring and preservation as well as in fragrance industries. The antimicrobial properties of essential oils have been known for many centuries [7]. In recent years, a large number of essential oils and their constituents have been investigated for their antimicrobial properties against some bacteria and fungi [8,9]. It is reported that essential oils provide a gentle and inexpensive way of treating acne, clearing infections and healing acne scarring [10,11].

India has a rich heritage of traditional remedies. In India Spices are used extensively for adding aroma and taste to food. They are used widely in Ayurvedic preparations, flavour and perfume industries.

Clove consists of dried flower buds of *Eugenia caryophyllus* (Thunb.), (Syn. *Syzygium aromaticum* (Linn.) Merrill and Perry), belonging to family *Myrtaceae* [Fig. 1, Fig. 2].

Clove contains about 15–20% of volatile (essential) oil; 10%–13% of tannin (gallotannic acid), resin, chromone and eugenin. The essential oil of Clove bud contains eugenol (about 70–90%),

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Fig. 1. Dried Flower buds of clove.

Fig 2. Clove tree.

eugenol acetate (2–17%) and caryophyllene as its main constituents. Among the other constituents present, the most important is methyl-n-amyl ketone, to which the oil owes its fresh and fruity aroma. Other substances present in traces are methyl salicylate, methyl benzoate, methyl alcohol, benzyl alcohol, furfural, methyl furfural, dimethyl furfural, α-pinene, methyl-n-heptyl ketone, methyl-n-amyl carbinol (2-heptanol), methyl-n-heptyl carbinol and vanillin.

The Cloves are acrid, bitter, aromatic and refrigerant; used widely as culinary spice and as condiments in adding taste and aroma to food preparations.

Clove bud oil is reported to have antibacterial, antifungal, insecticidal and antioxidant properties and are used traditionally as flavouring agent and antimicrobial agent in food products. Clove oil is also reported as an anti-carcinogenic agent due to its antioxidant properties [12]. The high levels of eugenol content in Clove essential oil are responsible for strong antimicrobial activity. This phenolic compound can denature proteins and reacts with cell membrane phospholipids changing their permeability [13,14]. Clove oil also have several therapeutic effects, including anti-vommiting, analgesic, antiplasmodic, anti-carminative, kidney reinforcement, digestive, stimulant, appetiser, expectorant, anthelmintic, diuretic, tonic, rejuvenating, emollient and antiseptic. Clove oil is reported to have antimicrobial activity against acne causing microorganisms such as *Propionibacterium acnes*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Candida albicans* [15].

It is reported that, Clove oil is used in aromatherapy and is successfully used for asthma and various allergic disorders by oral administration [16]. The oil is used in perfumery, in the manufacture of vanillin [17], and as a general antiseptic in medical dental practices [18]. Externally Clove oil is used as a rubefacient and counterirritant [19]. Despite these properties, the common spice Clove is not popular in cosmetics, may be because of inadequate scientific evidence to support the claims made about the various properties of the Clove oil in cosmetic preparations.

Hence, the present work is aimed at scientific evaluation of the commonly used Clove oil as Antiacne agent to treat acne by incorporating it in cream formulations and evaluating the Antiacne cream formulations.

2. Materials and methods

2.1. Collection and authentication of clove

Dried flower buds of Clove, were collected from the local market of Nagpur, Maharashtra, India and authenticated from the Post Graduate Department of Botany, Rashtrasant Tukdoji Maharaj Nagpur University, Nagpur [Fig. 3] The authentication number is 9863.

2.2. Extraction of clove oil [20]

Dried flower buds of Clove, were subjected to size reduction and then subjected to steam distillation for the separation of volatile constituents. It was done by the distillation of 100 g of Clove with water (300 ml) by using Clevenger's apparatus [21,22]. Distillation was continued for 5 h and the oil thus obtained was dried over anhydrous silica gel by using desiccator to remove any traces of moisture and stored in a refrigerator at 4 °C until use. Extractive value is shown in (Table 1) in Result section.

Fig. 3. Authenticated herbarium sheet of clove.

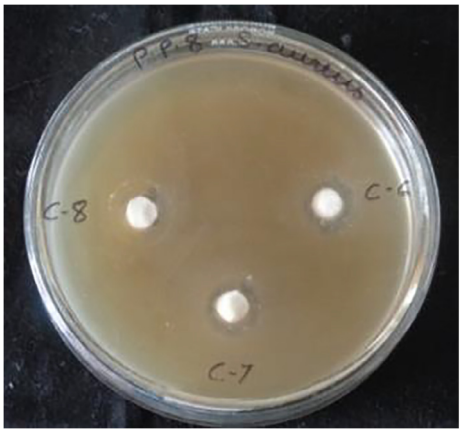


Fig. 4. Zone of inhibition of antiacne creams containing clove oil C6, C7. C8 against *S. aureus.*

Fig. 5. Zone of inhibition of antiacne creams containing clove oil C6, C7, C8 against *S. epidermidis.*

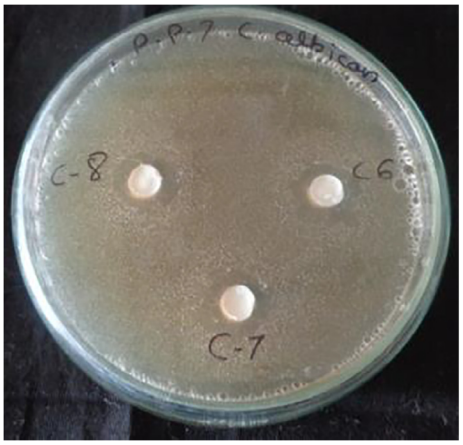


Fig. 6. Zone of inhibition of antiacne creams containing clove oil C6, C7, C8 against *C. albicans.*

2.3. Validation of clove oil

To validate the purity of extracted Clove oil the qualitative analysis of the extracted Clove oil was carried out for determination of organoleptic properties, specific gravity, refractive index, optical rotation and results were compared with the standard values. The results are shown in (Table 2) in Result section.

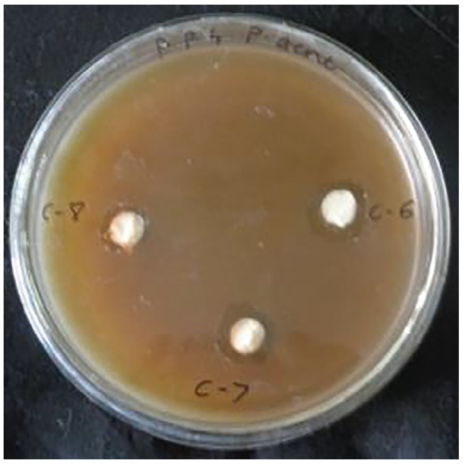


Fig. 7. Zone of inhibition of antiacne creams containing clove oil C6, C7, C8 against *P. acnes.*

2.4. Determination of organoleptic properties of clove oil [23]

Clove oil was subjected to organoleptic analysis for parameters like colour, odour, and taste and the results were recorded (Table 2).

2.5. Determination of specific gravity of clove oil [24]

The specific gravity of clove oil was determined by using pycnometer calibrated by boiled and cooled water at 25 °C. The weight per millilitre was determined by dividing the weight in air, in g, of the quantity of oil which fills the pycnometer at the temperature 25 °C, by the capacity expressed in ml, of the pycnometer at same temperature. The results were recorded (Table 2) and compared with standards.

2.6. Determination of refractive index of clove oil [25]

The Refractive Index of clove oil was determined by using Abbe's Refractometer, by placing a few drops of the liquid on the ground surface of the lower prism, fastening the prism box and focusing the cross –wires of the telescope by rotating the eyepiece and adjusting the mirror to get good illumination. Adjustments were done until sharp edge is in coincidence with the intersection of the cross-wires in the telescope and the refractive index was read off directly on the scale through the eyepiece. The results were recorded (Table 2) and compared with standards.

2.7. Determination of optical rotation of clove oil [26]

The Optical Rotation of clove oil was determined by using Polarimeter at 20 °C. Oils under study were placed in Polarimeter tube between the polarizer and analyser, at the proper setting by using sodium vapour lamp the direction of rotation was determined and results were recorded (Table 2) and compared with standards.

2.8. ***Formulation of antiacne cream using clove oil*** [27,28]

Antiacne cream base was formulated by using formulation given in (Table 3).

Table 1
Percent extractive value of clove oil.

S. N.	Name of oil	Wt. of crude drug	Wt. of oil obtained	% Extractive value	Standard extractive value
1.	Clove oil	100 gm	16.2466 gm	16.2466%	15–20% [16]

Table 2
Validation of spice oils.

S. N.	Parameter	Standards [16]	Laboratory extracted oil
1.	Color	Pale yellow	Pale yellow
2.	Odor	Strong aromatic, characteristic spicy	Strong aromatic, characteristic spicy
3.	Taste	Sweet, characteristic spicy	Sweet, characteristic spicy
4.	Specific gravity at 25 °C	1.038–1.060 wt/ml	1.040 wt/ml
5.	Refractive index at 20 °C	1.527–1.535	1.529
6.	Optical rotation at 20 °C	0^0 to −1.5′	$-1^0 33'$

All the ingredients of phase A and phase B were taken in separate beakers. Both the beakers were kept on water bath till the temperature reached 75 °C. At 75 °C, phase A was added to phase B and stirred keeping the beaker on the water bath itself. Contents in the beaker were then subjected to stirring with the help of mechanical stirrer. The speed of emulsification was slow in the initial stage and then increased gradually as emulsification of the cream progressed. Then the cream was allowed to cool down to the room temperature and transferred to suitable container.

Since, formulation Trial III (C-2) gave a satisfactory product as a cream base; it was selected as a suitable Antiacne cream base for incorporation of clove oil.

Three different concentrations of Clove oil (i.e. 1%, 0.75% and 0.5%) were incorporated in Cream base (C-2) to formulate three formulations of Antiacne creams (C-6, C-7, C-8) respectively (Table 4).

2.9. Evaluation of antiacne creams as per bureau of indian standards (BIS) guideline [29]

Antiacne cream base (C-2) and all the Antiacne cream formulations (C-6, C-7, C-8) was evaluated as per BIS Guidelines for the various parameters like determination of thermal stability, pH, Total Fatty Substance content, % by mass, Total residue, % by mass, Microbial content limit, the results were compared with standard and are summarized Result section in (Table 5).

Table 4
Formulation of Antiacne cream with Clove oil.

Ingredients	Cream formulation (quantity in %)		
	C-6	C-7	C-8
Cream Base C-2	99	99.25	99.50
Clove oil	1	0.75	0.5
Observations:	BW	BW	BW
Colour - Bright White – BW			
pH	pH 6.42	pH 6.39	pH 6.22
Consistency - Satisfactory - (S)	S	S	S

2.10. ***Stability study of antiacne creams*** [30]

The cream base C-2 and Antiacne creams (C-6. C-7, C-8) was subjected to stability studies. Changes in parameters like Colour, Odour, pH, Viscosity Particle size at three temperatures i.e. in oven at (45 ± 2 °C), in refrigerator at (4 ± 2 ^{0}C) and at room temperature were recorded for 45 days, at the interval of 4 days for colour, odour, pH and at the interval of 6 days for viscosity and particle size. Results showed that all the formulations were stable (See in Result section -Graph 1–12).

2.11. Evaluation of antimicrobial activity of Antiacne creams

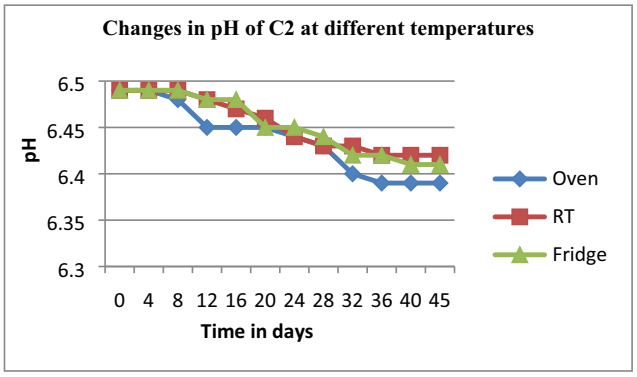
The method based on zones of inhibition – agar well diffusion technique is used in present investigations. In this method the

Table 3
Formulation of antiacne cream base.

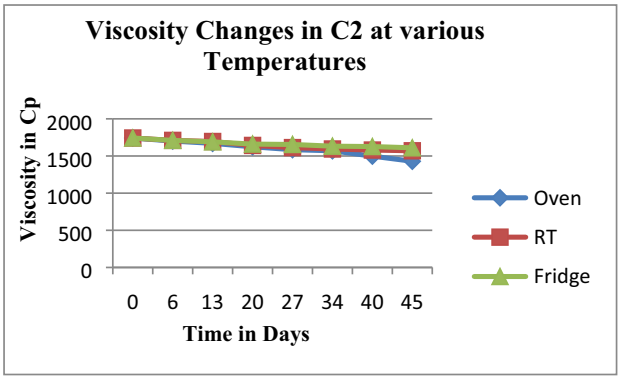
S. N.	Ingredients	Use	Trial I (Quantity in %)	Trial II (Quantity in %)	Trial III (Quantity in %)
	Phase A				
1	Stearic acid	Emulsifier	12	9	9
2	Cetyl alcohol	Emulsion stabilizer	2	1	1
3	Bees wax	Emollient	3	–	–
4	Glyceryl mono stearate	Self-emulsifier and emollient	–	2	1
5	Mineral oil	Occlusive film former	3	1	1
6	Propyl paraben	Preservative for oil phase	0.15	0.15	0.15
	Phase B				
7	Tri-ethanolamine	Emulsifier	1.5	0.5	0.5
8	Methyl Paraben	Preservative for water phase	0.15	0.15	0.15
9	Water	vehicle	Upto 100 (78.2)	Upto 100 (86.2)	Upto 100 (87.2)
	Observations				
			pH : 6.45 -Very Stiff, White Colour, Difficult to spread, -Not Satisfactory	pH : 6.04 -Slightly stiff, White colour, -Not Satisfactory	pH : 6.42 -Desired consistency, -Bright white colour, -Easily Spreadable, -Satisfactory

Table 5
Evaluation of Antiacne cream bases C-2 and Antiacne Formulations (C-6. C-7, C-8).

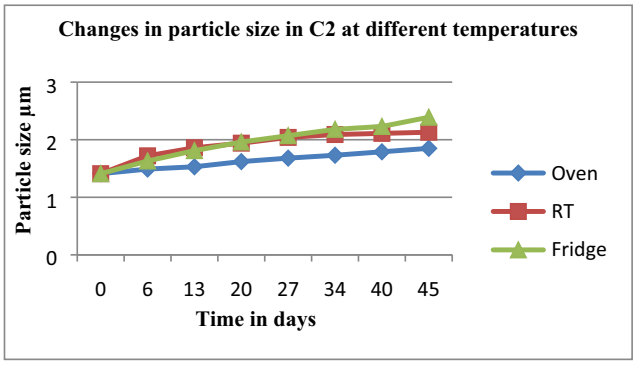
S. N.	Test	BIS requirement [28]	Observations for C-2	Observations for C-6	Observations for C-7	Observations for C-8	Inference
1	Thermal stability	No oil separation	No oil separation	No oil separation	No oil separation	No oil separation	Passes test.
2	pH	4.0 to 9.0	6.49	6.42	6.39	6.22	Passes test.
3	Total fatty substance content, % by mass	Min. 5%	10.86	11.06	10.89	10.78	Passes test.
4	Total residue, % by mass	Min. 10%	13.21	13.94	12.96	13.40	Passes test.
5	Microbial content limit	Not more than 1000 cfu/g	130 cfu/g	60 cfu/g	70 cfu/g	90 cfu/g	Passes test.



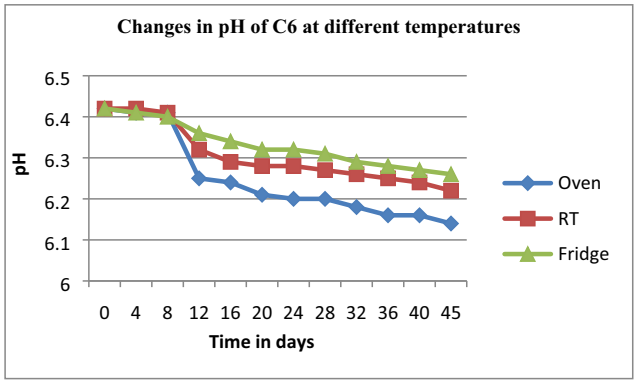
Graph 1. Graphical representation of changes in pH of C-2.



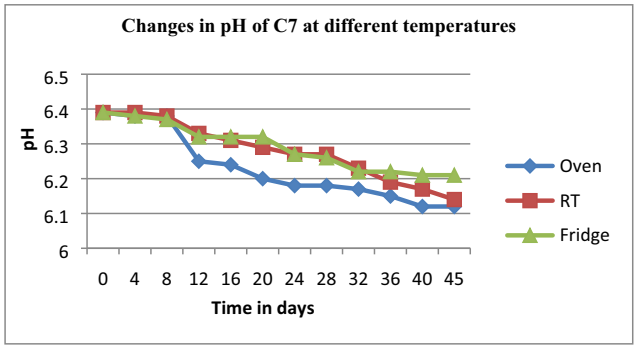
Graph 2. Graphical representation of viscosity changes of C-2.



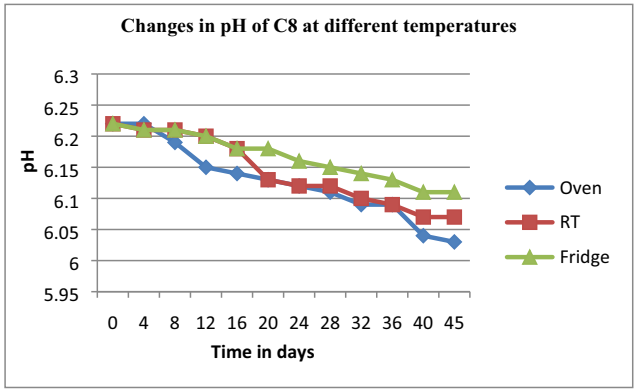
Graph 3. Graphical representation of changes in particle size of C-2.



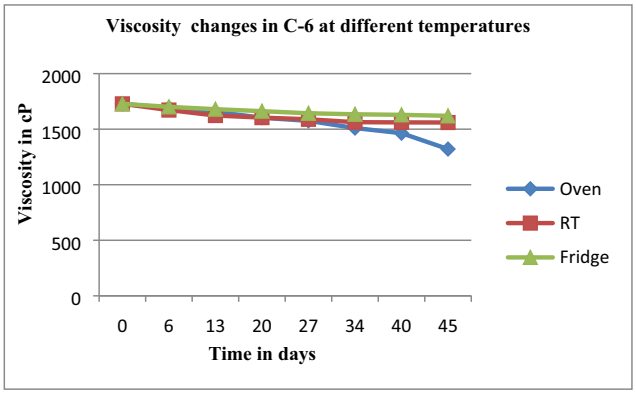
Graph 4. Graphical representation of changes in pH of C-6.



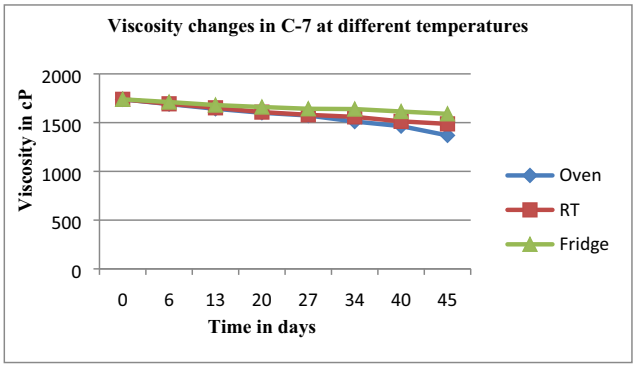
Graph 5. Graphical representation of changes in pH of C-7.



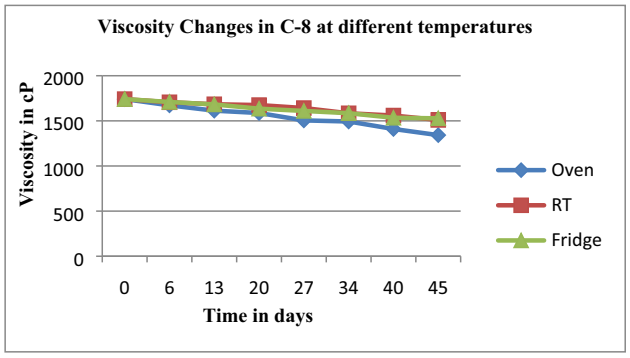
Graph 6. Graphical representation of changes in pH of C-8.



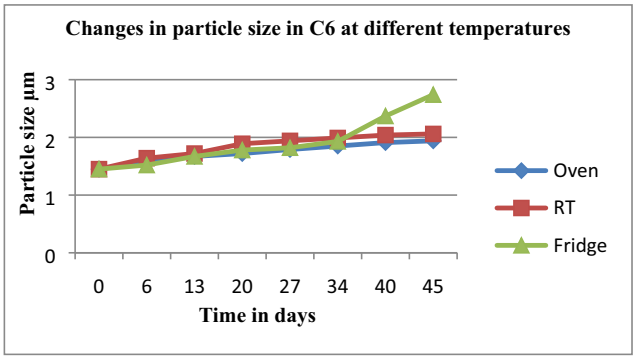
Graph 7. Graphical representation of viscosity changes in C-6.



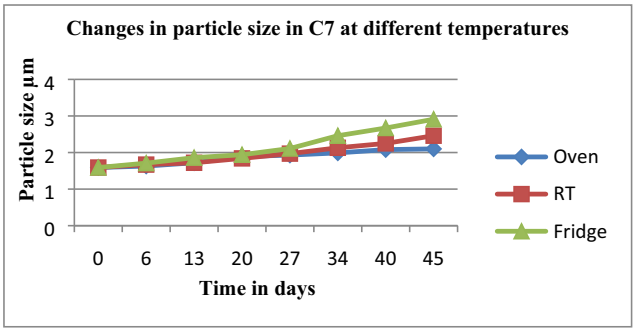
Graph 8. Graphical representation of viscosity changes in C-7.



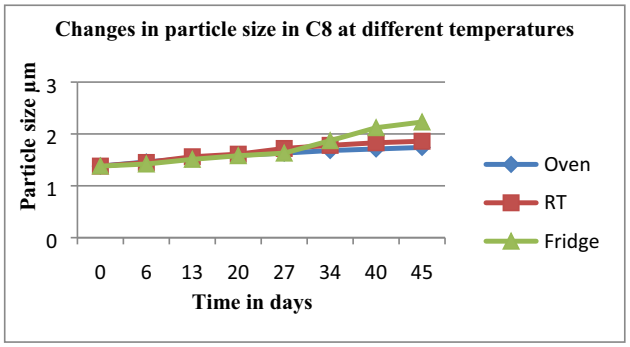
Graph 9. Graphical representation of viscosity changes in C-8.



Graph 10. Graphical representation of particle size changes in C-6.



Graph 11. Graphical representation of particle size changes in C-7.



Graph 12. Graphical representation of particle size changes in C-8.

activity of a compound is indicated by a clear zone around the 'cup', a hole is cut in the agar and filled with preparation under test. The zones of inhibition are measured. This method has the advantage of being reproducible and accurate comparisons between compounds can be made [31] and hence this method is selected for the present study.

Antiacne creams C-6, C-7, C-8 and Antiacne cream base C-2 were evaluated for their antimicrobial activity against pure cultures of *Pseudomonas aeruginosa* (MTCC 1688), *Escherichia coli* (MTCC 1687) , *Staphylococcus aureus* (MTCC737), *Candida albicans* (MTCC 227), *Staphylococcus epidermidis* (MTCC 6810) and *Propionibacterium acnes (MTCC 1951)* which were procured from Institute of Microbial Technology, Chandigarh, India.

2.12. Cultivation and maintenance of microorganisms [32]

All the microorganisms selected for study were cultivated and maintained on growth medium and growth conditions as recommended by Institute of Microbial Technology, Chandigarh, India; cultures of these microorganisms were used to evaluate antimicrobial activity. The details of conditions for cultivation and maintenance of microorganisms are summarized in Table 6.

The antimicrobial activity of Antiacne creams C-6, C-7, C-8 and Antiacne cream base C-2 was determined by the agar well diffusion technique. The melted and cooled media (approx. at 45 °C) seeded with the fresh cell suspension of microorganisms (i. e. 0.2 ml suspension used for 20 ml agar medium, concentration of organisms approx. 10^8 cells/ml) and allowed to solidify. The cups were bored using a sterile cork borer with 8 mm internal diameter. The cups were filled with 0.2 g of the cream formulations by using sterile spatulas. Plates were kept for diffusion in refrigerator at 4 °C for 30 min. The plates were then incubated at prescribed temperature, growth condition and time interval (Table 6). The experiments were performed in duplicates and mean values were recorded. The assessment of antimicrobial activity was based on the

Table 6
Conditions for cultivation and maintenance of microorganisms under study.

S. N.	Micro organisms	Growth medium used for cultivation and maintenance of stock culture (as recommended by Institute of Microbial Technology, Chandigarh, India)	Medium used for screening antimicrobial activity	Incubation period	Incubation temperature	Growth condition
1.	*P. aeruginosa* (MTCC 1688)	Medium No. 3 (Nutrient Agar Medium)	Muller Hinton Agar Medium	24 Hours	37 °C	Aerobic
2.	*E. coli* (MTCC 1687)	Medium No. 3 (Nutrient Agar Medium)	Muller Hinton Agar Medium	24 Hours	37 °C	Aerobic
3.	*S. aureus* (MTCC 737)	Medium No. 3 (Nutrient Agar Medium)	Muller Hinton Agar Medium	24 Hours	37 °C	Aerobic
4.	*C. albicans* (MTCC 227)	Medium No. 6 (Malt Yeast Agar)	Medium No. 6 (Malt Yeast Agar)	48 Hours	25 °C	Aerobic
5.	*S. epidermidis* (MTCC 6810)	Medium No. 3 (Nutrient Agar Medium)	Muller Hinton Agar Medium	24 Hours	37 °C	Aerobic
6.	*P. acnes* (MTCC 1951)	Medium No. 41 (Blood Agar Medium)	Medium No. 41 (Blood Agar Medium)	48 Hours	37 °C	Anaerobic

Table 7
Zone of Inhibition of Antiacne cream base C-2 and Antiacne creams C-6, C-7, C-8 against acne causing microorganisms.

Microorganisms	Antiacne cream base	Antiacne creams containing Clove oil		
	C-2	C-6	C-7	C-8
P. aeruginosa	–	15	13	11
S. aureus	–	16	15	12
S. epidermidis	–	14	13	11
E. coli	–	14	12	10
C. albicans	–	16	15	12
P. acnes	–	16	15	12

Diameter of Cork borer used – 8 mm, '-'indicates no zone of inhibition, All zones of inhibition in mm.

measurement of diameter of zone of inhibition in millimetre. The results are summarized in Table 7 in Result section.

2.13. Subjective evaluation [33] of antiacne creams containing clove oil

All the Antiacne cream formulations (C-6, C-7, C-8) were found to pass BIS specifications. However, there was variation in antimicrobial activity. Antiacne cream (C-6) showed maximum antimicrobial activity and hence was selected for subjective evaluation on panels of 20 human volunteers (age group between 18 and 35 years) having acne skin condition for the time period of 28 days. A systematic subjective evaluation study was carried out by instructing volunteers to use the C-6 cream on face twice daily so that the cream should remain on face for 3–4 h daily. The volunteers were instructed not to take any other treatment for acne and also to wash the face before applying Cream C-6. The observations were made before the starting of the study and after using the Antiacne cream i.e. at the end of 28 day. Assessment of the Antiacne cream was determined based on the functional parameters like Appearance of cream, ease of spreadability, Antiacne efficacy, Improvement in texture of skin, irritancy on application. The self-assessment questionnaires were filled by the volunteers and results were recorded.

3. Results and discussion

Acne is a common skin disorders which require antimicrobial agents for its treatment. Common microorganisms associated with acne skin conditions are *Propionibacterium acnes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *and Candida albicans*. It is reported that Clove oil possesses analgesic, antiseptic, antimicrobial and aromatic properties [34,35,36]. Despite these properties, the common spice Clove is not popular in cosmetics, may be because of inadequate documentation and non-availability of any scientific evidence regarding its activity in cosmetic preparations. Hence the present study was undertaken with the aim to formulate and develop Antiacne cream by using Clove oil and evaluation of Antiacne property of the cream formulations against microorganisms responsible for acne i.e., against *Propionibacterium acnes, Staphylococcus aureus, Staphylococcus epidermidis, Candida albicans* and also antibacterial activity of the Antiacne creams was evaluated against two common bacteria i.e., *Pseudomonas aeruginosa* and *Escherichia coli*.

In the present study, the dried flower buds of Clove were subjected to extraction by steam distillation with Clevenger's apparatus and extractive value was recorded in (Table 1). The percent extractive value was within the range of standard extractive value.

To validate the purity of laboratory extracted Clove oil, it was studied for organoleptic properties, specific gravity, refractive index and optical rotation. The results were compared with the Standard values which showed that the extracted oil is of standard quality. The results are recorded in the Table 2.

Evaluation of Antiacne creams C-6, C-7, C-8 and Antiacne cream base C-2 as per BIS Guideline showed that they comply with BIS Guidelines [Table 5].

It is a normal experience that whenever any formulation is to be developed containing essential oil, the stability of product possess challenge. The formulations in the present study were no exception to this. Complex nature of essential oil and its interaction with other ingredients are the normal causes of instability of the product. All this leads to changes in colour, odour, pH, viscosity and particle size. Thus the stability of Antiacne creams was studied by observing changes in parameters like colour, odour, pH, viscosity and particle size under extreme conditions and all Antiacne creams were found to be substantially stable [Graph 1–12].

3.1. Evaluation of antimicrobial activity of Antiacne creams

The Antiacne cream base C-2 and Antiacne creams C-6, C-7, C-8 prepared were subjected to agar-well diffusion technique to evaluate their antimicrobial activity; the zones of inhibition were measured and recorded [Table 7, Fig. 4–7].

Antiacne cream base C2 was non inhibitory to the growth of experimental microorganisms. All the Antiacne creams containing Clove oil (C-6, C-7, C-8) showed inhibitory activity against *P. aeruginosa, S. aureus, S. epidermidis, E. coli, C. albicans, P. acnes.*

From the evaluation of antimicrobial activity of Antiacne creams, it was observed that Antiacne cream [C-6 (containing Clove oil 1%)] showed maximum antimicrobial activity against

microorganisms under study hence was selected for subjective evaluation on panels of human volunteers. This study was designed to ascertain the Antiacne efficacy of Antiacne cream formulation in terms of reduction in the acne condition after application of the Antiacne cream. The main aim of the study was to evaluate the anti-acne effect of Clove oil by using cream base. The Antiacne creams were evaluated for functional parameters like, appearance, ease of spreadability, Antiacne efficacy, improvement in texture of skin and irritancy by panel of volunteers.

The results of subjective evaluation were quite promising. Antiacne cream C-6 was well appreciated by volunteers for its appearance, ease of spreadability and non-irritancy. Also C-6 showed remarkable reduction in acne condition and significant improvement in texture of skin.

4. Conclusion

In India there are many medicinal plants which are used from ancient times for skin care. Acne is a common skin problem associated with the microbial infections and many other causes also. Acne is not health threatening disorder but it definitely cast negative impact on one's personal self-image. The demand for more and more cosmetics from plant sources is continuously increasing. The prolonged chemical based treatments and the high rate of recurrence suggest the opportunity for alternative options. Despite of having reported antibacterial and antifungal properties, Clove oil is not popular as Antiacne agent for incorporation in cosmetic formulations may be because of inadequate documentation and non-availability of any scientific evidence regarding their activity in cosmetic formulations. So this study was undertaken with the aim to formulate, develop and evaluate Antiacne cream by using three different concentrations of clove oil. From the results of the present study it can be concluded that Antiacne cream containing 1% Clove oil (C-6) was acceptable in view of improvement in acne skin condition and contains all good characters of skin cream. Clove oil is effective Antiacne agent hence can prove to be beneficial for incorporating in Antiacne preparations. The optimum concentration of Clove oil to be used as Antiacne agent can be 1% for Antiacne cream formulations.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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