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Preparation and Characterization of Natural Antioxidant Emulgels Loaded with *Annona squamosa* L. Extract with and without Penetration Enhancer

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Highly sustainable emulgels loaded with *Annona squamosa* L. extract with and without penetration enhancer were formulated. Characterization evaluations of prepared emulgels formulations were done at various storage conditions. Acetone (70%) was used to prepare *Annona squamosa* L. extract by using maceration technique. Emulgels were prepared by using 4% extract with and without penetration enhancer (clove oil 8%). Varying concentrations of carbapol 940 (0.5-2%) were used in the development of emulgels. All the formulations were kept first at 25°C and then stressed at 40°C to sort out the most stable formulation. The *in vitro* characterization of the optimized formulation was done including pH, conductivity, phase separation, and mean droplet size. These parameters were monitoring over a period of 90 days at 8°C, 25°C, 40°C and 40°C with 75 % RH.

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1. INTRODUCTION

The word “natural” is devoted to something found and extracted directly from natural plants or animal products [1]. Natural plant extracts are exploring new eras of research but their use require special consideration to their active ingredients, method of extraction and solvent ratio [2]. Sources of these natural ingredients consist of herbs, leaves, fruits, minerals, flowers, water and land. The *in vitro* and *in vivo* activity and the base in which these natural ingredients are incorporated determine the efficacy of these ingredients in skin care [3]. In general, botanical products consist of variety of bioactive components like vitamins, antioxidants, terpenoids, proteins, essential oils and oils, hydrocolloids etc. Depending upon the composition of these extracts, their properties and activities vary [4].

Emulgel is a novel drug delivery system consisting of emulsion (oil-in-water or water in oil) combined with a gelling agent. Emulgel is stable preparation as compared to traditional emulsion. They have better patient acceptability due to possessing the activities of both emulsions and gels [5]. Both hydrophilic and lipophilic drugs can be incorporated by selecting the oil-in-water or water-in-oil system. Emulgel permits dual control of the release of drug from the emulsion as well as from the gel [6]. Emulgels in dermatology comprise numerous constructive properties such as being transparent, emollient, thixotropic, greaseless, easily spreadable & having longer shelf life [7].

To facilitate the absorption of drugs, penetration enhancing ingredients are incorporated in the formulation. These ingredients temporarily change the skin barrier, modify the partitioning of the drug into skin structures and ultimately enhance drug penetration into skin. e.g. clove oil 8%. They should be non-toxic, non-irritating and non- allergenic and compatible with both excipients and drugs [8].

Annona squamosa L. (Annonaceae), commonly known as custard apple, Shariffa and sweetsop is an indigenous to west Indies, it is also widely grown throughout the tropics in India, Pakistan and popularly cultivated in the north eastern parts of Thailand, mainly for its edible fruit. The plant is deciduous and small; reaching a maximum of 6 m in height with many lateral branches grows well in regions of medium humidity [9]. Screening for diverse biological compounds have been performed by means of various solvent which indicates the occurrence of steroid, ascorbic acid, terpenoids, alkaloid, flavonoid, saponin and phenolic compounds. The occurrence of these biologically active compounds depends upon the choice of solvent for extraction and the part of the plant used for the study [10]. The fruit has Analgesic and anti-inflammatory activity, Anti-bacterial and cytotoxic activity, Anti-oxidant and anti-lipidimic activity, Anti-ulcer and Anti-tumour activity, hepato protective activity and Anthelmintic activity [11].

The main objective of this study was to formulate a stable emulgel with *Annona squamosa* L. extract with and without penetration enhancer and evaluate the stability of these formulations at various storage conditions.

2. MATERIALS AND METHODS

2.1 Plant Material

Annona squamosa L. was purchased from the neighbouring market of Bahawalpur, Pakistan and then identified from the department of life sciences, The Islamia University of Bahawalpur, Pakistan with voucher number 7687/LS.

2.2 Chemicals

Acetone (Merck KGaA Darmstadt, Germany), DPPH (Sigma, USA), Distilled water (Department of Pharmacy, IUB, Pakistan), Carbopol 940 (Sigma, USA), Triethanolamine (Merck KGaA Darmstadt, Germany), Liquid paraffin (Merck KGaA Darmstadt, Germany), Span 80 (Sigma, USA), Tween 20 (Sigma, USA), Propylene glycol (Merck KGaA Darmstadt, Germany), Methylparaben (Acros Organics, USA), *Annona squamosa* L. (Bahawalpur, Pakistan)

2.3 Preparation of Fruit Extract

Annona squamosa L. extract was prepared by using cold maceration technique. 100 g of chopped fruit (pulp, peel and seed) was macerated in 500 ml of acetone (70%) for 72 hr at room temperature. 30 minutes stirring was done each day. The residues were separated by first passing the extract from different layers of muslin cloth and then by filtering through Whatman filter no. 1. The volume of filtrate was reduced to the

1/3 of the initial volume by evaporating it under reduced pressure with the help of rotary evaporator at 45°C. The concentrated extract was stored at 4°C for further studies.

2.4 Antioxidant Activity

2, 2-diphenyl-1-picryl-hydrazyl (DPPH) method was used to evaluate the radical scavenging potential of the *Annona squamosa* L. extract with slight modified previously reported method [12]. The basic principle of this method is that DPPH is reduced by the presence of antioxidant and changes its colour from purple to yellow. 100 µL volumes were used for the assay (10 µL of test solution and 90 µL of 100 µM DPPH solution) in a 96 well plate. The reaction mixtures were stirred and incubated at 37°C in the dark for 30 minutes. The reduction in absorbance was measured photo metrically at 517 nm by using microplate reader (Synergy HT BioTek, USA). L-ascorbic acid was used as reference standard antioxidant. The percent inhibition was calculated with the formula.

$$\text{Inhibition (\%)} = \{(A_0 - A_1)/A0\} \times 100$$

Where,

A_0 = absorbance of control,
A_1 = absorbance of test extract.

2.5 Formulation of Emulgels

The emulgels were prepared by the previously reported method [13]. This method includes first the formulation of emulsion, then the formulation of gel base, then finally incorporating the emulsion into the gel base to develop emulgel.

The different formulations were formulated by varying the amount of gelling agent i.e. carbapol-940(0.5%, 1%, 1.5%, and 2%). The preparation method of emulsion (o/w) was same in all the formulations. The gel bases were prepared by dispersing different concentrations of Carbopol in distilled water with constant stirring at a moderate speed. The pH of all the formulations was adjusted to 5 - 6.5 using tri ethanol amine (TEA).

For the preparation of emulsion, the oily phase was prepared by dissolving Span 20 in light liquid paraffin and clove oil (penetration enhancer) was also mixed in oily phase where necessary. The aqueous phase was prepared by first dissolving Tween 20 in purified water and Methyl paraben in propylene glycol (being hydrophobic) then by mixing the both. Both the oily and aqueous phases were separately heated to 70° to 80°C, then both the phases were mixed by continuous stirring until it was cooled to room temperature. The prepared emulsion was incorporated into the gel in 1:1 ratio with gentle stirring to formulate the emulgel.

The optimized formulation was selected by first storing the formulation at 25°C for a period of 28 days. Formulations were further stressed at 40°C to sort out the most stable emulgel preparation. Two formulation emulgels were prepared one with extract FA (formulation A) and second with extract FB (formulation B) along with penetration enhancer as shown in the formula in Table 1.

Table 1. Formulations ingredients of emulgels

Ingredients (%w/w)	Formulation A (FA)	Formulation B (FB)
Aqueous Phase		
Tween 20	0.5%	0.5%
Propylene glycol	5%	5%
Methyl paraben	0.03%	0.03%
Distilled water	q.s to 100 gm	q.s to 100 gm
Oily Phase		
Liquid paraffin	7.5%	7.5%
Span20	1%	1%
Gel Phase		
Carbapol	2%	2%
Distilled water	q.s	q.s
Extract		
Annona squamosa L. extract	4%	4%
Penetration enhancer		
Clove oil	-	8%

Where q.s = Quantity sufficient

2.6 Stability Studies

Stability studies were performed on the optimized emulgels by storing the emulgels at different storage conditions 8°C ± 0.1°C, 25°C ± 0.1°C, 40°C ± 0.1°C and 40°C ± 0.1°C & 75 ± 1% relative humidity for a period of 90 days. The samples were withdrawn at regular intervals of 15 days, 30 days, 45 days, 60 days, 75 days and 90 days of investigation and evaluated for change in pH, conductivity and phase separation on centrifugation.

2.6.1 pH determination

The pH of fresh samples and samples kept at different storage temperatures (8°C, 25°C, 40°C and 40°C & 75 ± 1% relative humidity) was measured by a digital pH meter Ino. Lab pH7110 pH meter (WTW, Germany). The measurements were done at regular intervals of 15 days, 30 days, 45 days, 60 days, 75 days and 90 days of investigation. All the measurements were performed in triplicate.

2.6.2 Conductivity measurement

Conductivity analysis of fresh samples and samples kept at different storage conditions (8°C, 25°C, 40°C and 40°C & 75 ± 1% relative humidity) was performed to examine the release of the electrolyte initially entrapped in the internal phase [14]. The specific conductivity of the emulgels was measured directly using a InoLab cond7110 conductivity meter (WTW, Germany). The measurements were done at regular intervals of 15 days, 30 days, 45 days, 60 days, 75 days and 90 days of investigation. All the measurements were performed in triplicate.

2.7 Centrifugation and Phase separation

Centrifugation test was carried out at 25°C by placing 2 g of each of prepared formulations and base, stored at different conditions (8°C, 25°C, 40°C and 40°C & 75 ± 1% relative humidity), in the 15 ml centrifuge tube and centrifuged at 5000 rpm for 10 minutes in centrifugation machine (Hettich EBA 20, Germany). The tubes were investigated macroscopically for the presence of any possible phase separation. The measurements were done at regular intervals of 15 days, 30 days, 45 days, 60 days, 75 days and 90 days of investigation.

2.8 Microscopic Analysis

The morphology and particle size of prepared emulgels was determined by using an optical microscope Nikon E200, Nikon, Japan) attached with a high resolution digital camera. Images were collected with the image analysing software Minisee version 1.1. A small quantity of emulgel was spread on the glass slide after diluting it with external phase. A cover slip was applied carefully to avoid air bubbles and sample damage by shear stress. The observations were made of fresh samples then after 30 days, 60 days and 90 days. All the observations were made at 100X magnification power.

2.9 Statistical Evaluation

Results of stability studies were subjected to statistical analysis by SPSS 20 software on the computer. One-way ANOVA test at the 5% level of confidence was applied to check the variation in results at different time and storage conditions.

3. RESULTS AND DISCUSSION

3.1 Antioxidant Activity

The antioxidant activity of the fruit extract was measured by the change in its absorbance after the reduction of DPPH. *Annona squamosa* L. extract showed excellent radical scavenging activity i.e. 90% when compared to the radical scavenging activity of ascorbic acid (standard) i.e. 92%.

3.1.1 pH determination

The pH of the freshly formulated emulgels was 5.92 and 5.83 of formulation A and formulation B respectively which is within the skin pH range. At the end of study, the pH values of formulation A at different storage conditions (8°C, 25°C, 40°C and 40°C & 75 ± 1% relative humidity) were 5.15, 5.13, 5.06 and 4.99 respectively while that in case of formulation B were 5.08, 5.06, 4.99 and 4.79. The changes occurred in the pH values of both the formulations at different storage conditions are noted in Table 2-3. The pH values of both the formulations kept on decreasing with the passage of time but that change was within the acceptable range except at accelerated storage condition of 40°C & 75 ± 1% relative humidity.

3.1.2 Conductivity measurement

In this study, the conductivity values of the freshly formulated emulgels were 210 and 289 of formulation A and formulation B respectively. At the end of study, the conductivity values of formulation A at different storage conditions

(8°C, 25°C, 40°C and 40°C & 75 ±1 % relative humidity) were 301, 320, 389 and 398 respectively while that in case of formulation B were 349, 362, 419 and 439 respectively. The changes occurred during different time intervals have been shown in the Table 2-3. The conductivity values of both the formulations kept on increasing with the passage of time with a slight change on lower temperatures and a marked change on higher temperatures.

3.1.3 Centrifugation and phase separation

In this study, the centrifugation test was performed for both the formulations kept at different storage conditions (8°C, 25°C, 40°C and 40°C & 75 ± 1% relative humidity) up to a period of 90 days at definite time intervals. No phase separation on centrifugation was seen in any of the samples of formulation B and slight phase separation was observed in formulation A at 90th day of observation at 40°C & 75 ± 1% relative humidity. This indicated that the emulgels showed long term stability at all the storage conditions.

3.1.4 Microscopic analysis

The droplet size of freshly formulated emulgels was 11.6 µm and 14.2 µm of formulation A and B respectively. At the end of study, the droplet size of formulation A at different storage conditions (8°C, 25°C, 40°C and 40°C & 75 ± 1% relative humidity) were 12.2 µm, 12.5 µm, 13.5 µm and 13.8 µm respectively while that in case of formulation B were 15.1 µm, 15.7 µm, 16.5 µm and 16.8 µm respectively. The extract loaded emulgel showed changes in only narrow ranges of droplet diameter that indicates that the formulation is fairly stable under study conditions. The increase in mean droplet diameter was greater when formulation was stored at higher temperatures (40°C and 40°C ± 75% RH) as shown in Table 4-5. Statistically non significant differences ($p > 0.05$) were observed regarding differences in globule sizes.

Table 2. pH values, mean conductivity and centrifugation of Formulation A kept at different storage conditions

Time	8°C	25°C	40°C	40°C with 75%RH
			pH	
0 days	5.92	5.92	5.92	5.92
15 days	5.81	5.74	5.64	5.61
30 days	5.66	5.58	5.43	5.49
45 days	5.42	5.39	5.36	5.35
60 days	5.29	5.27	5.29	5.24
75 days	5.21	5.20	5.12	5.08
90 days	5.15	5.13	5.06	4.99
			Conductivity (µS/cm)	
0 days	210	210	210	210
15 days	221	237	252	259
30 days	244	267	287	293
45 days	263	280	302	316
60 days	272	298	334	354
75 days	296	309	356	375
90 days	301	320	389	398
			Centrifugation and phase separation	
0 days	S	S	S	S
15 days	S	S	S	S
30 days	S	S	S	S
45 days	S	S	S	S
60 days	S	S	S	S
75 days	S	S	S	S
90 days	S	S	S	US

Where S = Stable after centrifugation and US = slightly unstable

Table 3. pH values, mean conductivity and centrifugation of formulation B kept at different storage conditions

Time	8°C	25°C	40°C	40°C with 75%RH
			pH	
0 days	5.83	5.83	5.83	5.83
15 days	5.49	5.54	5.56	5.42
30 days	5.29	5.32	5.31	5.28
45 days	5.21	5.25	5.26	5.01
60 days	5.19	5.19	5.11	4.93
75 days	5.11	5.14	5.04	4.87
90 days	5.08	5.06	4.99	4.79
			Conductivity (µS/cm)	
0 days	289	289	289	289
15 days	307	321	336	340
30 days	318	330	359	362
45 days	329	338	376	389
60 days	337	349	389	399
75 days	342	357	402	415
90 days	349	362	419	439
			Centrifugation and phase separation	
0 days	S	S	S	S
15 days	S	S	S	S
30 days	S	S	S	S
45 days	S	S	S	S
60 days	S	S	S	S
75 days	S	S	S	S
90 days	S	S	S	S

Where S = Stable after centrifugation

Table 4. Mean droplet size (µm) of formulation A Emulgels

Temperature	Fresh	After 30 days	After 60 days	After 90 days
8°C	11.3± 0.40	11.6± 0.13	11.8± 0.20	12.2 ± 0.60
25°C	11.3 ± 0.40	11.6 ± 0.70	11.9 ± 0.40	12.5 ± 0.40
40°C	11.3 ± 0.40	11.5 ± 0.50	12.2 ± 0.50	13.5 ± 0.60
40°C with 75% RH	11.3 ± 0.40	11.9 ± 0.90	12.4 ± 0.60	13.8 ± 0.30

Mean± SD, RH= 75 ± 1% relative humidity, (for number of droplets n = 20).

Table 5. Mean droplet size (µm) of formulation B Emulgels

Temperature	Fresh	After 30 days	After 60 days	After 90 days
8°C	14.2 ± 0.18	14.4 ± 0.42	14.8 ± 0.60	15.1 ± 0.50
25°C	14.2 ± 0.18	14.5 ± 0.40	15.2 ± 0.20	15.7 ± 0.40
40°C	14.2 ± 0.18	14.8 ± 0.50	15.8 ± 0.55	16.5 ± 0.50
40°C with 75% RH	14.2 ± 0.18	14.9 ± 0.50	15.3 ± 0.15	16.8 ± 0.42

Mean± SD, RH= 75 ± 1% relative humidity, (for number of droplets n = 20).

The presence of antioxidant phytochemicals like polyphenols and carotenoids contributes to the antioxidant properties of plants [15]. Polyphenols are powerful antioxidants and helpful in the prevention of certain neurodegenerative diseases, cardiovascular diseases and cancers etc. [16]. The presence of such ingredients is responsible for its antioxidant activity and thus makes the extract a potential ingredient for skin care preparations.

The pH determination is an important parameter for the stability and effectiveness of topical formulations [17]. The pH of the topical emulgels should be within the range of skin pH i.e. 5-7 to avoid any kind of skin irritation [18]. The decrease in the pH of the formulations in the present study may be due to the hydrolysis reaction or oxidation of any of the ingredients of the extract while the marked change in formulation B may be due to the production of

acidic products by additional presence of clove oil [19].

Centrifugation test is used for evaluating the shelf life of emulsions and works on the principle of centrifugal force for separating the substances of different densities. The cream volume or the separation of two phases at a specific time period is used to evaluate the physical stability of emulsion[20]. In this study, both the formulations were stable at all the storage conditions except FA at the 90th day at accelerated temperature and humidity. This stability might be either due to less density difference between the internal and external phase or due to strong interfacial film at the interface [21]. At higher temperatures slight phase separation might be because of lowering of viscosity of oil phase that resulted in the sedimentation of the heavier phase under centrifugal force [22]. The stability of FB may also be related to the presence of additional clove oil having preservative activity [23] The emulsions having water in the continuous phase are good conductors of electricity as compared to emulsions with oil as continuous phase [24]. The increase in the conductivity value during storage is contributed to many factors like diffusion of electrolyte, coalescence of internal and aqueous phase or destruction of oil phase by osmotic pressure [17].

In this study, the conductivity values increased slightly at low temperatures while moderate increase was observed at high temperatures. But pH and centrifugation analysis showed that the emulgels were stable at all storage conditions so this change does not lead to instability.

Droplet size is one of the important characteristic of the topical formulation that contributes to the physical stability of dermal and cosmetic products. The small droplet size prevents the droplet coalescence and sedimentation against gravitational force [25]. In this study, the droplet size of tends to increase of both the formulations which is more at higher temperatures. When one way ANNOVA was applied, the results were statistically non significant. It has been stated that at higher temperatures, the viscosity of the continuous phase decline that may allow more frequent collision between the droplets and result in increased droplet size [26]. Our results showed that the conductivity values increased with the passage of time while pH values decreased but remained within the skin range. Centrifugation test did not show phase separation at low temperatures but slight change was observed at 40°C & 75% RH. Microscopic analysis showed increase in mean droplet size but the change was insignificant ($p<0.05$) at normal storage conditions.

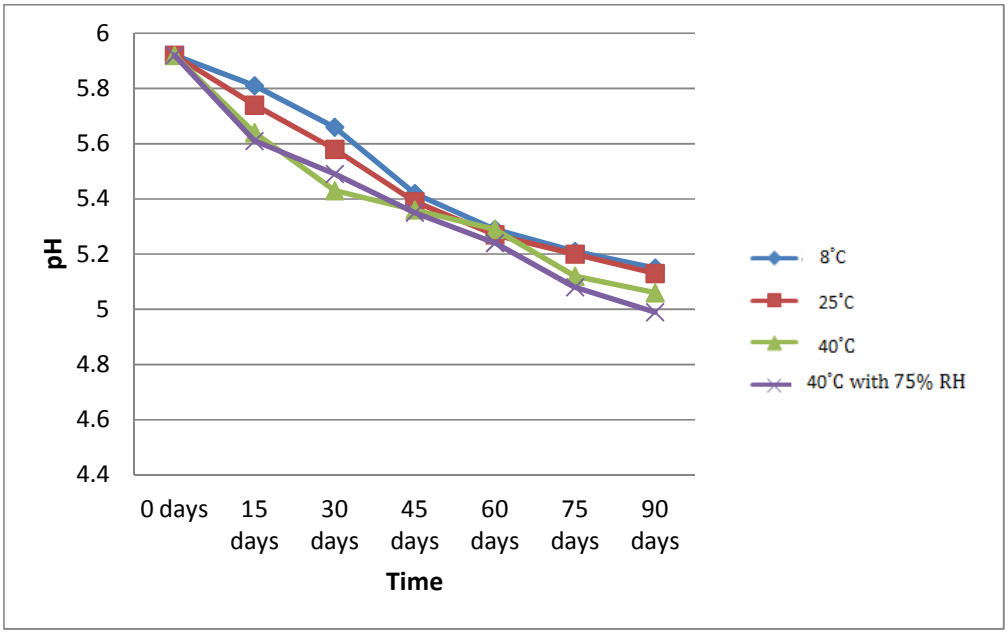

Fig. 1. Graph showing pH values of formulation A kept at different storage conditions

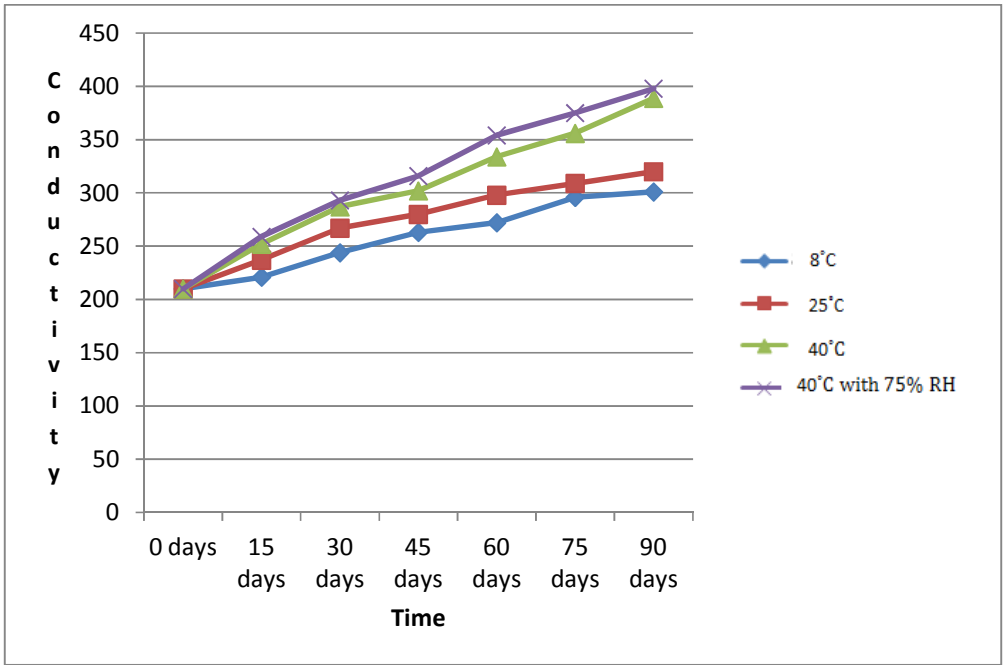

Fig. 2. Graph showing mean conductivity of formulation A kept at different storage conditions

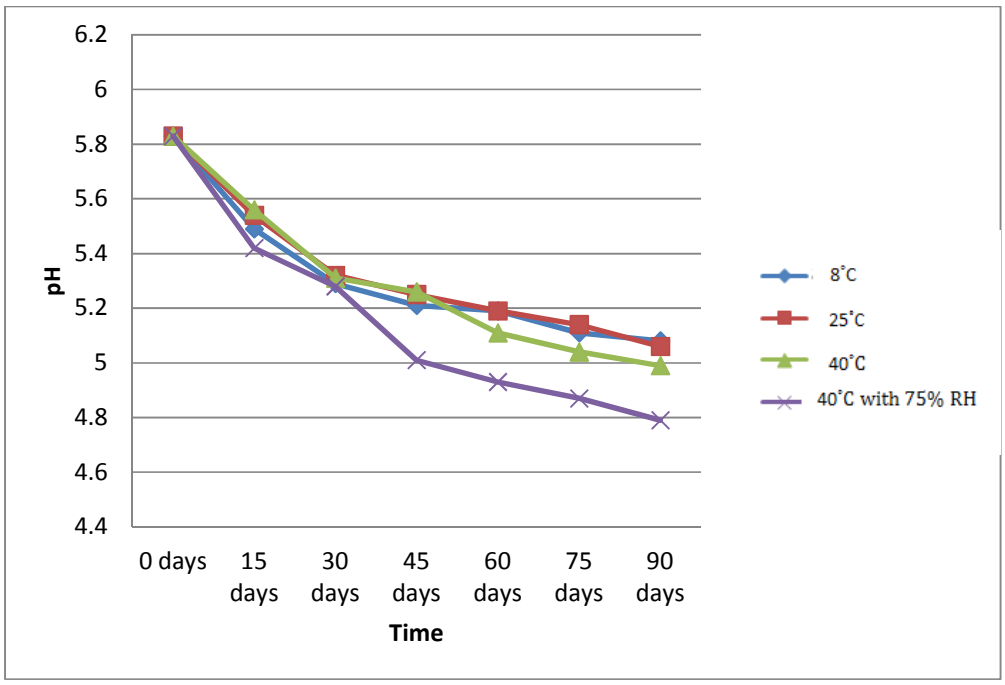

Fig. 3. Graph showing pH values of formulation B kept at different storage conditions

From the present study, we conclude that the emulgels containing *Annona squamosa* L. extract with and without penetration showed good physical characteristics and pharmaceutical stability. The use of additional penetration enhancer is useful not only in improving penetration enhancing activity but also improved stability of emulgels. Thus, the developed topical formulation can be used for the delivery of natural antioxidants in pharmaceutical and cosmetic formulations.

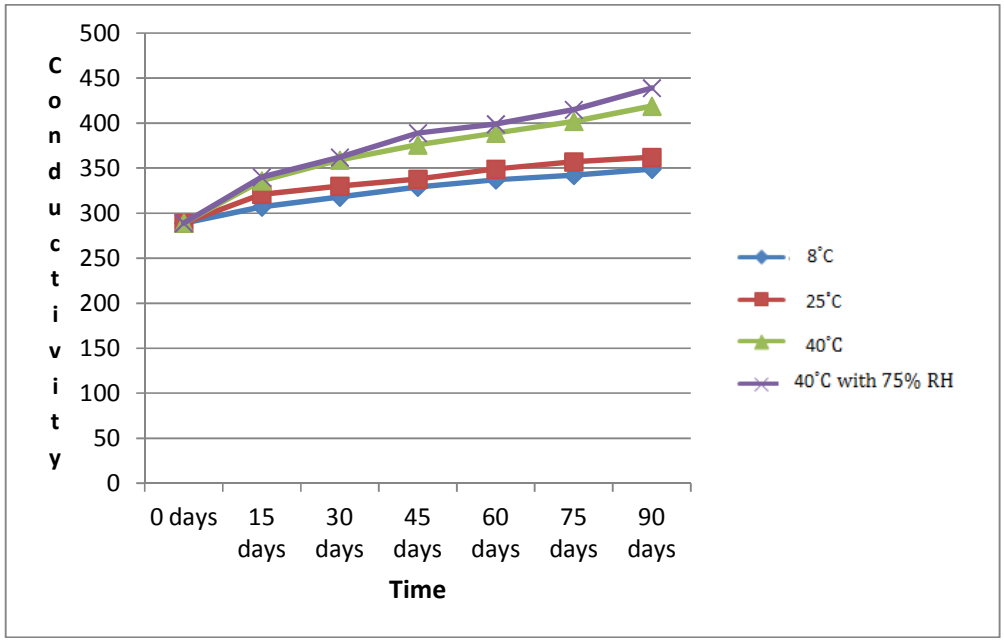

Fig. 4. Graph showing mean conductivity of formulation B kept at different storage conditions

4. CONCLUSION AND RECOMMENDATIONS

It is concluded that the developed emulgels are stable and hence may be used for cosmetic and skin Medicare purposes. Further *in vivo* studies will also be performed to explore different skin parameters like sebum, melanin and hydration level.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ribeiro AS, et al. Main benefits and applicability of plant extracts in skin care products. Cosmetics. 2015;2(2):48-65.
2. Aburjai T, Natsheh FM. Plants used in cosmetics. Phytotherapy Research. 2003; 17(9):987-1000.
3. Fowler JJ, et al. Innovations in natural ingredients and their use in skin care. Journal of Drugs in Dermatology: JDD. 2010;9(6 Suppl):S72-81,quiz s82-3.
4. Dubey N, Kumar R, Tripathi P. Global promotion of herbal medicine: India's opportunity. Current Science. 2004;86(1): 37-41.
5. Joshi B, et al. Emulgel: A comprehensive review on the recent advances in topical drug delivery. Int Res J Pharm. 2011;2(11): 66-70.
6. Kumar S, Singh N, Arora SC. Emulgel an Insight. European Journal of Pharmaceutical and Medical Research. 2015;2(4):693-698.
7. Haneefa KM, Mohanta GP, Nayar C. Emulgel: An advanced review. J. Pharm. Sci. & Res. 2013;5(12):254-258.
8. Hardenia A, Jayronia S, Jain S. Emulgel: An emergent tool in topical drug delivery. International Journal of Pharmaceutical Sciences and Research. 2014;5(5):1653.
9. Mariod AA, et al. Antioxidant activity of different parts from *Annona squamosa*, and *Catunaregam nilotica* methanolic extract. Acta Scientiarum Polonorum Technologia Alimentaria. 2012;11(3):249-258.
10. Sahz R, Pharmacognosy and pharmacology of *Annona squamosa*: A review. Int J Pharm Life Sci. 2011;2(10): 1183-1189.
11. Gajalakshmi S, et al. Pharmacological activities of *Annona squamosa*: A review.

International Journal of Pharmaceutical Sciences Review and Research. 2011; 10(2):24-29.
12. Ratshilivha N, et al. The variation in antimicrobial and antioxidant activities of acetone leaf extracts of 12 *Moringa oleifera* (Moringaceae) trees enables the selection of trees with additional uses. South African Journal of Botany. 2014;92: 59-64.
13. Mohamed MI, Optimization of chlorphenesin emulgel formulation. The AAPS Journal. 2004;6(3):81-87.
14. Vasiljević DD, et al. Rheological and droplet size analysis of W/O/W multiple emulsions containing low concentrations of polymeric emulsifiers. Journal of the Serbian Chemical Society. 2009;74(7): 801-816.
15. Zhang YJ. et al. Antioxidant phyto-chemicals for the prevention and treatment of chronic diseases. Molecules. 2015; 20(12):21138-21156.
16. Florence A, et al. Preliminary phytochemical studies of select members of the family Annonaceae for bioactive constituents. Biosci Discov. 2014;5(1):85-96.
17. Khan H, et al. Physical and chemical stability analysis of cosmetic multi-ple emulsions loaded with ascorbyl palmitate and sodium ascorbyl phosphate salts. Acta Poloniae Pharmaceutica. 2016;73(5): 1339-1349.
18. Johnsey Joseph DPA. Boby Johns George, Praveen Raj R, Noby Thomas, Betty Carla. Emulgel: A novel trend in topical drug delivery system. World Journal of Pharmaceutical And Medical Research. 2017;3(4):35-39.
19. Mahmood T, et al. Fabrication, physicochemical characterization and preliminary efficacy evaluation of a W/O/W multiple emulsion loaded with 5% green tea extract. Brazilian Journal of Pharmaceutical Sciences. 2013;49(2): 341-349.
20. Akhtar N, et al. Formulation and *in vitro* evaluation of a cosmetic emulsion containing apple juice extract. Asian Journal of Chemistry. 2010;22(9): 7235.
21. Mohsin S, et al. Formulation and stability of topical water in oil emulsion containing corn silk extract. Tropical Journal of Pharmaceutical Research. 2016;15(6): 1115-1121.
22. Herschel WH. The change in viscosity of oils with the temperature. Industrial & Engineering Chemistry. 1922;14(8):715-722.
23. Shahavi MH, et al. Clove oil nanoemulsion as an effective antibacterial agent: Taguchi optimization method. Desalination and Water Treatment. 2016;57(39):18379-18390.
24. Akhtar N, et al. Formulation development and pharmaceutical evaluation of aw/o emulsion of *Coleus* extract. Indian Journal of Pharmaceutical Education And Research. 2011;45(3):236-241.
25. Yang Y, et al. Formation and stability of emulsions using a natural small molecule surfactant: Quillaja saponin (Q-Naturale®). Food Hydrocolloids. 2013;30(2):589-596.
26. Dhanorkar V, et al. Development and characterization of rosin-based polymer and its application as a cream base. Journal of Cosmetic Science. 2002;53(4): 199-208.

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