

***Annona muricata* extract containing pharmaceutical emulgels with and without penetration enhancer for depigmenting and antierythmic effects**

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Abstract: The basic purpose of this research work was to investigate the skin depigmenting and antierythmic effects of emugel containing *Annona muricata* L. fruit extract by comparing it with its control and the variation in these effects with the addition of penetration enhancer. The control (without extract and penetration enhancer i.e. clove oil 8%) and the two test formulations with 4% fruit extract FA and FB (without clove oil and with clove oil) were formulated and evaluated for *in vitro* characteristics (pH, conductivity and *in vitro* release). The emulgels were then applied on the cheeks of 26 healthy female human volunteers (n=26) for a study period of 12 weeks. Skin melanin and erythema contents were measured by Mexameter at base line and then after every 2 weeks. Both the test formulations showed significant decrease in melanin and erythema contents when compared to control but FB showed marked decrease in skin melanin when compared to the FA. While in case of skin erythema, the effects of FA were greater as compared to other formulation. When paired sample t test (5% level of significance) was applied, the test formulations showed significant results. This study reveals that the *Annona muricata* L. fruit extract naturally contains some important phenolic compounds and can be effectively used in topical preparations for the treatment of skin hyperpigmentation and dermatitis. Skin whitening effects can be increased by the addition of penetration enhancer.

Keywords: *Annona muricata* L., emugel, penetration enhancer, depigmentation.

INTRODUCTION

There is an extensive range of colours (white to black) and gradations in human skin. This variation is due to the occurrence of melanin (chemically inert and stable pigment) which is produced within the skin (Costin and Hearing, 2007). Increased production of melanin can cause different hyperpigmented conditions like melasma, post-inflammatory hyperpigmentation, drug-induced hyperpigmentation and erythema dyschromicum which become prominent with age. To treat such disorders, topical formulations containing tretinoin, hydroquinone, azelaic acid, kojic acid etc are being employed (Stratigos and Katsambas, 2004).

Herbal ingredients are widely being used in cosmetics because of the poor image of animal-derived products (Patil *et al.*, 2014). Plant-derived extracts having compounds which inhibit melanin synthesis may be a good choice for skin whitening and protection against skin darkening (Baurin *et al.*, 2002).

Annona muricata L. family *Annonaceae* is one of the tropical fruits possesses strong antioxidant properties. These properties are associated with the presence of natural antioxidants like phenolic acids, vitamin C and E, carotenoids and flavonoids, which prevent free radical damage (Akomolafe and Ajayi, 2015). Epidemiological

studies have always shown a significant positive correlation between consumption of fruits and vegetables containing antioxidant phytochemicals and reduced chances of heart diseases mortality, other degenerative diseases, common cancers and ageing (Kaur and Kapoor, 2001)

Emugel, a novel drug delivery system has better patient acceptability due to possessing the activities of both emulsions and gels (Kumar *et al.*, 2015). They have the advantages of being transparent, thixotropic, easily spread able & having longer shelf life (Haneefa *et al.*, 2013). The presence of penetration enhancing ingredients in the topical formulation temporarily changes the skin barrier, modifies the partitioning of the drug into skin structures and ultimately enhances drug penetration into skin (Hardenia *et al.*, 2014).

This study depicts the depigmenting and anti-inflammatory effects of *Annona muricata* L. extract in a topical preparation and the change in these effects with the addition of penetration enhancer.

MATERIALS AND METHODS

Chemicals and apparatus

Acetone (Merck KGaA Darmstadt, Germany), DPPH(Sigma, USA), Carbopol 940 (Sigma, USA), Triethanolamine (Merck KGaA Darmstadt, Germany), Span 80 (Sigma, USA), Tween 20 (Sigma, USA),

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Propylene glycol (Merck KGaA Darmstadt, Germany), Liquid paraffin (Merck KGaA Darmstadt, Germany), Methyl paraben (Acros Organics, USA), *Annona muricata* L. (imported from Malaysia), Rotary evaporator (Eyela, Co. Ltd. Japan), homogenizer (Euro-Star, IKA D 230, Germany), pH-meter (WTW pH-197i, Germany), water bath (HH. S214. China), Mexameter MPA-5 (Courage & Khazaka, Germany)

Plant material

Annona muricata L. was imported from Malaysia and then identified from the department of life sciences, The Islamia University of Bahawalpur, Pakistan with voucher number 7686/LS.

Preparation of fruit extract and determination of antioxidant activity

Annona muricata L. extract was prepared by using cold maceration technique. 100 g of sliced whole fruit (peel, pulp and seed) was macerated in 500ml of acetone (70%) for 72hr at room temperature. The macerate was stirred daily for 30 minutes. The residues were collected 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 by using rotary evaporator at 45⁰C. The concentrated extract was stored at 4⁰C for further studies.

2, 2-diphenyl-1-picryl-hydrazyl (DPPH), a stable free radical was used to quantify the antioxidant activity with slight modification of method as described by Ratshilvha *et al.* Ascorbic acid was a reference standard antioxidant used in this measurement.

Preparation of emulgel

The emulgels were prepared by the method reported by Muhammad *et al.* This method involves three steps for the formulation of emulgel: first the formulation of emulsion, then the formulation of gel base, then finally incorporating the emulsion into the gel base to develop the final formulation.

One control emulgel (without extract) and two test formulation emulgels FA and FB were prepared one with extract and second with extract along with penetration enhancer. The aqueous phase of the emulsion contains Tween 20, Methyl Paraben, Propylene glycol and Distilled water while in oily phase liquid paraffin and span 20 was used. The gelling agent used was carbapol-940 (2%) and the penetration enhancer was clove oil (8%).

In-vitro characterization

pH determination

The pH of freshly prepared emulgels and emulgels kept at different storage temperatures (8⁰C, 25⁰C, 40⁰C and 40⁰C

& ± 75 % relative humidity) was measured with the help of a digital pH meter Ino-Lab pH7110 pH meter (WTW, Germany) 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.

In-vitro release study

Ascorbic acid release across rabbit's skin was determined using Franz diffusion cell with an effective diffusion area of 1.72cm². Prior to the use the rabbit's skin was rinsed with distilled water and soaked in receptor liquid (glycerine: water pH4) for at least 1 hr. The skin was then placed horizontally dividing the cell into receptor and donor compartments.

0.5g emulgel was applied homogeneously on skin surface. The experiment was done on thermostatically controlled water bath at 25±2⁰C with agitation. The aliquots of 1ml at regular intervals of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5hr were removed and ascorbic acid amount was determined by UV at 268nm (Farahmand *et al.*, 2006)

In-vivo studies

Study protocol

A total of 26 healthy female volunteers with a mean age of 25 y were selected and divided into two groups each comprising 13 volunteers (one for control and formulation A and second for formulation A and B). A single blinded study was designed to contrast the effects of different formulations and a consent form containing the terms and conditions of testing was signed from all volunteers before beginning of a study. Volunteers were examined by a specialist for any type of skin disease particularly on cheeks and forearms. Each volunteer was instructed to apply creams on cheeks twice daily during the entire study period and appear for measurement of effects on 2nd, 4th, 6th, 8th, 10th and 12th week. The measurements were taken by using Mexameter® MPA 5 (Courage + Khazaka, Germany) at controlled conditions of 25±1⁰C and 45±2% relative humidity.

Skin irritation assessment

A patch test was done on both forearms of every volunteer on the first day of *in vivo* study to evaluate primary irritation potential of formulations. A 5 × 4 cm region was marked on forearms of volunteers. For 1st group Patch for left forearm was applied with 1.0 g of formulation A while that of right forearm was applied with 1.0 g of the control after application on marked areas. Same was done with 2st group by choosing left forearm for formulation B and right forearm for formulation A. A surgical dressing was applied on the marked regions for patch test after application of formulations. The dressings were removed after 48 hrs and regions of forearms were rinsed with physiological saline. The skin was observed for any irritation by using Mexameter.

Ethical standards

The human and animal study was approved (Reference no. 33/S-2018-/PREC) by the Board of the Advanced Study and Research (BASAR), the Islamia University of Bahawalpur and the Institutional Ethical Committee, Faculty of Pharmacy and Alternative medicines, The Islamia University of Bahawalpur, Pakistan.

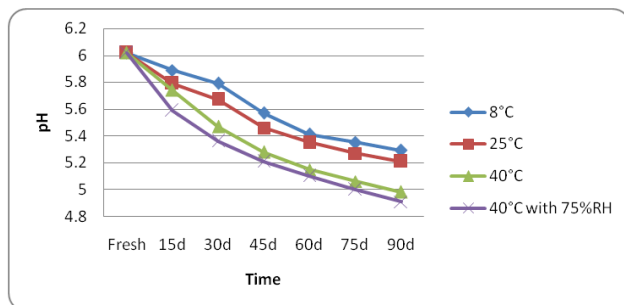


Fig. 1: Graph showing the pH changes in the control emulgel. Key: (d=day, °C =degree Celsius, RH = relative humidity)

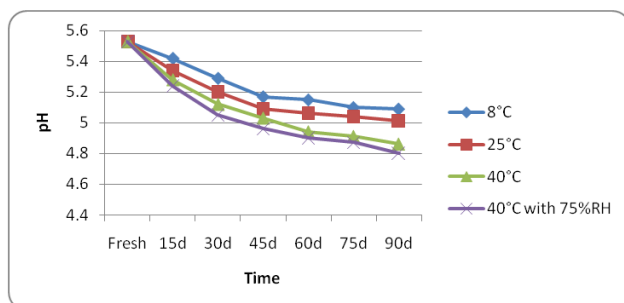


Fig. 2: Graph showing the pH changes in the Formulation A. Key: (d=day, °C =degree Celsius, RH = relative humidity)

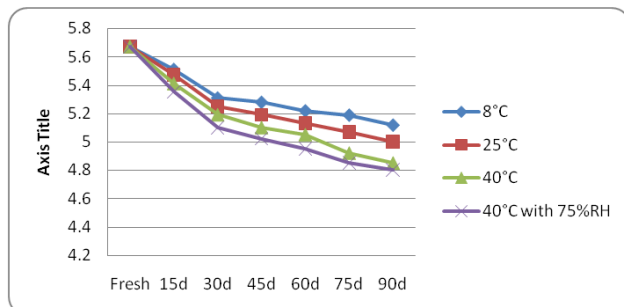


Fig. 3: Graph showing the pH changes in the Formulation B. Key: (d=day, °C =degree Celsius, RH = relative humidity)

Mathematical and statistical analysis

The percentage changes for the each value of different parameters of volunteers were determined by the following formula:

$$\text{Percentage change} = [(A-B)/B] \times 100 \quad (\text{Equation 1})$$

Where; A =Individual value of any parameter specific week, B = Zero hour value of that parameter.

Paired samples t-test for deviation between the two preparations and two-way ANOVA for deviation between different times intervals were analyzed using SPSS 15.0 using a 5% level of significance.

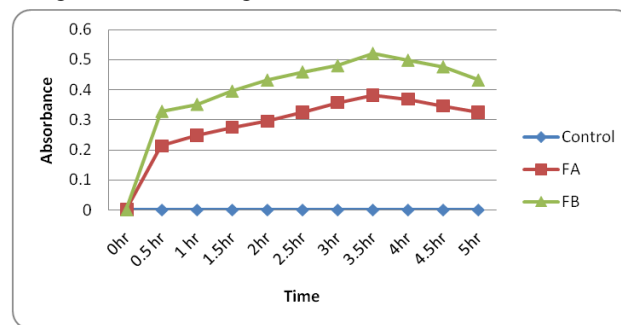


Fig. 4: Graph showing the release profile of control, formulation A (FA) and formulation B (FB)

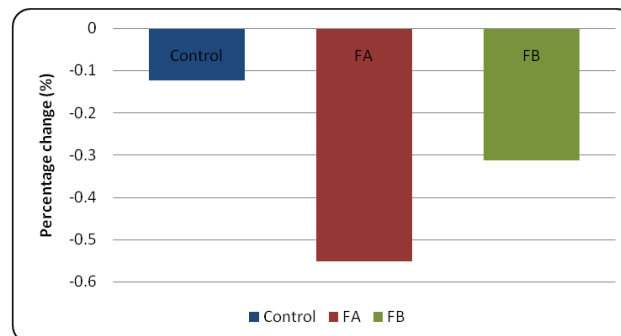


Fig. 5: Patch test showing the percentage change in skin erythema value after 48 hours with the use of control, formulation A (FA) and formulation B (FB).

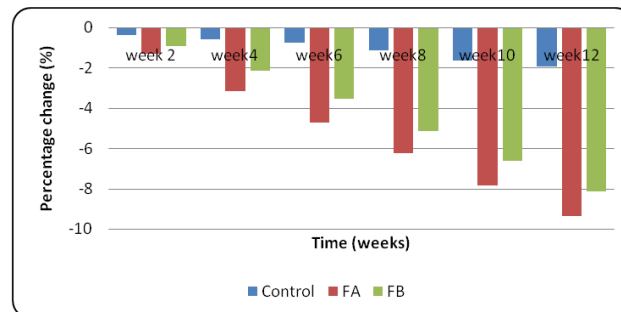


Fig. 6: Percentage change in skin erythema value after 12 weeks with the use of control, formulation A (FA) and formulation B (FB).

RESULTS

Antioxidant activity

The studied fruit extract showed a remarkable free radical scavenging activity (88%) against DPPH when compared to the free radical scavenging activity of the ascorbic acid as standard (92%).

pH determination

The pH of the freshly formulated emulgels was 6.02, 5.53 and 5.67 of control, formulation A and formulation B

respectively which is within the skin pH range. The changes occurred in the pH values of control and test formulations at different storage conditions are noted in figs. 1-3. The pH values of all 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 % relative humidity.

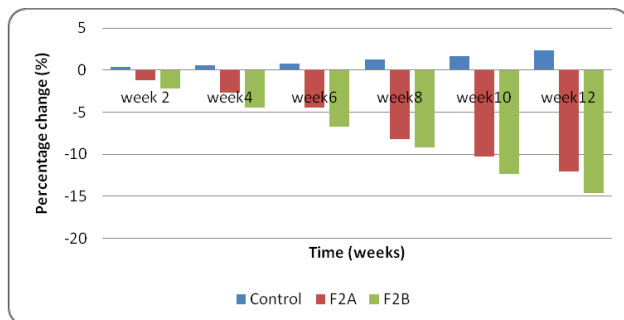


Fig. 7: Percentage change in skin melanin value after 12 weeks with the use of control, formulation A (FA) and formulation B (FB).

In vitro release study

When the release profile was observed by using Franz diffusion cell, it was noted that the control showed no release till the end of observation but the formulation A showed a gradual increase in release up till 3.5 hours and then a decrease in the last hour while that release profile was greater in case of formulation B when compared to formulation A. The release of ascorbic acid was increased from 0.213 (0.5hr) to 0.383 (3.5hr) in case of FA while in case of FB that release was 0.328 (0.5hr) to 0.521 (3.5hr) as shown in fig. 4.

Skin irritation assessment

Skin erythema contents were measured before application of emulgel (0 hour readings) and then after 48 hours by Mexameter MPA 5 (Courage and Khazaka GmbH). It was observed that after application of the control, the erythema level was decreased slightly while that after application of the formulations was decreased more after 48 h. But with the application of paired sample t-test it was obvious that the effects of the formulations and control were insignificant. The percent changes occurred were calculated by with the help of equation 1 and result is presented in fig. 5.

Skin erythema contents

Skin erythema content were measured prior to the application of emulgel (0 hour readings) and then at 2nd, 4th, 6th, 8th, 10th and 12th week of study period by using Mexameter MPA 5 (Courage and Khazaka GmbH). It was observed that there were slight variations occurred in erythema values after the use of control. However, with the use of formulation A, it was observed that there was a marked decrease in erythema values while the decrease in erythema value with the use of formulation B was greater

than control but less than formulation A. The percent changes occurred were calculated by the use of equation 1 and result is presented in fig. 6. With the use of the ANOVA, the changes in erythema values produced by the formulations were found to be significant and that by the use of control were found insignificant over time. After the application of the paired sample t-test it was found that the control and formulations showed significant variations as regards to erythema values over time.

Skin melanin contents

Skin melanin content were measured prior to the application of emulgel (0 hour readings) and then at 2nd, 4th, 6th, 8th, 10th and 12th week of study period by Mexameter MPA 5 (Courage and Khazaka GmbH). After the application of control, there was a slight increase observed in skin melanin contents, but in the case of the formulation A there was a continuous decrease in skin melanin content, and that decrease was more pronounced in case of formulation B throughout the study period. The percent changes occurred were calculated by using the equation 1 and result is presented in fig. 7.

When the ANOVA test was applied, it was found that changes in skin melanin values produced by the application of control were insignificant and by the test formulations were significant over time. After the application of paired sample t-test, the effects after the application of formulation were significant ($p \leq 0.05$) when compared to that of control.

DISCUSSION

The presence of antioxidant phytochemicals like polyphenols and carotenoids contributes to the antioxidant properties of plants (Zhang *et al.*, 2015). Polyphenols are powerful antioxidants and helpful in the prevention of certain neurodegenerative diseases, cardiovascular diseases and cancers etc. (Florence *et al.*, 2014). 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 essential parameter for the stability and efficacy of topical formulations. The pH of the topical emulgels should be inside the range of skin pH i.e. 5-7 to avoid any kind of skin irritation (Johnsey Joseph, 2017). 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 (Mahmood *et al.*, 2013).

In the development of topical formulations, the *in vitro* release of the drug from artificial membranes is necessary for predicting an suitable vehicle as they measure

drug/vehicle interaction (Özer *et al.*, 2007). In this study, the release of ascorbic acid was increased during first 3.5 hrs and then decreased from both the formulations FA and FB but the release of drug was greater in case of FB. Previous studies also supported such release pattern of ascorbic acid in multiple emulsion (Farahmand *et al.*, 2006). The increased release in case of formulation B is related to the presence of clove oil (penetration enhancer). The occurrence of eugenin (terpene) in clove oil is related to the increased release of the formulation containing clove oil (Kumar *et al.*, 2014). According to lipid partition theory, there are three different mechanisms of penetration enhancers, one of which is increasing the partitioning of drug into skin tissue (Fox *et al.*, 2011). Clove oil acts as penetration enhancer by increasing the partition of drug into the stratum corneum.

Initially, the patch test was performed to evaluate the safety of all the formulations. For the evaluation of acute irritation potential of topical formulation, the patch testing following a single application is an extensively used method (Gaspar *et al.*, 2008). Both the formulations as well as control showed no skin irritation during patch test, and decreased the skin erythema contents when observed after 48 hours. This shows that all the emulgels can safely be used to the human skin for cosmetic and therapeutic purposes.

Regarding the skin erythema contents, all the three emulgels decreased the skin erythema contents and the order of decrease was: FA>FB>Control. The inflammatory response subsequent of acute UV irradiation and the degenerative progressions related to chronic skin exposure to UV radiations are largely associated by the overproduction of reactive oxidative species and by destruction of the antioxidant defence system (Ali *et al.*, 2012). The presence of non-enzymatic antioxidants namely ascorbic acid, total carotenoids, lycopene, flavonoids, α -tocopherol, and reduced glutathione are crucial for the cellular systems in decreasing reactive oxygen species (ROS) (Rani *et al.*, 2004). The reduced skin erythema contents with the application of the formulation A and B may be related to the occurrence of antioxidants like carotenes, tocopherols and ascorbic acid in the fruit extract (Muthu and Durairaj, 2015). The decreased effect of formulation B as compared to formulation A may be associated to the components of clove oil i.e. eugenol and β caryophyllene which are slightly irritant (Chen *et al.*, 2015).

In case of skin melanin contents, the control emulgel increased the skin melanin contents and the test formulations decreased the skin melanin contents with an order of decrease: FB>FA. The colour of human skin is due to the existence of melanin pigment in skin. It is also present in bacteria, plants and fungi. Tyrosinase is the major enzyme responsible for the biosynthesis of melanin

(Nerya *et al.*, 2003). Hyper-activity of this enzyme results in the over production of melanin which leads to hyper-pigmentation of the skin and hypo-activity of this enzyme results in certain disorders like vitiligo (depigmentation spots occurring on the skin) and hair whitening (Mapunya and Lall, 2011). So, tyrosinase enzyme inhibition can lead to decreased melanin production.

The reduction in skin melanin contents can be related to the phenolics and flavonoids present in *Annona muricata* L. fruit like quercetin, caffeic acid, cinnamic acid, syringic acid, ferulic acid and *p*-coumaric acid etc. (Jiménez *et al.*, 2014, Adefegha *et al.*, 2015). The inhibition of tyrosinase enzyme, associated with flavonoids might be due to chelating the active center of tyrosinase enzyme leading to decreased melanin synthesis (Saewan *et al.*, 2011). The skin depigmenting and antioxidant activity of *Annona muricata* L. fruit can also be related to presence of ascorbic acid (Singh *et al.*, 2014). The increased effects of formulation B may be associated with the penetration enhancement effect of clove oil (Jiang *et al.*, 2017). Eugenol in the clove oil is reported in increasing the partitioning of the drug to the stratum corneum (Aggarwal *et al.*, 2013).

CONCLUSION

From the present findings, it can be concluded that the *Annona muricata* L. extract was successfully incorporated into a topical emulgel and showed its skin depigmenting activity at 4% concentration. The depigmenting effect was increased with the addition of penetration enhancer but that effect is not seen in case of anti-inflammatory action. So, this extract can be successfully used as a natural alternative treatment for certain skin diseases. A targeted study is needed in the future to explore the actual potential of this plant in patients with melasma and psoriasis.

REFERENCES

- Adefegha SA, Oyeleye SI and Oboh G (2015). Distribution of phenolic contents, antidiabetic potentials, antihypertensive properties and antioxidative effects of soursop (*Annona muricata* L.) fruit parts *in vitro*. *Biochemistry Research International.*, 2015.
- Aggarwal S, Agarwal S and Jalhan S (2013). Essential oils as novel human skin penetration enhancer for transdermal drug delivery: A review. *Int. J. Pharm. Bio. Sci.*, **4**: 857-868.
- Akomolafe S and Ajayi O (2015). A comparative study on antioxidant properties, proximate and mineral compositions of the peel and pulp of ripe *Annona muricata* (L.) fruit. *IFRJ*, **22**(6): 2381-2388
- Ali A, Akhtar N and Khan MS (2012). *In vivo* evaluation: the effects of a cream containing Acacia bark extract

- on skin melanin and erythema content. *Postepy Dermatol. Alergol.*, **29**: 369.
- Baurin N, Arnoult E, Scior T, DO Q and Bernard P (2002). Preliminary screening of some tropical plants for anti-tyrosinase activity. *J. Ethnopharmacol.*, **82**: 155-158.
- Chen J, Jiang QD, WU YM, Liu P, Yao JH, LU Q, Zhang H and Duan JA (2015). Potential of essential oils as penetration enhancers for transdermal administration of ibuprofen to treat dysmenorrhoea. *Molecules*, **20**: 18219-18236.
- Costin GE and Hearing VJ (2007). Human skin pigmentation: Melanocytes modulate skin color in response to stress. *The FASEB Journal*, **21**: 976-994.
- Farahman S, Tajerzadeh H and Farboud E (2006). Formulation and evaluation of a vitamin C multiple emulsion. *Pharm. Dev. Technol.*, **11**: 255-261.
- Florence A, Joselin J, Brintha T, Sukumaran S and Jeeva S (2014). Preliminary phytochemical studies of select members of the family Annonaceae for bioactive constituents. *Biosci Discov.*, **5**: 85-96.
- Fox LT, Gerber M, Plessis JD and Hamman JH (2011). Transdermal drug delivery enhancement by compounds of natural origin. *Molecules*, **16**: 10507-10540.
- Gaspar L, Camargo JR F, Gianeti M and Campos PM (2008). Evaluation of dermatological effects of cosmetic formulations containing *Saccharomyces cerevisiae* extract and vitamins. *Food Chem. Toxicol.*, **46**: 3493-3500.
- Haneefa KM, Mohanta GP and Nayar C (2013). Emulgel: an advanced review. *J. Pharm. Sci. & Res.*, **5**: 254-258.
- Hardenia A, Jayronia S and Jain S (2014). Emulgel: An emergent tool in topical drug delivery. *IJPSR*, **5**: 1653.
- Jiang Q, WU Y, Zhang H, Liu P, Yao J, Yao P, Chen J and Duan J (2017). Development of essential oils as skin permeation enhancers: Penetration enhancement effect and mechanism of action. *Pharm. Biol.*, **55**: 1592-1600.
- Jimenez VM, Gruschwitz M, Schweiggert RM, Carle R and Esquivel P (2014). Identification of phenolic compounds in soursop (*Annona muricata*) pulp by high-performance liquid chromatography with diode array and electrospray ionization mass spectrometric detection. *Food Res. Int.*, **65**: 42-46.
- Johnsey Joseph DPA, Boby Johns George, Praveen Raj R, Noby Thomas and Betty Carla (2017). Emulgel: A novel trend in topical drug delivery system. *WJPMR*, **3**: 35-39.
- Kaur C and Kapoor HC (2001). Antioxidants in fruits and vegetables the millennium's health. *Int. J. Food Sci. Technol.*, **36**: 703-725.
- Kumar A, Aggarwal G, Singh K and Harikumar S (2014). Comparison of vegetable and volatile oils as skin permeation enhancers for transdermal delivery of losartan potassium. *Der. Pharmacia. Lettre.*, **6**: 199-213.
- Kumar S, Singh N and Arora SC (2015). Emulgel an Insight. *EJPMR*, **2**: 693-698.
- Mahmood T, Akhtar N, Khan BA, Rasul A and Khan HMS (2013). Fabrication, physicochemical characterization and preliminary efficacy evaluation of a W/O/W multiple emulsion loaded with 5% green tea extract. *BJPS*, **49**: 341-349.
- Mapunya, Manyatja and Lall Namrita (2011). Melanin and Its Role in Hyper-Pigmentation. Current Knowledge and Future Trends in Research. 10.5772/21159.
- Muthu, S. and Durairaj, B. (2015). Evaluation of antioxidant and free radical scavenging activity of *Annona muricata*. *Eur. J. Exp. Biol.*, **5**: 39-45.
- Nerya O, Vaya J, Musa R, Izrael S, Ben-arie R and Tamir S (2003). Glabrene and isoliquiritigenin as tyrosinase inhibitors from licorice roots. *J. Agric. Food. Chem.*; **51**: 1201-1207.
- Ozer O, Kivcak B, Mutlu B, Akay S, Saglam H and Tömek S (2007). Open access article *in vitro* release studies on multiple and simple emulsions of α -Tocopherol with Pistacia leaves. *Sci. Pharm.*, **75**: 97-146.
- Patil SS, Phutane KR, Adnaik RS, Mohite SK and Magdum CS (2014). Novel cosmeceutical herbal emulgel for skin care. *WJPPS*, **3**: 801-811.
- Rani P, Unni KM and Karthikeyan J (2004). Evaluation of antioxidant properties of berries. *Indian Journal of Clinical Biochemistry*, **19**: 103.
- Saewan N, Koysoomboon S and Chantrapromma K (2011). Anti-tyrosinase and anti-cancer activities of flavonoids from *Blumea balsamifera* DC. *J. Med. Plants Reseach*, **5**: 1018-1025.
- Singh D, Singh S and Banu V (2014). Phytochemical composition, antioxidant activity and sensory evaluation of potential underutilized fruit soursop (*Annona muricata* L.) in Bay Islands. *J. Andaman Sci. Assoc.*, **19**: 30-37.
- Stratigos AJ and Katsambas AD (2004). Optimal management of recalcitrant disorders of hyperpigmentation in dark-skinned patients. *American Journal of Clinical Dermatology*, **5**: 161-168.
- Zhang Y-J, Gan R-Y, LI S, Zhou Y, LI A-N, XU D-P and LI H-B (2015). Antioxidant phytochemicals for the prevention and treatment of chronic diseases. *Molecules*, **20**: 21138-21156.