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Development of grape seed extract based formulations by using non-invasive biophysical technique and its impact on skin aging

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Abstract: Given the substantial benefits of grape seed extract (GSE) in reducing oxidative stress, the study aimed development, characterization and comparative analysis of GSE-based formulations. The development entailed extraction of GSE from *Vitisvinifera L*. HPLC confirmed catechin, epicatechin, gallic acid, epicatechingallate and procyanidin dimers. Storage of Formulations observed, Stability & rheological parameters determined. Olive oil used as a permeability enhancer. Presence of the highest oleic acid content (65-86%) in Olive oil, skin permeability within the stratum corneum was enhanced hence better transdermal skin absorption. Using two-way ANOVA, and T-test, efficacy of formulations and impact on slowing down skin aging by countering exogenous factors of oxidative stress determined. Non-invasive biophysical technique showed emulgel substantially reduced roughness, scaliness, winkles, and sebum content by 55%, 26 %, 23.9% and 30.3% respectively enhancing elasticity and hydration by 50% and 32.2% respectively. Emulsion reduced roughness, scaliness, winkles and sebum content 14%, 13%, 21% and 26.13% respectively enhancing elasticity and hydration 45.3% and 29.85% respectively. The formulations significantly offset exogenous factors of aging and impact on free radicals and oxidative stress and may be safe to incorporate bio-active botanical antioxidants for evaluation of derma cosmetic benefits in management of dehydrated and aged facial skin.

Keywords: Grape seed extract (GSE), skin-aging, oxidative stress, photo-aging, non-invasive biophysical technique, reactive oxygen species (ROS), relative humidity (RH).

INTRODUCTION

Aging is an inevitable process in all living organisms. In this process, oxidative stress damages skin cells and tissues (Magenta *et al*., 2020). Excessive ROS production contributes to aging. The skin provides the frontline defense against pathogens and it helps in the anchoring of cells (Yadav *et al*., 2019). It protects internal structures and organs from detrimental environmental agents, chemicals, and pathogens.

Skin aging is a continuous and multifaceted phenomenon. It is caused due to internal and external factors. Internal factors chiefly include the DNA metamorphosis, hormones, cellular metabolism, genetics, mitochondrial dysfunctions, and disparity in redox & telomere shortening (Tripathi *et al*., 2019, Saluja and Fabi, 2017). External factors include lifestyle, diet, pollution, smoking, UV-light, and other environmental factors. Excessive UV exposure causes photoaging and affects genetic metamorphosis by targeting the dermal matrix (Singh *et al*., 2019, Zarbafian *et al*., 2020). Skin aging has been mainly attributed to degeneration, development of wrinkles, and decline in tactile power.

Photoaging causes functional and mechanical changes in the skin (Peres *et al*., 2011). UV radiations cause the production of ROS. Besides UV-light, visible light, especially blue light (400-495nm), also causes photodamage (Rinnerthaler *et al*., 2015). Efficient photo-protective and chemo-preventive strategies have been introduced to overcome photo-damage of skin (Lim *et al*., 2017). Photosynthesis generates ROS in the whole range of light (Nakashima *et al*., 2017). ROS cause skin aging by oxidative stress (Callaway and Jiang, 2015).Oxidative stress causes DNA damage, inflammation, and neoplastic conditions. Exogenous and endogenous antioxidants have the potential to counterbalance oxidative stress which has a positive outcome on slowing down the aging process (Zarbafian *et al*., 2020). A variety of skincare products containing diverse antioxidants can prevent skin aging when used topically on the skin.

Grape is the world's third most valued cultivated crop (Alston and Sambucci, 2019). GSE can be used in anti-aging cosmetics because it is an efficient and viable natural resource for preventing UV-induced pre-mature aging. Its effectiveness is more than any other reducing agents like vitamins and carotenoids (Camera *et al*., 2009). It increases cell vitality and protects them from UVA impairment. (Hama *et al*., 2012) The strong antioxidant activity of GSE makes it a value-addition to cosmetics (Yarovaya *et al*., 2021).

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MATERIALS AND METHODS

GSE and Determination of Phenolic Content

After drying, grape seeds powder was obtained using a comminutor. The powder was soaked into 03 parts of 95% ethanol for 7 days. Subsequently, through a vacuum evaporator at the temp of 40°C, the filtrate was concentrated. Residue filtrate was dried for three days in an oven. It was done to get the constant weight of the percent yield of extract from the dried powder. The same procedure was repeated thrice. The spectrophotometric method determined the concentration of phenolic contents in GSE. Key phenolic compounds found in GSE include flavonoids (flavones, flavonols, proanthocyanidins), phenolic acid, tannins, stilbenes and coumarins (Morin *et al*., 2008). The concentration of phenolic content was measured in mg/mL. Phenolic content in the extract is usually stated in mg GAE/g DW. The antioxidant properties of GSE were determined through DPPH radical scavenging technique & FRAP assay and calculated antioxidant index (%).

Preparation of Formulations

Different formulations of emulsion and emulgel using different compositions of ingredients were prepared and further investigation was performed on stable formulations.

Preparation of Emulsion

W/O emulsion was prepared. Oily phase had propylene paraben as a preservative, paraffin oil, and Abil-EM 90 as an emulsifier. The distilled water was heated at 85±0.2ºC and then 5% of the GSE was incorporated into it. After that, Methylparaben was added to the resultant formulation. Using a mechanical mixer, the formulations were stirred at 2000 rpm for 15 minutes and aqueous phase &olive oil were also mixed into it. For pleasant fragrance, 2-3 drops of lemon oil were mixed into formulations. Soon after adding the water phase, the mixer's speed was further decreased at 1000 rpm to homogenize both phases for 5 minutes. For good homogenization, speed was further slowed down to 500 rpm and the emulsion was placed at room temperature. The whole procedure was done thrice (n=3).

Preparation of Emulgel

Emulgel was prepared by mixing a gelling agent with emulsion. The oily phase was incorporated drop-wise along with constant stirring with an aqueous phase of emulsion containing GSE. The gel phase was formulated by dispersing Carbapol 940 into water. pH was regulated after dispersion by dint of Triethanolamine. Homogenizer was used to mix emulsion with gel while stirring at 2000 rpm for 15 minutes. Speed was by and by slowed down to 50 % for five minutes and then reduced to $1/4^{th}$for another five minutes to get good homogenization. After a while, the emulgel was placed at room temperature. A few drops of lemon were also mixed to get the fragrance. The whole procedure was done thrice (n=3).

HPLC (Determination of Antioxidant Properties)

The antioxidant ingredients (Phenolic and flavonoids) were analyzed by using the technique employed by Caponio with slight modifications (Caponio *et al*., 1999). 20 ml acetone-water solution (80:20 v/v) was mixed with approx. 2g GSE in a flask. After 30 minutes, the upper layer was parted away. The same procedure was repeated twice with a fresh solution and the supernatants were separated. Under vacuum conditions, acetone was removed at 40°C. With help of a 0.45µm membrane filter, the residue was filtered. Lastly, 20µL of the solution was kept in an HPLC analyzer (Shimadzu, Japan). The analysis was conducted thrice (n=3). HPLC Autosampler (SIL-40) was used for the identification and quantification of phenolic content. Elution solvents include water and acetic acid (92:8) at pH 2.29 & 100% acetonitrile.

For sample extraction and separation, gradients included (a) 0-15-minute 15% acetonitrile, (b) 15-30 minutes 45% acetonitrile, and (c) 30-40 minutes 100% acetonitrile were used. The flow rate kept at 01mL/min. Quantities of phenolic content were estimated from peak areas and their concentrations were reflected in mg/100g of the dry weight (DW)±S.D.

Characterization of Formulations

Sample formulations of emulsion and emulgel were characterized for physical appearance, stability, pH, viscosity, electrical conductivity, and phase separation.

Rheological Study

Shear stress, viscosity, and a shear rate of emulgel formulations were studied through Brookfield DV III ultra-model 220 and Rheolac V2.6 software. All measurements were taken thrice (n=3) at room temperature with spindle speed ranging from 10-100 rpm. Viscosity measurements must comply with Power-law, known as $\tau=\kappa\gamma^{n}$(Ferreira *et al*., 2010).

Where

τ= shear stress, n= flow index, γ= shear rate and k=consistency index

Rheological parameters of fresh sample formulations at room temperature and of samples kept at different storage conditions were determined during the study period.

In Vivo Study of Formulations using Non-Invasive Biophysical Techniques

In Vivo Study of sample formulations of emulsion and emulgel was performed**.** Different skin evaluation parameters like skin moisture content, Sebum content, elasticity, and SELs (scaliness, wrinkles, roughness) were evaluated.

Ethical Considerations

Ethical review committee of faculty of Pharmacy, Bahauddin Zakariya University had approved the research vide letter No 189/2020.

Clinical Study Design

To compare the effects of GSE-based formulations (emulsion & emulgel), the details of active ingredients in formulations were not shared with volunteers. With free consent, 40 healthy females participated in the experiments. The volunteers with skin sensitivity, pregnancy, hormonal disorder, or hypersensitivity with any ingredient of formulations did not take part in the experiments. A dermatologist examined the skin (cheeks and forearm) of the volunteers before they participated in the experiments. A patch test was conducted to see if the possibility of any reaction. Afterward, half of the volunteers were provided emulsions and the other half were provided emulgel for application on the left and right cheeks. The volunteers were advised to use these formulations on their respective areas of cheeks for twelve weeks.

To avoid the effect of direct sunlight, sweating and humidity, all measurements were calculated at room temperature with 40-50% RH. Measurements in triplicate were conducted at different time intervals during 12 weeks.

STATISTICAL ANALYSIS

By using SPSS Version 17.0 (Chicago), Two-way ANOVA with a significance level of 5% and paired t-test were used to measure the percentage changes concerning initial values for every volunteer to calculate different parameters.

RESULTS

Antioxidant Activity Studies (DPPH Assay)

This study showed 93% antioxidant activity of GSE with acetone-water (80:20 v/v) consumption. This high antioxidant activity was attributed to numerous polyphenolic Phyto-constituents (Fu *et al*., 2015).

Macroscopic Observations of Formulations

During the study, macroscopic parameters of formulations were observed. Formulations remained stable and viscosity did not change during the period of study. The pH of formulations remained within the range of 4.6 to 5.8. The pH values suit perfectly for topical use on skin as normal skin pH is 5.5 (Korać and Khambholja, 2011). No centrifugation or phase separation was found in any formulation. Key results are mentioned in table 1.

Rheological Study

Rheological parameters of a formulation have different effects on spreadability and ease of application on the skin(Gaspar and Maia Campos, 2003). Flow curves are shown in fig. 1-4. Shear stress change shear rate determines rheological behavior of formulation i.e. whether is Newtonian or otherwise. An increase in shear stress causes increases in the shear rate. The fig. 1-4 shows that formulations exhibited flow like plastic at 25°C. With an increase of shear rate, viscosity decreases which confirms the properties of non-Newtonian pseudoplastic fluids. Results show that emulsion exhibited lower viscosity than emulgel as emulgel contained carbapol 940.

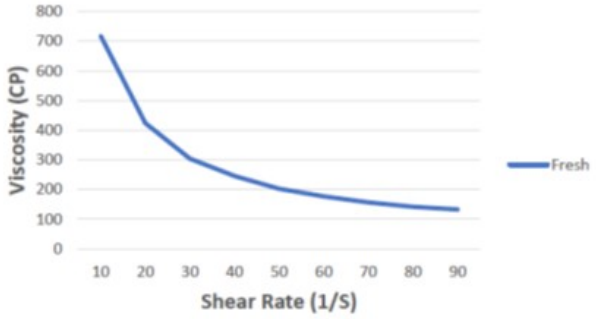

Fig. 1: Viscosity profile of emulsion formulation at T_0

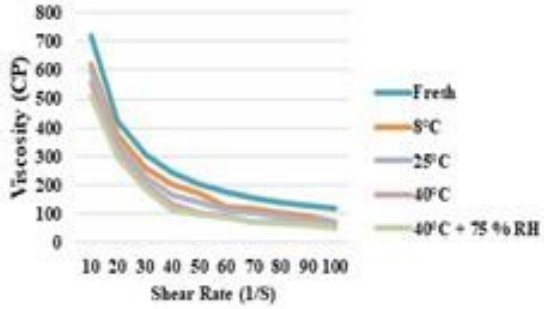

Fig. 2: The changes in CP of emulsion formulation at different storage conditions after 90 days

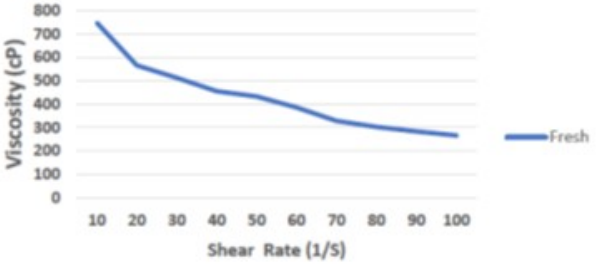

Fig. 3: Viscosity profile of emulgel formulation at T_0

Table 1: Macroscopic observations of formulations

Formulation	Macroscopic Observation	pH
GSE-based Emulsion	Light pink color, No phase separation or liquefaction, homogeneous	5.38
GSE-based Emulgel	Light pink color, No phase separation or liquefaction, homogenous	5.13

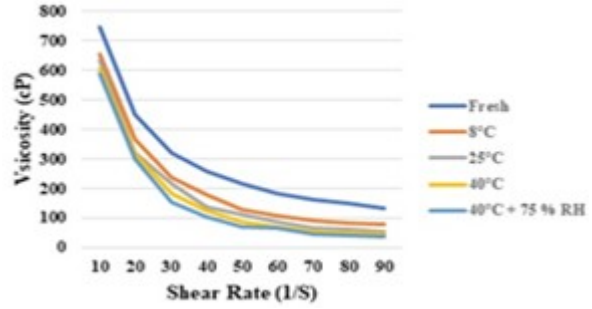

Fig. 4: The change in CP of emulgel at different storage conditions after 90 days

HPLC (Determination of Antioxidant Properties)

HPLC studies confirmed the presence of antioxidants in GSE. Chromatogram of GSE with peaks of Catechin (290.3 MW), Epicatechin (16.1 MW), Gallic acid (3.0 MW), Epicatechingallate (22.7 MW), and procyanidin dimers (B-1(578.5), B-2 (578.5), B-3 (576.5) at different retention time (min) (11.5, 14.5, 5.8, 23.5 and (9.7, 13.3, 10.7) respectively has been depicted in the fig. 5. Catechin in GSE is responsible for the bitterness and astringency of grapes (Gaspar and Maia Campos, 2003). Gallic acid possesses anti-inflammatory and anti-microbial properties. Likewise, epicatechin in extract oppose UV-induced oxidative damage of skin fibroblast and inhibit the tyrosinase activity (Domingo *et al*., 2010). The antioxidants' action of procyanidins dimers depends on the level of polymerization. The higher the level of polymerization, the greater the antioxidant activity (Perumalla and Hettiarachchy, 2011).

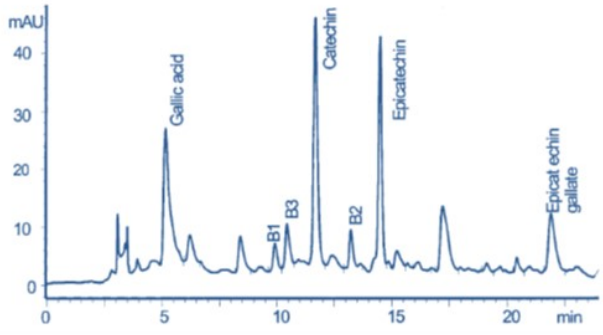

Fig. 5: HPLC profile of GSE by 80:20 Acetone/water solution at room temp

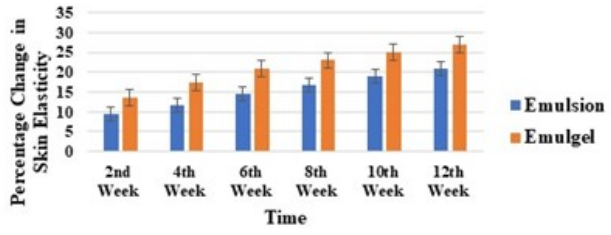

Fig. 6: Comparative analysis between GSE based emulsion and emulgel on skin elasticity (n=40)

Skin elasticity

The measurement of skin elasticity has been carried out through Elastometer EM25 provided with a single probe. Calculation of cutaneous elasticity permits monitoring of skin's visco-elastic properties regarding immediate deformation and its recovery. After application of GSE-based emulsion and emulgel, the percentage increase in skin elasticity at T4, T8 and T12 were 11.78±0.73, 16.81±0.94 and 20.89±1.05 (for emulsion) and 17.5± 1.47, 22.99±1.52 and 26.94±1.42 (for emulgel). At T12, emulsion and emulgel caused a 45.38% and 50% increase in skin elasticity shown in fig. 6. Two-way ANOVA test showed that formulations greatly improved skin elasticity ($p \leq 0.05$).

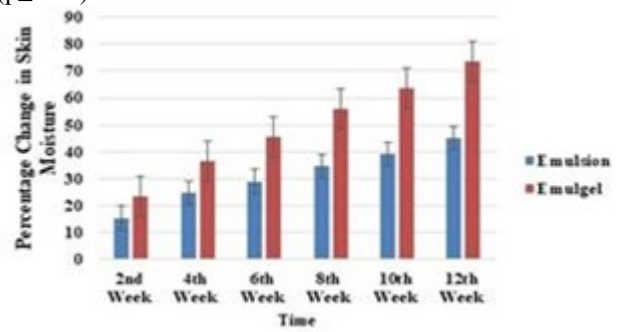

Fig. 7: Comparative analysis between GSE based emulsion and emulgel on skin moisture (n=40)

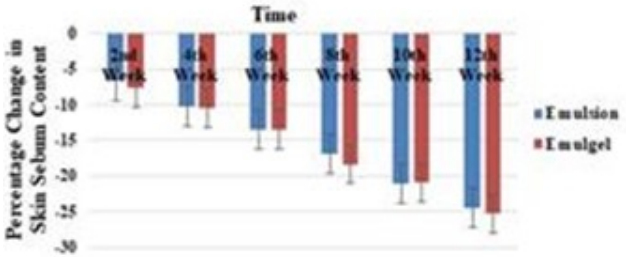

Fig. 8: Comparative analysis of formulations on skin sebum content (n=40)

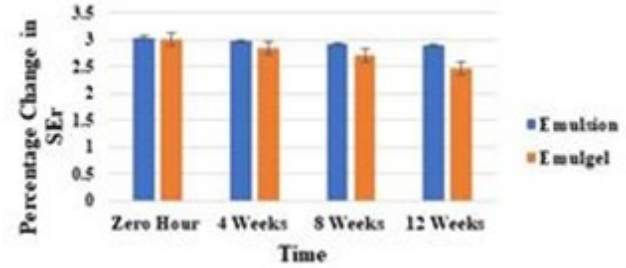

Fig. 9: Comparative analysis between GSE based emulsion and emulgel on SEr (n=40)

Skin moisture

It is an important factor to maintain the epidermis layer of skin and its biomechanical properties. Corneometer CM825 was used to measure skin moisture at a different time interval after application of emulsion and emulgel. This device used a capacitor sensor to monitor the dielectric constant of skin and its layers. Skin hydration

level was measured during the study. The percentage increase in skin moisture level at T4, T8 and T12 were 24.72±2.04, 34.82±2.25 and 45.29±2.64 (for emulsion) and 36.48±2.32, 55.98±2.09 and 73.47±1.87 (for emulgel) respectively. GSE-based emulsion and emulgel indicate a continuous increase in moisture content of skin which indicates the water retention feature of formulation as shown in fig. 7. At T12, emulsion and emulgel increase skin moisture by 29.85% and 32.19% respectively. Two-way ANOVA test showed significant improvement in skin moisture over time ($p \leq 0.05$).

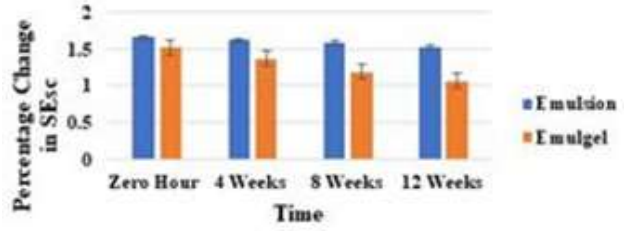

Fig. 10: Comparative analysis between GSE based emulsion and emulgel on SEsc (n=40)

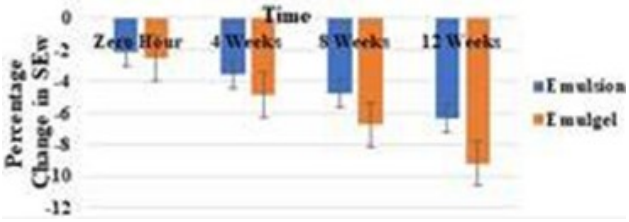

Fig. 11: Comparative analysis between GSE based emulsion and emulgel on SEw (n=40)

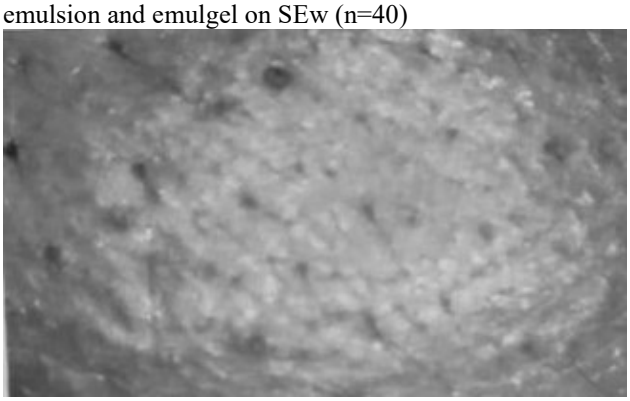

Fig. 12: Surface of the skin of a volunteer before application of emulsion formulation

Skin sebum content

The impact of both formulations on sebum secretion was analyzed using a sebum meter. A decrease in sebum content was observed by application of emulsion and emulgel.

The percentage decrease in skin sebum content at T4, T8 and T12 were -10.24±1.67, -16.94±2.03 and -24.56±2.53 (for emulsion) and -10.45±1.50, -18.33±1.83 and -25.19± 2.57 (for emulgel) respectively. A two-way ANOVA test confirmed a decline in skin sebum content for time ($p \leq 0.05$). After three months, emulsion and emulgel decreased skin sebum content by 26.13% and 30.03% respectively which is shown in fig. 8.

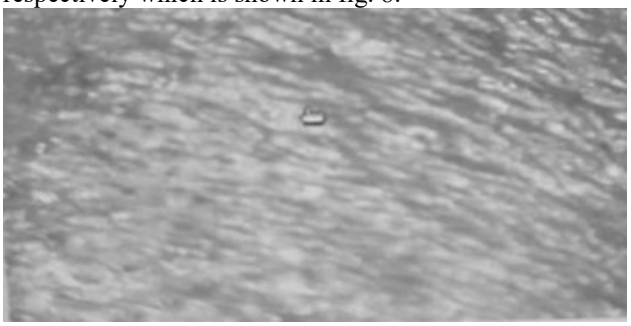

Fig. 13: Surface of the skin of a volunteer after application of emulsion formulation after 12 weeks

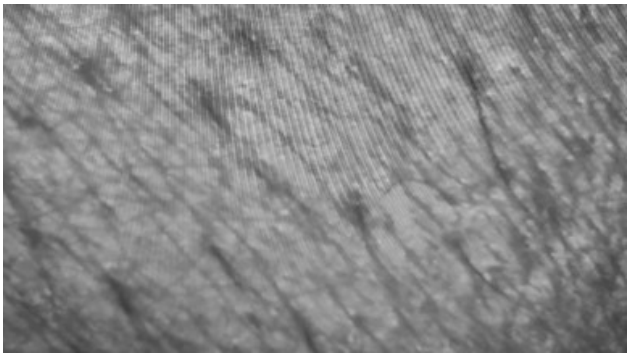

Fig. 14: Surface of the skin of a volunteer before application of emulgel formulation

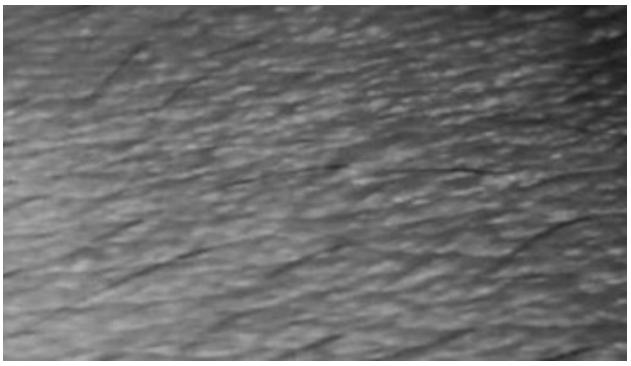

Fig. 15: Surface of the skin of a volunteer after application of emulgel formulation after 12 weeks

Surface Evaluation of Living Skin (SELS)

Skin parameters like roughness, scaliness and wrinkles were evaluated by using Visioscan VC98. High-resolution images of a specific portion of skin where formulations were applied, were taken at different time intervals. MATLAB software was used to analyze the skin images for different parameters. Results showed in fig. 9-11 illustrated that emulsion reduced skin roughness, scaliness and wrinkles by 14%, 13% and 21% respectively whereas emulgel reduced the same parameters by 55%, 26% and 23.9% respectively after 12 weeks. The surface of the skin of a volunteer before application of emulsion and

emulgel formulations have been shown in fig. 12 and fig. 14. The surface of the skin of the same volunteers after application of emulsion and emulgel formulations have been shown in fig. 13 and fig. 15. It could be seen that before the application of formulations, the surface of the skin of volunteers had wrinkles, roughness, scaliness and less smoothness. With the application of formulations on the skin surface, wrinkles, roughness and scaliness have substantially reduced and smoothness increased.

DISCUSSION

DPPH scavenging activity determined antioxidants in the sample formulations. An increase in the degree of hydroxylation of the phenolic compound increases DPPH scavenging activity. The results showed that GSE-based formulation counterbalanced the skin damage caused by photoaging through oxidative stress. Antioxidants of GSE have caused skin rejuvenation by decreasing oxidative stress (Zhou *et al*., 2016). The main ingredients of GSE are polyphenols and proanthocyanins. Polyphenolic content in GSE is higher than the apple, spinach, lemon verbena, black tea, pomegranate, olives, peach, orange, broccoli, redcurrant, apricot (Joseph, 2019). These components work as antioxidants and protect cells from free radicals induced by UV and VIS light radiation (Zhou and Raffoul, 2012). The GSE with good UV absorption capacity, when used in formulations, may provide safe protection against photoaging. Therefore, GSE has been strongly considered as a key additive to anti-aging formulations with a wide scale of protection (Greene, 2001).

The aging process causes elastosis. Catechinepi-gallocatechingallate and cinnamic acid derivatives have anti-elastosis properties (Kim *et al*., 2004, Löser *et al*., 2000). Antioxidantsstimulate collagen synthesis and decrease the enzymatic decline of collagen matrices (Kishimoto *et al*., 2013). Topical formulations loaded with bioactive phenolic ingredients particularly GSE enhanced the water level of the skin and make the skin smoother (Khan *et al*., 2015). Proanthocyanidins increased the rate of convertibility of solubility of collagen into insoluble collagen and also decreased the rate of enzyme degradation (Han *et al*., 2003). An increase in collagen synthesis caused an increase in hydration level. Peroxisome proliferation activated receptor helped maturing of sebocytes in sebaceous glands. It has 3 isoforms α, β, γ. Cosmetic formulation containing polyphenol reduces sebum production, by retarding maturing of sebocytes which affect sebum production (Ali *et al*., 2012).

Due to enhanced permeability, phenolic compounds in GSE-based emulsion and emulgel improved SELS indicators. Phenolic compounds have redox qualities and quench oxygen by donating hydrogen. In this way, they offset the negative impact of UV radiations on the skin by counterbalancing oxidative stress. Proanthocyanidins scavenge free radical effect more than vitamin C & E and prevent UV-B, UV-C induced lipid peroxidation (El Gharras, 2009).

Being safe, Olive oil was used to increase the spreadability and permeability of formulations into the skin. It contains more oleic acid (76%) than avocado oil (68%), crocodile oil (40%), and coconut oil (8%). The unsaturated fatty acids cause oil to spread easily. It offset moisture evaporation from the skin (Viljoen *et al*., 2015). Thus, it enhanced the diffusion of GSE into the skin and give better results.

When the results were compared with other studies, it could be seen that GSE-based formulations produced better results than Tamarindusindica seeds extract (Waqas *et al*., 2017) and carrot extract (Dias, 2014).

CONCLUSION

GSE-based emulsion and emulgel were successfully prepared and tested for their anti-aging potential on the skin. The tested formulations are effective and safe. Notably, polyphenols in GSE have hydrating, anti-inflammatory and anti-wrinkle properties that promote skin hydration, elasticity and reduce roughness, scaliness, and winkles. Emulgel produced better results on skin aging as compared to emulsion because of its two-fold better-controlled release effect than emulsion. In post-COVID-19 scenarios, GSE-based cosmetics may be beneficial to offset the aging effect of the virus. However, experiments need to be undertaken to show its effectiveness on post-COVID-19 scenarios.

REFERENCES

Ali A, Naveed A, Muhammad H and Khan HMS (2012). Cream containing acacia bark extract significantly reduces skin sebum content in healthy volunteers. *J. Sci. Ind. Res.,* **71**: 678-681.

Alston JM and Sambucci O (2019). Grapes In The World Economy. *In:* Cantu D and Walker MA (Eds.) The Grape Genome. Cham: Springer International Publishing, pp.1-24.

Callaway DA and Jiang JX (2015). Reactive oxygen species and oxidative stress in osteoclastogenesis, skeletal aging and bone diseases. *J. Bone. Miner. Metab.,* **33**(4): 359-70.

Camera E, Mastrofrancesco A, Fabbri C, Daubrawa F, Picardo M, Sies H and Stahl W (2009). Astaxanthin, canthaxanthin and beta-carotene differently affect uva-induced oxidative damage and expression of oxidative stress-responsive enzymes. *Exp. Dermatol.,* **18**(3): 222-231.

Caponio F, Alloggio V and Gomes T (1999). Phenolic compounds of virgin olive oil: Influence of paste preparation techniques. *Food Chem,* **64**(2): 203-209.

Dias J (2014). Nutritional and health benefits of carrots and their seed extracts. *Food Sci. Nutr.,* **05**: 2147-2156.

Domingo DS, Camouse MM, Hsia AH, Matsui M, Maes D, Ward NL, Cooper KD and Baron ED (2010). Anti-angiogenic effects of epigallocatechin-3-gallate in human skin. *Int. J. Clin. Exp. Pathol.,* **3**(7): 705-9.

El Gharras H (2009). Polyphenols: Food sources, properties and applications a review. *Intl. J. Food Sci. Technol.,* **44**: 2512-2518.

Ferreira M, Santiago R, Souza T, Egito S, Oliveira E and Soares L (2010). Development and evaluation of emulsions from *Carapa guianensis* (Andiroba) oil. *AAPS PharmSci.Tech.,* **11**(3): 1383-1390.

Fu R, Zhang Y, Peng T, Guo Y and Chen F (2015). Phenolic composition and effects on allergic contact dermatitis of phenolic extracts *Sapium sebiferum* (L.) Roxb. leaves. *J. Ethnopharmacol.,* **162**: 176-80.

Gaspar LR and Maia Campos PM (2003). Rheological behavior and the spf of sunscreens. *Int. J. Pharm.,* **250**(1): 35-44.

Greene JAS Ca). (2001). Composition for protecting skin from damaging effects of ultraviolet light. United States Patent Application 6235272.

Hama S, Takahashi K, Inai Y, Shiota K, Sakamoto R, Yamada A, Tsuchiya H, Kanamura K, Yamashita E and Kogure K (2012). Protective effects of topical application of a poorly soluble antioxidant astaxanthin liposomal formulation on ultraviolet-induced skin damage. *J. Pharm. Sci.,* **101**(8): 2909-2916.

Han B, Jaurequi J, Tang BW and Nimni ME (2003). Proanthocyanidin: A natural crosslinking reagent for stabilizing collagen matrices. *J. Biomed. Mater. Res. A.,* **65**(1): 118-24.

Joseph M (2019). The Top 100 Foods High In Polyphenols [Online]. Available: https://www.nutritionadvance.com/nutrition/top-food-sources-polyphenols/#:~:text=1%20black%20chokeberry%201756%20mg%202%20black%20elderberry,mg%209%20sweet%20cherry%20274%20mg%2010%20blackberry [Accessed].

Khan B, Naveed A, Menaa A and Menaa F (2015). A Novel *Cassia fistula* (L.) - Based emulsion elicits skin anti-aging benefits in humans. *Cosmetics,* **2**(4): 368-383.

Kim YJ, Uyama H and Kobayashi S (2004). Inhibition effects of (+)-catechin-aldehyde polycondensates on proteinases causing proteolytic degradation of extracellular matrix. *Biochem. Biophys. Res. Commun.,* **320**(1): 256-61.

Kishimoto Y, Saito N, Kurita K, Shimokado K, Maruyama N and Ishigami A (2013). Ascorbic acid enhances the expression of type 1 and type 4 collagen and svct2 in cultured human skin fibroblasts. *Biochem. Biophys. Res. Commun.,* **430**: 579-584.

Korac RR and Khambholja KM (2011). Potential of herbs in skin protection from ultraviolet radiation. *Pharmacogn Rev,* **5**(10): 164-173.

Lim HW, Arellano-Mendoza MI and Stengel F (2017). Current challenges in photoprotection. *J. Am. Acad. Dermatol.,* **76**(3S1): S91-S99.

Loser B, Kruse SO, Melzig MF and Nahrstedt A (2000). Inhibition of neutrophil elastase activity by cinnamic acid derivatives from *Cimicifuga racemosa*. *Planta Med.,* **66**(8): 751-3.

Magenta A, Florio MC, D'agostino M and Sileno S (2020). Chapter 1 - Oxidative stress and Mir-200c. *In:* Preedy VR and Patel VB (Eds.) Aging (Second Edition), Academic Press, pp.3-10.

Morin B, Narbonne JF, Ribera D, Badouard C and Ravanat JL (2008). Effect of dietary fat-soluble vitamins A And E and proanthocyanidin-rich extract from grape seeds on oxidative dna damage in rats. *Food Chem. Toxicol.,* **46**(2): 787-96.

Nakashima Y, Ohta S and Wolf AM (2017). Blue light-induced oxidative stress in live skin. *Free Radic. Biol. Med.,* **108**: 300-310.

Peres PS, Terra VA, Guarnier FA, Cecchini R and Cecchini AL (2011). Photoaging and chronological aging profile: Understanding oxidation of the skin. *J. Photochem. Photobiol B.,* **103**(2): 93-7.

Perumalla A and Hettiarachchy N (2011). Green tea and grape seed extracts potential applications in food safety and quality. *Food Res. Int.,* **44**(4): 827-839.

Rinnerthaler M, Bischof J, Streubel MK, Trost A and Richter K (2015). Oxidative stress in aging human skin. *Biomolecules,* **5**(2): 545-589.

Saluja SS and Fabi SG (2017). A holistic approach to antiaging as an adjunct to antiaging procedures: A review of the literature. *Dermatol. Surg,* **43**(4): 475-484.

Singh B, Malhotra H, Kumar D, Mujtaba SF and Upadhyay AK (2019). Understanding cellular and molecular events of skin aging and cancer: An integrative perspective. *In:* Dwivedi A, Agarwal N, Ray L and Tripathi AK (Eds.) Skin Aging and Cancer: Ambient Uv-R Exposure. Singapore: Springer Singapore, pp.11-28.

Tripathi SS, Singh S and Singh AK (2019). Human skin stem cells, aging, and possible antiaging strategies. *In:* Dwivedi A, Agarwal N, Ray L and Tripathi AK (Eds.) Skin aging and cancer: Ambient UV-R exposure. Singapore: Springer Singapore, pp.309-331.

Viljoen JM, Cowley A, Du Preez J, Gerber M and Du Plessis J (2015). Penetration enhancing effects of selected natural oils utilized in topical dosage forms. *Drug. Dev. Ind. Pharm.,* **41**(12): 2045-2054.

Waqas MK, Khan BA, Akhtar N, Chowdhry F, Khan H, Bakhsh S, Khan S and Rasul A (2017). Fabrication of *Tamarindus indica* seeds extract loaded-cream for photo-aged skin: Visioscan(®) studies. *Postep. Derm. Alergol.,* **34**(4): 339-345.

Yadav N, Parveen S, Chakravarty S and Banerjee M (2019). Skin Anatomy And Morphology. *In:* Dwivedi A, Agarwal N, Ray L and Tripathi AK (Eds.) Skin aging and cancer: Ambient UV-R exposure. Singapore: Springer Singapore, pp.1-10.

Yarovaya L, Waranuch N, Wisuitiprot W and Khunkitti W (2021). Effect of grape seed extract on skin fibroblasts exposed to uva light and its photostability in sunscreen formulation. *J. Cosmet Dermatol.,* **20**(4): 1271-1282.

Zarbafian M, Dayan S and Fabi SG (2020). Teachings from Covid-19 and aging-an oxidative process. *J. Cosmet Dermatol.,* **19**(12): 3171-3176.

Zhou K and Raffoul JJ (2012). Potential anticancer properties of grape antioxidants. *J. Oncol.,* 803294.

Zhou Z, Huang Y, Liang J, Ou M, Chen J and Li G (2016). Extraction, purification and anti-radiation activity of persimmon tannin from *Diospyros kaki* L.f. *J. Environ Radioact.,* pp.162-163, 182-188.