Dermocosmetic emulgels for anti-aging effects: Evidence from chromatographic and non-invasive biophysical techniques

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Abstract: Persimmon Fruits (*Diospyros kaki* L.f, *Ebenaceae*) and its active principles have long been used in traditional medicines for various cosmetics and skin conditions, however clinical efficacy on various facial skin parameters like roughness, scaliness, hydration, elasticity and wrinkles have not yet been reported. Current study was aimed to analyse polyphenolic constituents of *Diospyros kaki* fruit extract (*DK*FE) and to clinically evaluate dermocosmetic emulgels loaded with bioactive phytoconstituents from persimmon fruits, using non-invasive *in-vivo* evaluation techniques. HPLC analysis established the presence of quercetin, gallic acid, chlorogenic acid, ferulic acid, p-coumeric acid, catechin and cinnamic acid. Results revealed that test formulation produced significant and control showed insignificant ($p>0.05$) effects on moisture contents and elasticity. Surface evaluation of living skin (SELS) index values were reduced significantly ($p\leq0.05$) for the emulgels loaded with *DK*FE, represented by reduction in skin wrinkles (-12.40%), roughness (-11.76%) and scaliness (-18.59%). Conclusively, a safe and compatible dermocosmetic emulgel formulation loaded with antioxidant enriched *DK*FE, revealed promising anti-aging attributes that may be due to presence of vital polyphenolic constituents as presented by HPLC analysis.

Keywords: Anti-aging, *Diospyros kaki*, skin elasticity, skin moisture contents, SLES.

INTRODUCTION

Cosmetic industry is undergoing through a major shift from inorganic to natural regimen because of lower risk attached to natural phytoconstituents based cosmetics and beauty-care products. Skin care products, stands first with 36% of total share in global cosmetic industry. In 2016, natural beauty products worth more than 11 billion U.S.D, which is forecasted to double in 2024 (statista, 2018). Global market share for natural cosmetics have doubled from 7 billion USD in 2007 to fifteen Billion-USD in 2017, which indicates improved customer acceptance for organic beauty-care products.

Persimmon fruits, widely grown in Asia and Europe including China Japan, Korea and Brazil. *D.kaki* is most important specie from genus *Diospyros* because of yielding exotic fruits. For many years this fruit has been used in Chinese traditional medicines to treat various skin ailments like pimples, skin eruptions and eczema (Kashif, Akhtar *et al.*, 2017). The fruits are shown to be enriched with many bio-active phytoconstituents of cosmetic and dermatological interest including phenolic acids, flavonoids, terpenoids, carotenoids, tannins, proanthocyanidins (PAs), ascorbic acid and other vitamins.

Skin aging being a vibrant process is controlled by both intrinsic and extrinsic factors which may contribute at both aesthetic and structural levels. Chronological aging process is determined by genetic make-up, while photoaging because of continuous ultraviolet (UV) exposure leads to microscopic changes in *stratum corneum* (SC). Major set-back by UV exposure is production of reactive oxygen species (ROS), which accelerate oxidative stress and hence fastens the aging process. Oxidative damage targets various bio-functional molecules under skin including lipids, proteins, carbohydrates and DNA resulting in redox imbalance. Excessive exposure to UV radiations is a leading factor in photoaging. In both chronological and photoaging, the skin aging process can be slowed down by avoiding UV exposure and/or improving anti-oxidant levels in the skin.

Emulsion based gels (emulgels) are relatively a newer class of dermocosmetic preparations with added advantage of being non-greasy, and more stable than traditional creams and emulsion. Emulgels have more flexibility of loading wider class of organic and chemical moieties (hydrophilic and lipophilic) for transdermal delivery through *SC*. This study was aimed to formulate and clinically evaluate cosmetics emulgels loaded with antioxidant enriched extract from persimmon fruits by using non-invasive *in-vivo* evaluation tools in Asian volunteers.

MATERIALS AND METHODS

Extraction technique

Persimmon fruits were identified from Department of life Sciences (Herbarium N0-2318/2), The Islamia University of Bahawlapur, Pakistan. Fresh fruits after tape washing,

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were sliced into 4 pieces and shade dried for 4-weeks. Dried material was milled to fine powder and macerated (100-grams) with methanol. After 72 hours of maceration, coarse filtration was done and filtrate was passed through whatman NO-42 filter paper. Filtrate was subjected to rotavapor under reduced pressure until 20% was remained. This was further evaporated to dryness at 40^{o}C in hot air oven and stored at 2-8^{o}C until further use.

DPPH free radical scavenging activity

Free radical capturing tendency of the extract was performed using procedure described by Mohsin *et al*, (Mohsin, Akhtar *et al.*, 2016). Briefly 90µL of freshly prepared solution of 1,1-Diphenyl-2-Picrylhydrazyl (100µM in methanol) was mixed with 10µL of extract to a final volume of 100µL, in a 96-well microplate. A negative control was prepared by adding 90µL of DPHH solution added to 10µL of ethanol. L-ascorbic acid was used as standard. The reaction mixtures were incubated at 37^{0}C in darkness. Half hour latter decrease in absorbance was measured photometrically at 517nm with a microplate reader (synergy-HT. BioTek, USA). Data obtained was plotted in EZ-Fit software and DPPH scavenging activity was measured by following formula. Scavenging activity (%) = $(A_0\text{-}A_1)/A_0 \times 100$. Where A_0 = absorbance of control, A_1 = absorbance of test extract.

Estimation of total phenolic and flavonoid contents (TPC and TFC)

TPC in methanolic extract were determined by Folin-Ciocalteu Reagent (FCR) method as described in literature (Ali *et al.*, 2018). Calibration curve was prepared with different concentrations of gallic acid. 1mL aliquots of 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09 and 0.10mg/mL gallic acid solution in methanol was mixed with 5mL FCR (diluted ten folds) and 4mL of sodium carbonate (20% w/v). The absorbance was noted after 1 hour at 765nm and the calibration curve was plotted by taking absorbance as a function of concentration. 1mL of extract (0.001g/mL) was mixed with the same reagent as described above and after 1 hour the absorbance of the resulting blue colour complex was measured at 765nm. All determinations were performed in triplicates. Quantification was done with respect to the standard (gallic acid). TPC in extracts was measured as gallic acid equivalents (mg GAE/g) using following formula.

$$TPC = \frac{C \times V}{M} \ldots\ldots\ldots (1)$$

Where; C = Gallic acid concentration calculated from calibration curve in mg/mL, V = Extract volume in mL, M = Plants extract weight in grams.

TFC were calculated using reported method (Martinez-Las *et al.*, 2017) with little modifications. Briefly, 0.5mL of plant extract was mixed with 2mL of distilled water and 0.15mL of 5% $NaNO_2$ solution. Reaction mixture was incubated for 6-minutes. After that 0.15mL of 10% $AlCl_3$ solution was added to that and again incubated for 6 minutes followed by the addition of 4% NaOH solution. Methanol was added up to final volume of 5mL and mixed thoroughly. Finally Absorbance was taken at 510nm after incubation for 15minutes. Total flavonoid contents (TFC) of the extracts were expressed as µg catechin equivalents per g (µg CE/g) of extract from the linear regression curve of catechin.

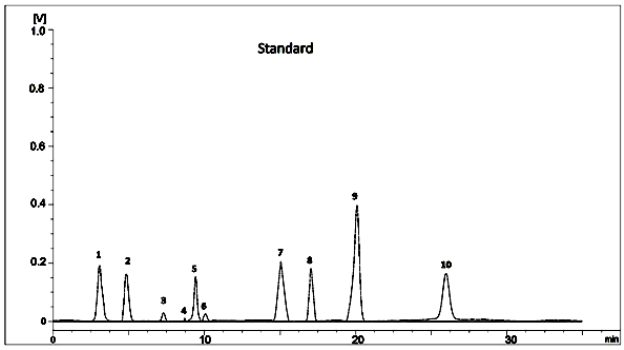

Fig. 1: HPLC chromatogram of reference standards for polyphenols. 1: quercetin (RT 2.736), 2: gallic acid (RT 4.893), 3: chlorogenic acid (RT 7.381), 4: catechin (RT 8.702), 5: caffeic acid (RT 9.231), 6: ferrulic acid (RT 10.092), 7: cinnamic acid (RT 15.136), 8: m-coumeric acid (RT 16.981), 9: p-coumeric acid (RT 22.559) and10: sinnapic acid (RT 26.129)

HPLC analysis of phenolic constituents

Phenolic and flavonoid compounds were analysed using method reported by (Caponio *et al.*, 1999) with minor variation. 20mL of acetone-water (80:20 v/v) solution was mixed with approximately 2.0g of botanical extract in a volumetric flask. This sample mixture was incubated at 50^{o}C for 30-minutes. Upper layer was separated. Whole extraction process was repeated twice with fresh 20mL of acetone-water solution and all supernatants were combined. Acetone was removed under vacuum at 40^{o}C and remainder was filtered through 0.45µm membrane filter. Finally 20µL of filtered solution was injected into an HPLC analyser (Shimadzu, Japan) equipped with photodiode array detector (SPD-10AV, λ_{max}=278nm) using Shim-pack CLC-ODS C-18 reverse-phase column (25cm×4.6mm, 5µm) at room temperature. The analysis was repeated in triplicates. Detection and quantification of phenolics was performed using SCL-10A system control unit, SIL-10AD autosampler and LC-10AT pump and a DGU-14 degasser. Elution solvents were (A) Water and acetic acid (94:6) at pH 2.27 and (B) 100% acetonitrile. Following gradient was used for sample extraction and separation. 0-15 minute 15% B, 15-30 minutes 45% B and 30-40 minutes 100% B. Flow rate was maintained at 1mL/Min. Phenolic and flavonoid standards used in current analysis include quercetin, gallic acid, chlorogenic acid, catechin, caffeic acid, ferrulic acid, cinnamic acid, m-coumeric acid, p-coumeric acid and sinnapic acid.

Standard solution (1mg/mL stock) was prepared in acetone water (80:20 v/v). Peak identification was done in-comparison to that of standards used. Quantities were calculated from peak areas. The concentrations of polyphenolic constituents were expressed as mg/100g of dry weight (DW)±S.D.

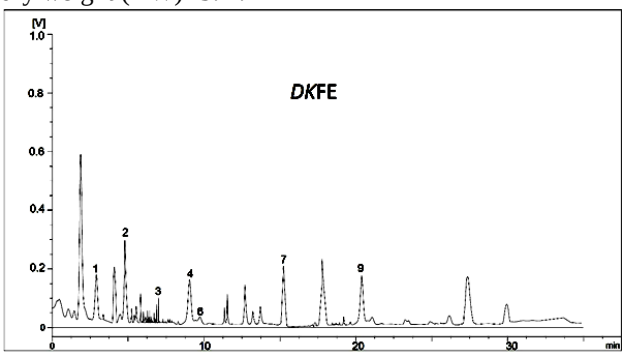

Fig. 2: HPLC chromatogram of DKFE representing peaks comparable to retention time of 1: quercetin (RT 2.736), 2: gallic acid (RT 4.893), 3: chlorogenic acid (RT 7.381), 4: catechin (RT 8.702), 6: ferrulic acid (RT 10.092), 7: cinnamic acid (RT 15.136), 9: and p-coumeric acid (RT 22.559)

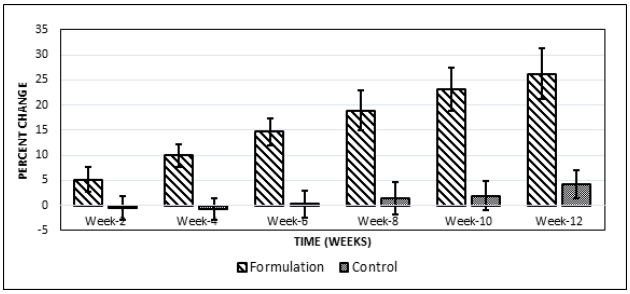

Fig. 3: Percent change in skin elasticity after application of test formulation and the control (n = 13)

Formulation of emulgels

Dermocosmetic emulgel was prepared into two separate steps including preparation of primary emulsion (step-1) and then dispersing this primary emulsion into prepared gel (step-2). Both oil phase and aqueous phase (see table 1) were heated around 70-80^{0}C in separate beakers and then oily phase was added gradually into aqueous phase at same temperature using lab homogenizer (Eurostar) at 2000-rpm for 30-minutes. Homogenizer's speed was then reduced to 1000-rpm for 10-minutes and then to 500-rpm for 10-minutes to ensure homogeneity of preparation. Methylparaben was dissolved in small amount of propylene glycol before it was added to aqueous phase.

Gel phase was prepared separately by gradually dispersing weighed amount of carbopol-940 into deionized water, preventing any lump formation. Prepared gel was left overnight for completeness of carbopol hydration. The pH was then adjusted to 5.5-6.5 by adding triethanolamine drop-wise. Finally, primary emulsion prepared in step-1 was dispersed into gel phase with continuous stirring to obtain emulgel. (All prepared formulations were subjected to accelerated stability testing as reported by (Mohsin, 2016), data not presented here).

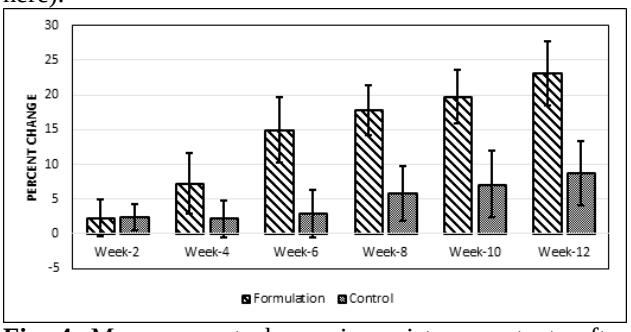

Fig. 4: Mean percent change in moisture contents after application of test-formulation and control (n=13)

Non-invasive in-vivo biophysical evaluation on human skin

Test and control formulations were evaluated for their effects on various skin micro-relief parameters using VisioScan VC-98. Visioscan® is equipped with a unique high resolution UVA camera allowing investigation of living skin directly. Index values for surface evaluation of skin roughness (SEr), scaliness (SEsc) and wrinkle (SEw) were measured at baseline, 2nd, 4th, 8th and 12th weeks. Skin elasticity was measured with elastomer® EM-25. Corneometer® CM825 was used for determination of moisture contents (MC).

Study protocol

Thirteen healthy male human volunteers of Asian origin, aged between 25-37 years were recruited after signing informed consent for this study. Single blinded split face study design with placebo control trial was used to evaluate any difference between studied parameter for test and control formulations. All volunteers were inspected by an expert dermatologist to ensure that they are free from any serious skin conditions on the cheeks and forearms. Volunteers were given proper training on how to apply formulations during the study period. Formulations were labelled with bold letter "R" and "L" indicating their respective site for application. Volunteers were advised to apply appropriate amount of formulations twice a day for a period of 12-weeks. During this study period, no other cosmetic and/or medical treatment was used for any topical application by any study subjects. Values of different test parameters were measured at baseline, 2nd, 4th, 6th, 8th and 12th-week intervals at controlled room temperature and relative humidity (25^{0}C±2^{0}C and 45±2% RH) from whole cheek area. Each measurement was taken in triplicates (n = 3) and results were averaged

Ethical standard

Study protocol for clinical efficacy evaluation on human volunteers for this research was approved from Board of Advance Studies and Research (BASR, Ref. 30/AS/RB),

The Islamia University of Bahawalpur, Pakistan and its ethical committee for *in-vivo* studies. Study was conducted following the ethics principles of international guidelines of Helsinki Declaration.

STATISTICAL ANALYSIS

Studied parameters were presented as percent change from baseline values measured at each time interval using following formula: Percent change = [(A-B)/B] x 100 where, A = observed value for parameter, B = base-line value at 0-weeks. SPSS version 17.00 was used to apply two-way ANOVA at 5.0% level of significance to observe variation in observed values at different time interval, for test and control formulations. Paired sample t-test was applied to determine any difference between studied parameters. Standard error of means (SEM) was calculated for any mean value.

RESULTS

Extraction yield and antioxidant activity

The extraction yield from persimmon fruits was 23.64%. Antioxidant activity as measured by using DPPH method for *DK*FE equalled to 79.98%. This high antioxidant activity is attributable to numerous polyphenolic phytoconstituents.

Total phenolic and flavonoid contents

Total phenolic and flavonoid contents in *DK*FE was 20.06 ± 0.31(mg GAE/g ± S.D) and 50.18 ± 0.13 (µg CE/g ± S.D) respectively. These values for TPC and TFC are in agreement with previous reports (Chen *et al.*, 2016, Martinez-Las 2017, Zhou *et al.*, 2016).

HPLC analysis of phenolic compounds

Phenolic and flavonoid reference standard compounds namely quercetin, gallic acid, chlorogenic acid, catechin, ferrulic acid, cinnamic acid, m-coumeric acid, p-coumeric acid and sinnapic acid were eluted at 2.736, 4.893, 7.381, 8.702, 9.231, 10.092, 15.136, 16.981, 20.186, and 26.129 min respectively (fig. 1). The chromatogram of *DK*FE represented numerous peaks overlapped closely, however retention time comparison specified presence of following seven polyphenolic compounds (mg/100g±S.D of DW) as shown in fig. 2. (1) Quercetin (18.17±0.16), (2) gallic acid (88.92±0.27), (3) chlorogenic acid (34.56±0.37), (4) ferulic acid (3.45±0.11), (6) p-coumeric acid (31.93±0.14), (7) catechin (26.93±0.23) and (9) caffeic acid (16.73±0.24). It is worth noting that alcoholic crude extract of persimmon fruits have shown to possess antityrosinase activity (*in-vitro*) comparable to that of arbutin (Fukai *et al.*, 2009, Tiechi *et al.*, 1999). We have cited in our previous report (Kashif, 2017) that most of these phenolic components possess useful activities for cosmetics and dermatological uses. For example, Gallic acid from the fruit pulp tested in eosinophil-dermal fibroblast exhibited anti-inflammatory, anti-microbial, and anti-elastase effects. Similarly epicatechin, and chlorogenic acid present in extract can help reverse UV induced oxidative damage to human skin fibroblast with inhibition of tyrosinase activity (Domingo *et al.*, 2010, Jeon *et al.*, 2010).

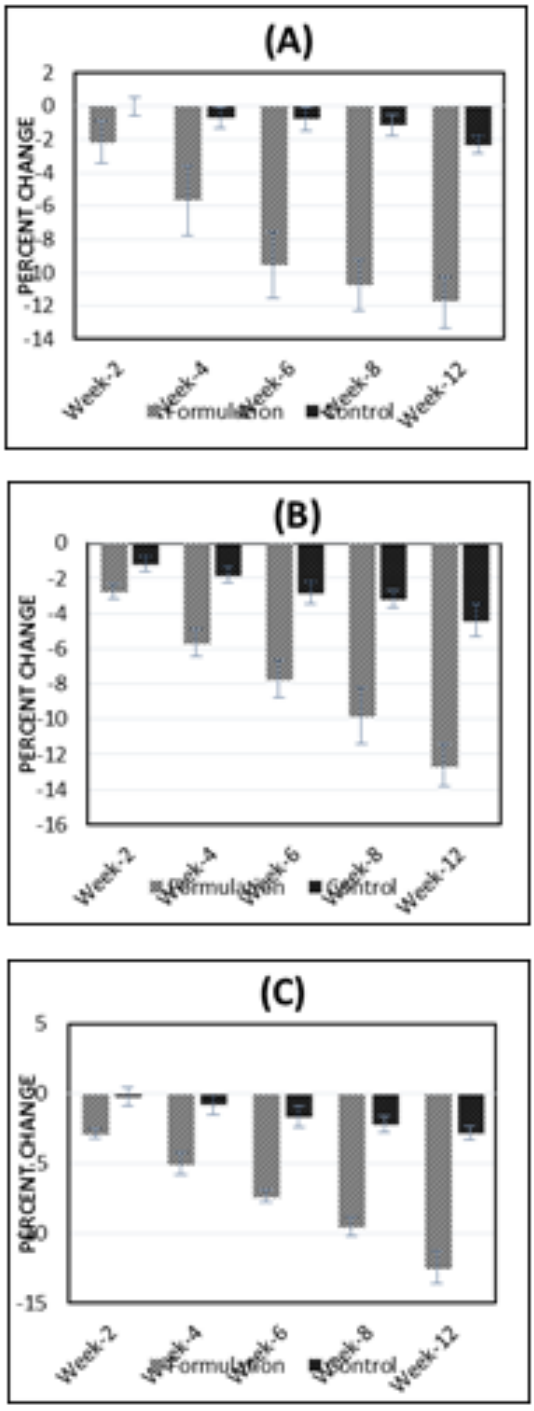

Fig. 5: Mean percent change in A = Skin roughness, B = Skin Scaliness and C = Skin wrinkle for test formulation loaded with Persimmon fruit extract and Control over a period of 12-weeks (n=13)

Table 1: Formula for Dermocosmetic emulgel containing antioxidant enriched extract of persimmon fruits

Oil Phase	Liquid paraffin	15.0%	Primary emulsion
	Span-80	0.85%	
Aqueous Phase	Tween-80	2.16%	
	Methylparaben	0.05%	
	Fruit extract (in test formulation only)	10.0%	
	Water (q.s)	100%	
Gel phase	Carbopol-940	1.0%	Gel phase

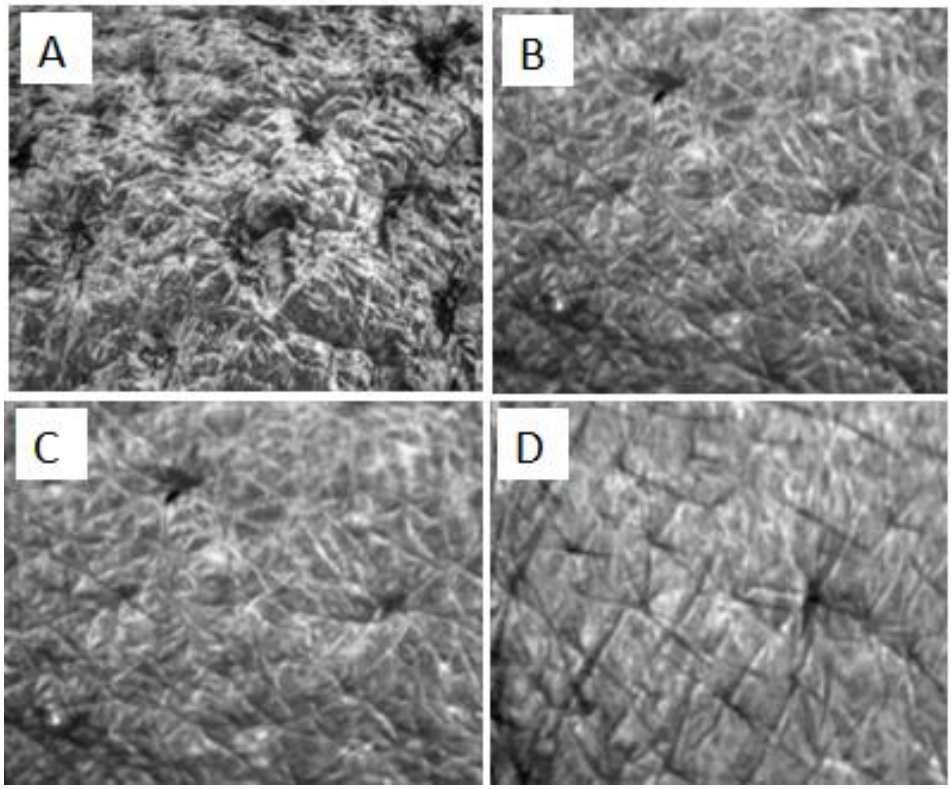

Fig. 6: Facial skin topographic images by Visioscan VC98 UV-light assisted CCD camera. (A) = before treatment, (B) = after 6-weeks, (C) after 8 weeks and (D) at 12-weeks of treatment with test-formulation

Facial skin elasticity

Elastometer EM25 is based on suction technique implementing 400-mbar pressure for 6 seconds (3 seconds for suction and 3 for relaxation), through its 2.0 mm probe opening. Results are presented in percentage values at a scale from 0-99. Control formulation showed trivial alteration in skin elasticity over first 6-weeks of study, however little improvement was observed after week-6. In contrast with control, continuous application of test formulation has resulted in noticeable enhancement of facial skin elasticity (mean percent change 26.254±5.103) in volunteers as depicted in fig. 3. Two way ANOVA test confirmed that formulation has significantly ($p \leq 0.05$) improved elasticity in human skin while this effect was non-significant in control formulation ($p > 0.05$) with respect to time.

Moisture contents of skin

Corneometer CM825 is based on measuring a change in capacitance in a dielectric medium which is directly linked with skin hydration level. MC can be determined at approximately 10-20µm of *stratum corneum* (*SC*) avoiding any interference with deeper skin layers (blood vessels). The results of current study revealed a regular increase in MC over a period of 12-weeks as presented in fig. 4. Persimmon fruits extract loaded emulgel formulation indicates a continuous increase in the MC of facial skin indicating water retention feature of the formulation. MC of the facial skin was increased to 15% on 6^{th}-week and 23% on 12^{th}-week (fig. 4). The ANOVA indicates that effect of formulation was significant ($p < 0.05$) whereas control showed insignificant effects on MC with respect to time. Paired sample t-test indicates

that there were significant differences in MC for control and test-formulation.

Surface evaluation of living skin (SLES)

Visioscan VC98 was used to capture high resolution images of facial skin (area 6x8mm) at baseline, 2nd, 4th, and 6th, 8th and 12th-week. These images were processed using a sophisticated SLES-2000 software, utilizing image histogram for measurement of SEr, SEsc and SEw. Images were captured in 255 grayscale levels, with 0 representing black and 255 indicating white colour distribution in the image. Measuring roughness and wrinkles is based on drawing virtual calculation lines on horizontal (x) and vertical (y) directions on images. Now average number (Fax and Fay) and the average width (Fmx and Fmy) of wrinkles between these lines are quantified.

Skin roughness

After the application of formulation SEr value was reduced by 11.76%±1.54 (fig. 5A). Control formulation also exhibited a reduction in SEr parameter however the effect was insignificant ($p \leq 0.05$) as revealed by two way ANOVA.

Skin scaliness

Mean percent change in SEsc index values for a treatment course of 12th weeks is presented in fig. 5B. Significant reduction (-18.59%±1.54) was observed in SEsc absolute value. A minute but statistically insignificant ($p \leq 0.05$) reduction in placebo formulation was also observed at end of 12th week.

Skin wrinkles (SEw)

SEw is anticipated using average numbers (Fmx+Fmy) and average width (Fmx+Fmy) of horizontal and vertical wrinkles. Formula used to calculate SEw = [(Fmx+Fmy) / (Fax and Fay)] x100. More prominent (broad and deep) wrinkles results in higher SEw index value (GmbH, 2016). Test formulation showed a total of 12.40%±1.14 reduction at end of 12th-week in SEw index value, indicating a reduction in depth and width of wrinkle on facial skin. Fig. 5C represents mean percent change in SEw parameters at various time interval of treatment. Fig. 6 (A-D) represents superficial changes observed in facial skin before treatment and after 6, 8 and 12-weeks of treatment.

DISCUSSION

DPPH scavenging activity is usually used to estimate antioxidants in a given sample. DPPH reagent radical's deep violet colour gives intensive absorption spectra in range of 515-528nm. DPPH has capability to interact with hydrogen donating molecules such as polyphenols. On accepting a proton it loses its colour and turns yellow. In a given sample, as number of phenolic compounds or degree of hydroxylation of phenolic compounds increases, their DPPH scavenging capability also increases, and hence antioxidant potential of various plant material can be predicted. As these radicals are very reactive to existence of proton donors even at very low concentration, making this process capable to analyse large number of samples in very short time interval. The DPPH activity of *D.kaki* extract was found to be 79%, indicating existence of numerous antioxidant in extract which is in close approximation with already reported data in literature (Chen, Fan *et al.*, 2008, Fukai, Tanimoto, Maeda, Fukuda, Okada and Nomura, 2009, Nisar, Shah *et al.*, 2015). Antioxidant potential of persimmon fruit extract have shown to be higher than that of grapes, white apples and tomatoes as reported previously, which is attributable to high contents of phenolics in the extract (Chen, 2008, Moure *et al.*, 2001). Thirty two low molecular weight phenolics have been separated from pulp of persimmon and among them gallic acid has highest antioxidant activity. These multiple polyphenolic compounds have potential role in reversing oxidative stress damage by capturing ROS (Fu *et al.*, 2015, Zhou, 2016) and inhibiting lipid peroxidases (Toschi *et al.*, 2000), which can contribute in various skin pathological conditions. TPC assay is based on transfer of an electron from phenolic entity to FCR and is used commonly to quantify antioxidant activity because of its simplicity and reproducibility (Fu *et al.*, 2011). Persimmon fruits extract have shown to possess ferulic acid, tannic acid, protocatechuic acid, vanillic acid, epicatechin gallate, and catechin gallate. Similarly, persimmon fruits contains high molecular weight condensed proanthocyanidins in vacuoles of tannin cells that causes astringency in unripe fruits. Proanthocyanidins have been reported to have better radical scavenging activity than vitamin C and E (Hatano *et al.*, 2002).

Biomechanical strength of human skin is assessed by three key feature including strength, pliability and elasticity. Skin is a largest viscoelastic organ of the body composed of elastic (collagen and elastin fibres) and viscous (tissue fluid) components. As skin aging proceeds, these characteristics components are altered with loss of skin elasticity being more noticeable (Bonaparte and Ellis, 2015). The tendency of human skin to restore to its original position when subjected to a stretch is called elasticity. Elastosis is a natural phenomenon, presented by loss of skin elastic nature as result of aging process. Catechin, epi-gallactocatechin gallate (Kim *et al.*, 2004) and cinnamic acid derivatives like caffeic acid (Löser *et al.*, 2000) have been reported to possess potent anti-elastase activities. Ascorbic acid (AA) is a vital constituents of persimmon fruit extract and it acts as a co-factor for polyl and lysyl hydroxylases, which are required for collagen synthesis. AA also leads to provocation of collagen gene expression. Human skin fibroblasts have shown to produce higher amounts of

collagen type I and IV, on prolong exposure to AA (Kishimoto *et al.*, 2013). Polyphenolic compounds, terpenoids and other antioxidants can promote collagen synthesis, reduce enzymatic degradation of collagen matrices thus promoting skin elasticity.

Moisture is a key player in maintenance of epidermal skin barrier and biomechanical attributes of the skin. Topically applied formulations are subjected to corneometry at developmental stage for their moisture retention feature. Bioactive phenolic constituents loaded into topical formulations have shown to enhance hydration level of the skin thus imparting skin a more smoother and even feel (Khan *et al.*, 2015). Collagen synthesis can be enhanced by antioxidants, as they stimulate dermal fibroblasts. Flavano-ellagitannin, lycopene, chlorogenic acid, epi-gallactocatechin and β-carotene found in fruit pulp have potent antioxidant activities. Similarly caffeic acid, gallic acid, quercetin, kaempferol, apigenin and leutin, found in persimmon have been cited in literature to possess sufficient anti-elastase activity (Thring *et al.*, 2009). Mineral oil present in emulgels can get deposited forming a lipid film on *SC*, restoring a physical barrier function, which retains moisture leading to reduced water loss from *SC*.

Surface roughness of living skin is measured by calculating grayscale levels beyond the threshold, in comparison to the roughness of whole image (Mahmood, 2014). As a gold standard this parameter is described as "smaller the SEr, the less rougher is the skin (Dabrowska *et al.*, 2018). Surface scaliness gives the measure of desquamation of the *SC*. "The smaller the SEsc index value, less is the scaliness of the skin". Hydration of *SC* prevents desquamation of the skin, reducing its scaliness. Reduction in facial skin roughness, wrinkles and scaliness can be safely correlated with existence of bioactive polyphenols in *DK*FE. All these parameters refers to various attributes of facial skin such as depth of wrinkles, levels of dryness, desquamation of skin, number and length of horizontal of vertical lines. These attributes can represent aging of human skin. An improvement in skin roughness, wrinkles and scaliness results in a younger, healthier and moisturized skin.

CONCLUSION

The clinical data reveals that dermocosmetic formulations fabricated with *DKFE* containing bio-active antioxidants exhibited promising anti-aging effects upon topical application. The formulated emulgels were found to enhance MC and elasticity of facial skin leading to a smoother and younger feel. Improvement observed in skin topography after application of test formulation may be attributable to presence of (I)-polyphenolic acids (like gallic acid, catechin, epicatechin, epi-gallactocatechin, ferulic acid, chlorogenic acid, caffeic acid), (II)-Bioflavonoids (high molecular weight proanthocyanidins and their subunits), (III) carotenoids (such as β-carotene, leutin, xeaxanthin, and cryptoxanthin), and other vitamins, minerals and antioxidants. Moreover, emulgels can be a good choice to incorporate bio-active botanical antioxidants for evaluation of their dermocosmetic benefits with no pronounced side effects for the management of dehydrated, and aged facial skin. Persimmon fruit extract can serve as a valuable cosmetic ingredient for its anti-aging effects, for incorporation into dermocosmetic formulations, so that maximum potential of *Diospyros kaki* can be attained in future.

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