CURRENT TOPICS IN NUTRACEUTICAL RESEARCH Vol. 20, No. 2, pp. 1–8, 2022
doi: https://doi.org/10.37290/ctnr2641-452X.20:1-8
ISSN 1540-7535 print, ISSN 2641-452X online, Copyright © 2022 by New Century Health Publishers, LLC
www.newcenturyhealthpublishers.com
All rights of reproduction in any form reserved

Research Article

Antiaging Effects of Oleuropein Combined with *Helichrysum Italicum* or Kumquat Essential Oils in Cosmetic Lotions

[1]Shirin Tarbiat, [2]Fatma Gulgun Yener, [2]Ashkan Kashefifahmian and [3]Ali Reza Mohseni

[1]*Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Uskudar University, Istanbul, Turkey;*
[2]*Department of Pharmaceutical Technology, Faculty of Pharmacy, Istanbul University, Istanbul, Turkey and*
[3]*Department of Medicine, Cerrahpaşa Medical Faculty, Istanbul University – Cerrahpaşa, Istanbul, Turkey*

Received September 25, 2021; Accepted February 7, 2022

Communicated By: Dr. Antonios E. Koutelidakis

Skin aging as a complex process involves innumerable genetic, biochemical, and environmental mechanisms. This in vitro study delves deep into whether topical cosmetic formulations containing oleuropein combined with essential oils of *Helichrysum italicum* or kumquat prevent skin aging. Two separate cosmetic lotions were formulated and evaluated by their physical and bioactive properties. The oil-in-water emulsions were prepared, antiaging lotions were formulated with the addition of oleuropein combined with either of the two essential oils as active components with the equal total doses of 1.5%, and lotions containing vitamin C or only oleuropein were used as controls. Findings revealed that pH, viscosity, and appearance of the lotions were stable and that the essential oils did not influence the physical properties. The lotion containing the combination of oleuropein and *H. italicum* exhibited the highest antioxidant and antimicrobial properties. In contrast, the oleuropein and kumquat combination exposed the highest inhibitory effect on all three hyaluronidase, lipoxygenase, and tyrosinase enzymes. It can be inferred that the combination of oleuropein and *H. italicum* is the most potent active ingredient of antioxidant in skin care products and that the oleuropein and kumquat combination is the better candidate as an active ingredient against skin inflammation, pigmentation, and loss of collagen.

Keywords: Cosmetic products, Essential oil, *H. italicum*, Kumquat, Lotions, Oleuropein, Skin aging

Abbreviations Used: Analysis of variance, ANOVA; 2,2-Diphenyl-1-picrylhydrazyl, DPPH; Dynamic light scattering, DLS; Minimum inhibitory concentration, MIC; Oil-in-water, O/W; Polydispersity index, PDI; Reactive oxygen species, ROS; Reducing power, RP; Standard deviation, SD; Zeta potential, ZPot

Corresponding Author: Dr. Shirin Tarbiat, Molecular Biology and Genetics, Uskudar University, Istanbul, Turkey;
E-mail: shirin.tarbiat@uskudar.edu.tr

INTRODUCTION

Skin aging is a complex process involving genetic, biochemical as well as environmental mediators (Nikolakis et. al., 2013). This process is often differentiated into intrinsic or chronologic aging and extrinsic or environmental aging, both occurring concurrently and overlapping each other (Trojahn et al., 2015; Boismal et al., 2020). Skin aging, whether extrinsic or intrinsic, leads to changes in the skin characterized by wrinkling, sagging, decreased elasticity, and pigmentation, and it is influenced by diet and lifestyle (Trojahn et al., 2015). Free radicals contribute to the development of both intrinsic and extrinsic aging (Poljšak and Dahmane, 2012). During the intrinsic aging process, free radicals are naturally produced through normal human metabolism, whereas in the extrinsic aging process, they are brought about by exogenous factors such as alcohol intake, cigarette smoking, ultraviolet light (UV) exposure, and other environmental factors (Baumann, 2007). While the biochemical process of oxidation is essential to aerobic life, it also generates oxidative stress and free radical surges that must be kept in balance. Cellular respiration, an endogenous metabolic process taking place in the mitochondria, can be named as a primary source of intracellular ROS (Nohl et. al., 2005). One way to counteract the surplus of ROS is the utilization of antioxidants that can scavenge for free radicals providing protection from both endogenous and exogenous oxidative stresses (Kandola et al., 2015; Gromkowska-Kepka et al., 2021; Lohan et al., 2021; Percoco

et al., 2021). That being the case, topical antioxidants, available in multivariate combinations through over-the-counter skin care products, may be an option for skin protection against the clinical signs of aging. Phytochemicals constitute a large and important group of naturally occurring antioxidants; for instance, flavonoids, phenolic acids, tocopherols, alkaloids, monoterpenes flavonoid glycosides, catechins, proanthocyanidins, flavanolignans, and phenylpropanoids (Soobrattee et. al., 2005). These phytochemicals exhibit antioxidant effects by quenching superoxide anions, oxidative radicals, and lipid peroxides (Merecz-Sadowska et al., 2021). Therefore, they are widely included in the formulations of antiwrinkle topical cosmetic products (Lacatusu et al., 2020).

Hyaluronic acid, the most prevalent glycosaminoglycan to extracellular matrix of soft connective tissues of skin binds a volume of water in the dermis up to 10,000 times of the size of the molecule itself (Masson, 2010). Because of these water-attracting properties, it may be responsible for maintenance of skin hydration and the transport of nutritional material in the matrix (Rinaldi et. al., 2021). In aging skin, there is a steady decline of the synthesis of hyaluronic acid (Sano et. al., 2018). It is possible to control the degradation of the HA from premature degradation by inhibition of the enzyme hyaluronidase (Buhren et al., 2020).

Tyrosinase is an oxidase that is the rate-limiting enzyme for controlling the production of melanin (Iozumi et al., 1993). Excess production of melanin leads to age spot. Suppression of melanin formation by inhibition of tyrosinase in melanocytes results in lightening of skin and prevention of brown spots caused by solar exposure (Goenka et al., 2021). Dermal lipoxygenases have been shown to be a key step in the progression of anti-inflammatory reaction. This family of enzymes is responsible for the release of active inflammatory mediators such as leukotrienes by catalyzing the conversion of polyunsaturated fatty acids to biologically active metabolites (Rhodes et al., 2009).

While most cosmetic preparations in the market today contain a wide array of synthetic chemicals (Becker et al., 2015; Fiume et al., 2015a–c; Elmore, 2003) generally judged safe by "The Cosmetic Ingredient Review Expert Panel," there is a negative perception in consumer's mind (Zota and Shamasunder, 2017; Ross, 2006; Mohammed et al., 2021). The search for natural cosmeceuticals has gained an increasing demand due to their fewer side effects and become more prevalent in cosmetic formulations (Juhász et al., 2018). Plant sources contain numerous natural compounds, which can be used as whitening, antiaging, and antiwrinkle ingredients (Thiagarajan et al., 2020). Hence, cosmeceuticals are a growing market segment, driven by the rising desire of people to maintain healthy skin without using chemicals (Romes et al., 2021). Turkey holds a treasure of high biodiversity, which has potential applications for use in cosmetic industry (Ceylan et al., 2019).

The olive tree *Olea europaea*, a member of the family of *Oleaceae*, has long since been an integral part of the Mediterranean basin with its leaves extensively utilized as folk medicine in regions around the Mediterranean Sea (Hassen et. al. 2015). Oleuropein, the main phenolic component of *O. europaea* L., is known for its numerous health-beneficial properties; namely, antioxidant, anti-inflammatory, cardio- and neuroprotective, and anticancer properties making it appealing for scientific attention (Caruso et al., 2000). These advantages could be related to the abundance of phytochemicals and their strong ROS scavenging activity and strong anti-inflammatory activities (Musa et al., 2021).

Recently, incorporation of essential oils as cosmeceutical ingredients in cosmetic products has come to be of increasing importance (Lohani et al., 2018). Features such as aroma, natural preservative action against microbial infection, powerful source of antioxidants, humectant capacity, guarding against UV-B damage, skin discoloration, wound healing, antimutagenic, and antiaging, which essential oils possess, give them a great potential for application in the cosmetic industry (Sharmeen et al., 2021).

Essential oil from *Helichrysum italicum*, which belongs to the family Asteraceae is represented by approximately 300 species in the world (Pljevljakušić et al., 2018). This genus is represented in Turkish flora by 18 taxa, of which 9 are endemic. *H. italicum* is known to be used in folk medicine owing to its anti-inflammatory and antiallergic properties attributed to the presence of flavonoids. Due to versatile biological activities and applications, the interest in *H. italicum* scientific research and its use has increased in recent years (Nincevic et al., 2019; Gismondi et al., 2020). Essential oil from peels of the citrus fruit, *Fortunella japonica Swingle*, commonly known as kumquat, possesses a wide number of flavonoid derivatives and exhibits remarkable antioxidant properties (Lin et al., 2021); however, only a limited number of studies have reported the bioactivity and topical evaluation of kumquat in any cosmetic product.

Selecting a suitable essential oil gains importance in cosmetic product claims. In view of the diverse biological properties of natural extracts presented earlier, the present research intended to investigate whether topical cosmetic formulation containing these extracts can prevent skin aging. The researchers, in the present research, formulated cosmetic products containing oleuropein with two essential oils; namely, *H. italicum* and kumquat, into two separate cosmetic lotions and evaluated their physical and bioactive properties such as antimicrobial activity, antioxidant activity, and the effect on enzymes such as 5-lipoxyganase, tyrosinase, and hyaluronidase. Here, a potential application for these extracts in antiaging formulations is reported.

MATERIAL AND METHODS

Chemicals, Reagents, and Test Materials

Oleuropein was collected from Sankara Brain and Biotechnology Research Center (Istanbul University) and identified by Professor. Dr. Ihsan Kara. According to the supplier, the extracts were 80% pure oleuropein and the other 20% consisted of various other phytochemicals of ethanolic leaf extracts. Hydrodistilled *H. italicum* and kumquat essential oil was obtained from Helens Herb in Balıkesir Bigadiç Kozpınar village in Turkey and identified by Phytotherapy Education and Research Center, Bezmialem University, Istanbul. We were provided with the analysis results of the extracts done by Phytotherapy Education and Research Center, Bezmialem University. Essential oil extracts of *H. italicum* flowers consisted of alpha pinene, limonene, alpha copaene, italicene, linalool, alpha trans-Bergamotene, alpha cis-Bergamotene, caryophyllene, gamma selinene, gamma curcumene, alpha terpienol, beta selinene, alpha selinene, neryl acetate, beta cadinene, alpha curcumene, geranyl propionate, nerol, ltalidione I, and ltalidione 2.

Essential oil extracts of kumquat consisted of flavanone, apigenin C-glucoside, flavanone di-C-pentose–hexose, luteolin-8, luteolin 6-C-neohesperidoside, apigenin-8-C-neohesperidoside apigenin, margaritene, isomargaritene, fortunellin, and poncirin. All of other chemicals were purchased from Sigma–Aldrich (USA), and cosmetic raw materials for lotions preparation were purchased from cosmetic Yenifarmakon Ltd., Turkey.

Preparation of Cosmetic Lotions

The O/W emulsions used in this study were prepared depending on the solubility of the different components of the formulation: The lipid phase contained mineral oil stearic acid glyceryl stearate and cetyl alcohol. The aqueous phase contained poly acrylic acid (PAA; trade name is carbomer), triethanolamin, glycerin, and water. Both phases were heated to a temperature of 60–80°C, under slow agitation to melt the lipid phase, and then the two phases were mixed by homogenizing at 20,000 rpm for 30 min. The other active ingredients used in the study were separately mixed into the five sample batches. The first batch served as the negative control and did not contain any of the additives. The second batch contained vitamin C 1.5% (positive control). Further, 1.5% oleuropein was added to each of the remaining three batches, 0.5% *H. italicum* essential oil was added to the fourth batch, kumquat 0.5% essential oil was added to the fifth batch, and all creams were mixed and homogenized at 10,000 rpm for 5 min and were cooled down at room temperature. Finally, all samples were transferred to a stoppered flask and stored under refrigeration (5 ± 1°C for 24 h) prior to use.

A lotion consists typically of two phases: an oily internal phase and an aqueous external phase. The aqueous phase is of lighter weight than cream and is nongreasy, intended mainly for external use. Due to its emulsified nature to be applied on the skin surface, lotion interacts more effectively with skin and more readily penetrates through biological membranes, hence, the lotion form was chosen for the study. In this study, different active compounds were put in the lotion in an equal dosage. Antiaging lotions were designed as formulas F_1, F_2, F_3, F_4, F_5, and F_6 as shown in Table 1.

Physical Properties

Determination of Particle Size (Zeta Potential Measurement)

The droplet size and its distribution for each lotion were measured by DLS using a Zetasizer NanoS90 (Malvern Instruments Ltd., UK). Mean droplet size and size distribution of the samples were determined by photon correlation spectroscopy using a Malvern Zetasizer Nano ZS (Malvern Instruments, UK). Light scattering was monitored at room temperature (25°C) at a scattering angle of 90°. The samples were suitably diluted with distilled water (1:100 v/v). The samples were considered polydisperse when the polydispersity index (PDI) was higher than 0.2.

Zeta potential is measured by Malvern Zetasizer Nano ZS (Malvern Instruments, UK). For measuring zeta potential, the samples were diluted with distilled water (1:100 v/v), and their values were estimated from the electrophoretic mobility of oil droplets. The size distribution was described by PDI and the size distribution graph. Measurements were made in triplicate for each sample (Arancibia et al., 2016).

TABLE 1 | Antiaging lotions' (O/W) emulsion formulas.

Formulations	Contents
F_1	Base lotion (negative control)
F_2	Base lotion + Vitamin C (positive control)
F_3	Base lotion + 1.5% Oleuropine
F_4	Base lotion + 1% Oleuropine + 5% *Helichrysum italicum* essential oil
F_5	Base lotion + 1% Oleuropine + 5% Kumquat essential oil
F_6	Base lotion + 1% Oleuropine + 5% Phenoxyethanol

Rheological Characteristics

The viscosity of the samples was determined without any dilution using Brookfield cone and plate viscometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) at 25 ± 0.5°C. The viscosity (cP) at different shear rates (10, 20, 30, 40, 50, and 60 rpm) was also determined. The graphs were plotted with shear stress versus rate of shear to evaluate rheological characteristics of the samples (Arancibia et al., 2016).

Determination of the pH

The pH of the lotions was measured using pH meter (EDGE pH meter) at 21.7°C.

Stability Studies

To assess the formulation's stability, stability studies were carried out as per ICH guidelines. The cream-filled bottles were kept in a humidity chamber maintained at temperatures 8°C ± 0.1°C, 25°C ± 1°C, 40°C ± 1, and 65 + 5°C RH for 3 months. At the end of the studies, the samples were analyzed for the physical properties (Rodrigues et al., 2016).

Bioactive Properties

Determination of Antioxidant Activities

The antioxidant activity of active components of lotions was evaluated by assaying the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and reducing power (RP), following the methodology described in previous studies (Nakagawa et al., 2021). Both assays were repeated on the 14th and 28th days of preparation to determine the stability of active ingredient antioxidant activity through time. The results were expressed as IC_{50} (mg/mL) values.

Determination of Enzyme Inhibition Activity

Hyaluronidase inhibitory activity was determined according to the method described by Acikara et al. (2019). Sodium hyaluronate was used as a substrate. The tyrosinase inhibition activity was determined using mushroom tyrosinase and L-3, 4-dyhydroxyphenylalanine as the substrate according to the method of Farajzadeh et al. (2021). Soybean 5-lipoxygenase enzyme inhibition was studied with arachidonic acid substrate following the previously described method (Tarbiat 2018). Enzyme inhibitory effects of all formulations was expressed as IC_{50}, which is their ability to reduce the enzyme activity by 50%.

Antibacterial Activity

For the antibacterial activity, the tested bacterial strains were ATCC strains: Gram+ bacteria (*Staphylococcus aureus* ATCC 19213) and

Gram– bacteria (*E. coli* ATCC 10536). F6 formula containing phenoxyethanol as a preservative was used as a positive control according to the method described by Thomsen et al. (2011). MIC defined as the lowest concentration of drug that inhibits visible growth after 18 h of incubation. All determinations were performed in triplicate (Zaazaa et al., 2021).

Statistical Analysis

The results are presented as mean ± standard deviation (SD, $n = 3$). Statistical analyses were carried out by a one-way ANOVA using SPSS version 16.0. Significant differences were at $P < 0.05$.

RESULTS

Physical Properties

The results related to the PDI, zeta potential**,** and pH of lotions, are provided in Table 2. The PDI values varied significantly among all formulations except between F_1 and F_3 ($P < 0.05$). The difference of all zeta potential values among formulations was significant ($P < 0.05$) except between F_3 and F_4. The pH of F_2 was the only significantly different value in respect to other formulations.

The results from rheological studies, displayed in Figure 1, indicate that all formulations tested exhibited pseudoplastic type non-Newtonian flow properties, which are usually seen in creams. When the shear rates increased, viscosity values decreased in all lotions tested. The highest viscosity was detected with F_5 lotion followed by F_3, F_2, F_1, and F_4, respectively.

Biological Activities

The antioxidant abilities of lotions in different time intervals are illustrated in Table 3. Overall, all the formulations exhibited antioxidant activity in all three-time intervals; however, the effect of all formulations degraded significantly through time with IC_{50} values ranging from 20.83 ± 0.37 to 59.23 ± 0.99 mg/mL in the DPPH assay and 16.65 ± 0.42 to 49.41 ± 2.09 mg/mL in the RP assay. In all time intervals, F_4 showed the strongest antioxidant activity after F_2 (control), but the difference between the IC_{50} values of the two lotions was not significant in both assays through all time intervals.

Table 4 reports the enzyme inhibitory potential of the formulated lotions. F5 exhibited the strongest inhibition in all the three enzyme assays compared to F_3 and F_4; however, its inhibition was significantly weaker than that of F_2 in all the three enzyme assays. The antibacterial activities of the five formulated lotions are presented in Table 5. The reference lotion containing vitamin C (F_2) exhibited the highest antimicrobial activity against gram-positive *S. aureus* ATCC 19213 (MIC = 15.84 mg/mL) and gram-negative *E. coli* ATCC 10536 bacteria (MIC = 29 mg/mL) followed by F_4, F_5, F_3, and F_1 when compared with the lotion containing 0.5% phenoxyethanol as a preservative (F6).

TABLE 2 | PDI, zeta potential (ZPot), and pH values for O/W lotions with different active compounds. Values are the mean ± SD ($n = 3$); values within the same column followed by different superscript letters are significantly different ($P \leq 0.05$).

Formulations	PDI	ZPot (mV)	pH
F_1	0.497 ± 0.009^a	-59.4 ± 0.3^a	6.00 ± 0.17^a
F_2	0.614 ± 0.017^b	-54.5 ± 0.4^b	4.5 ± 0.02^b
F_3	0.518 ± 0.020^a	-58.1 ± 1.1^c	5.87 ± 0.06^a
F_4	0.748 ± 0.024^c	-59.6 ± 1.3^c	5.89 ± 0.10^a
F_5	0.344 ± 0.026^d	-54.5 ± 0.4^d	5.89 ± 0.05^a

DISCUSSION

The zeta potential is a physical property, which is exhibited by any particle in distribution. It can be used to optimize the formulations of emulsions with high accuracy and precision. Knowledge of the zeta potential provides an assessment of product stability. Strong negative Z-potential was observed in these lotions due to the presence of carbomer as a thickener, which is an anionic polymer. These values ranged from –54.5 to –60.7 mV. The general dividing line between stable and unstable formulations is generally taken at either +30 or –30 mV. Particles with zeta potentials more positive than +30 mV or more negative than –30 mV are normally considered stable. The zeta-potential values obtained with (vitamin C) F_2 and F_5 compared to other formulations were most stable. Depending on these data, F_5 lotion could be accepted as the most suitable formulation in terms of viscosity.

The PDI is an indication of how narrow a Gaussian size distribution would be that, in turn, could represent the fitted DLS data. It is a measure of the width of the particle size distribution. For DLS, the best PDI is 0.0 (infinitely thin delta function), and the worst PDI is 1.0. A PDI value of 1 indicates that the sample has a very broad size distribution and may contain large particles or aggregates that could be slowly sedimented. PDI values <0.1 are monodisperse and >0.1 are multimodal. The results showed that the PDI value of all the formulations was <1 and ranged from 0.344 to 0.748, which shows that droplets are homogenously distributed throughout the

TABLE 3 | Antioxidant capacity of O/W lotions with different active compounds. Values are the mean ± standard deviation ($n = 3$); values in the same column with the same superscript letters are not significantly different ($P < 0.05$). IC_{50} = inhibitor concentration causing 50% loss of enzyme activity. ND = not determined.

Formulations	IC_{50} (mg/mL)					
	DPPH radical-scavenging activity			Reducing power		
	0 Days	14 Days	28 Days	0 Days	14 Days	28 Days
F_1	ND	ND	ND	ND	ND	ND
F_2	20.83 ± 0.37^a	33.29 ± 0.86^b	48.88 ± 2.11^c	16.65 ± 0.42^d	26.53 ± 0.60^e	40.56 ± 0.97^f
F_3	23.85 ± 0.54	40.16 ± 1.30	59.23 ± 0.99	20.48 ± 0.50	32.76 ± 1.23	49.41 ± 2.09
F_4	21.63 ± 0.51^a	34.42 ± 0.58^b	51.21 ± 0.84^c	17.31 ± 0.43^d	27.58 ± 0.79^e	41.91 ± 1.20^f
F_5	22.80 ± 0.57	37.25 ± 0.61	54.76 ± 1.32	19.11 ± 0.38	30.11 ± 0.71	44.99 ± 1.37

dispersion media. As shown in Table 2, the most homogenously distributed droplets throughout the dispersion medium were found to be F_5 when compared with control lotions: F_1 and F_2.

Rheology is used as a very useful tool to evaluate the application behavior, manufacturing, processing, and thermal storage stability of cosmetic creams and lotions. There are several reasons to measure rheological properties of an emulsion. Flow and deformation parameters are important in pharmaceutical products. Some physical measurements give information about the structure of the lotions and make it possible to predict a sensory assessment of product's texture characteristics and performance. Regarding the rheological properties (Fig. 1), the spreadability of the prepared lotions was excellent, and the viscosities were within limits which indicated suitability of lotions for application on the skin.

Ideal and healthy skin pH is between 5.3 and 5.7, and one of the desired characteristics expected from skin care products is their pH compatibility to skin. In the present study, pH measurement results have shown that pH values of F_3, F_4, and F_5 formulations were around 5.9 which is in the ideal pH range for skin. The pH value of emulsion containing vitamin C was found to be 4.18, which is slightly acidic but still can be used for cosmetic purposes in skin care products, and it is possible to increase the pH up to 5.

Stability studies showed a stable homogenous appearance during the 3-month-storage period but no separation of phases (data not shown). The results indicated that the pH, viscosity, and appearance of the lotions were stable and that the essential oils did not affect the physical properties tested.

It is observed that the antioxidant potential of the lotions was in the following order: $F_2 > F_4 > F_5 > F_3$. The effectiveness of antioxidant combination in each formula is mediated by their interactions in surrounding environment.

Each active component is a combination of many individual and specific compounds; interactions between these contents within the mixture of the formulations might have a major impact on their antioxidant, antibacterial, and enzyme inhibitory activities.

All oleuropein-containing lotions exhibit strong antioxidant and antiradical activities, and the results are in line with reports of several studies on antioxidant capacity of olive leaf extracts and when oleuropein is incorporated into stable topical cosmetic formulations (Cádiz-Gurrea et al., 2021; Nunes et al., 2021); however, F_4 lotion, containing additional active compound of *H. italicum*, exhibits the highest antioxidant potential. This is most likely due to the occurrence of significant amounts of terpens and sesqueterpens such as alpha copaene and curcumene which are known to protect

TABLE 4 | Enzyme inhibition capacity of O/W lotions with different active compounds. Values are the mean ± standard deviation (n = 3); values in the same column are significantly different ($P < 0.05$). IC_{50} = inhibitor concentration causing 50% loss of enzyme activity. ND = not determined.

Formulations	IC_{50} (mg/mL)		
	Tyrosinase	5-Lipoxygenase	Hyaluronidase
F_1	ND	ND	ND
F_2	96.2 ± 0.9	22.4 ± 0.6	25.8 ± 0.5
F_3	622.7 ± 4.4	62.9 ± 5.2	38.3 ± 1.0
F_4	357.5 ± 1.3	42.9 ± 1.1	31.9 ± 0.8
F_5	156.0 ± 1.7	35.5 ± 0.8	27.6 ± 1.1

TABLE 5 | Minimum inhibitory concentrations of O/W lotions with different active components against ATCC bacterial strains. Values in the same row are significantly different ($P \leq 0.05$).

Strains	Minimum inhibitory concentration (mg/mL)					
	F1	F2	F3	F4	F5	F6
***Staphylococcus aureus* ATCC 19213 (Gram+)**	–	15.84	80	18.9	22.3	1.44
***E. coli* ATCC 10536 (Gram–)**	–	29	150	38.2	45	1.5

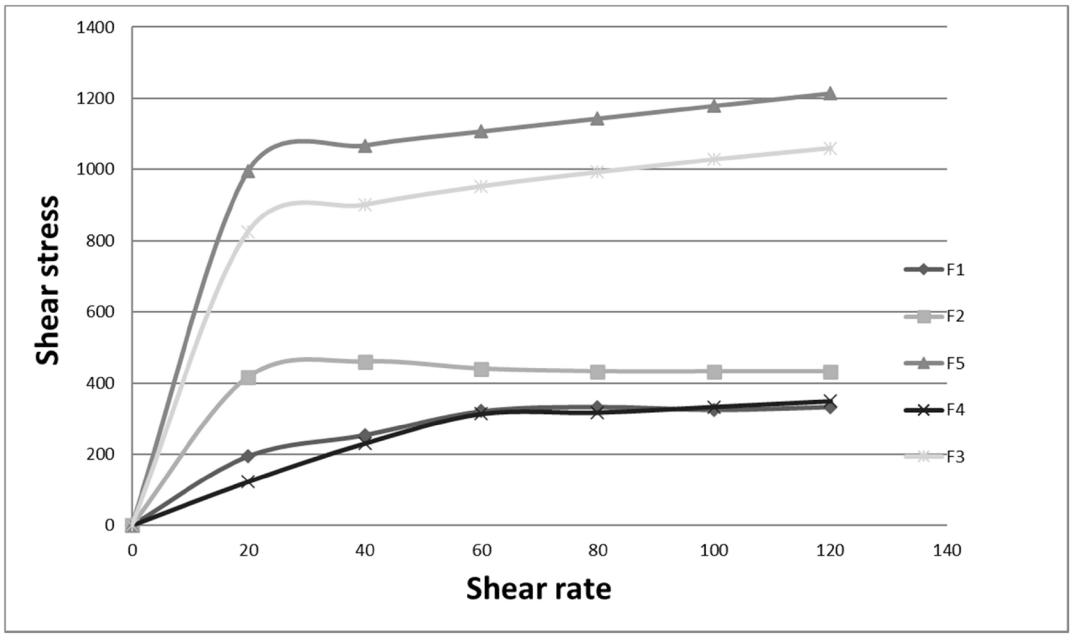


FIGURE 1 | Flow curves of O/W lotions with 1.5% concentrations of different types of active compounds.

skin against oxidants and microorganisms. Similar results revealed by different studies (Sala et al., 2002; Poli et al., 2003). In another study, Mari et al. (2014) determined the metabolite profile of the methanol extract of *H. italicum* flowers. In addition to 14 compounds belonging to flavonoid, phenylpropanoid and acylbenzofuran classes one novel drimane sesquiterpene was also identified.

F_5 formulation with additional active component of the kumquat also showed strong antioxidant properties, which can be attributed to compounds that exhibit a broad spectrum of biological activities; namely, fortunellin and aglycones of flavonoid C-glycosides such as phloretin and acacetin present in the kumquat (Barreca et al., 2014).

It is commonly known that the breakdown of hyaluronic acid by hyaluronidase can decrease hyaluronic acid levels in the skin, which consequently leads to appearing fine lines and wrinkles on the skin (Jung 2020). Various essential oils are shown to exhibit lipoxygenase inhibitory activity (85–96%) at a concentration of 0.5 µg/mL (Wei et al., 2010). Several studies also show that phytochemicals from different plant sources exhibit a tyrosinase inhibitory effect in cosmetic base carriers (Kang et al., 2012; Namngam et al., 2017). In the present study, the protective effect against activity of all understudy enzymes was provided by F_5 lotion. This might be associated with the synergic effects of phenolic composition in oleuropein and the presence of flavonoids such as fortunellin in the kumquat (Lou et al., 2017).

The results have shown that addition of 0.5% of either kind of essential oils enhances the antimicrobial effects of lotions (F_4 and F_5). Monoterpenes, as one of the main components of the essential oils, have been shown to compromise the integrity and function of the microorganism cell membranes leading to a significant inhibition of microorganism growth (He et al., 2019). Gismondi et al. (2020) report that *H. italicum* essential oil is composed of the different cyclic and acyclic monoterpenes and the terpenoids both of which contribute to the antibacterial property of the formulated lotion. Hence, it can be inferred that the antimicrobial effect of our formulations can be attributed to the presence of essential oils and their lytic action (Greay et al., 2015).

A major limitation of the study is that we have used extracts to test the biological effects and have not used individual components. Hence, we are unable to assign a specific bioactivity to a specific compound. Clinical studies in humans or animals with products identical to our product have not been published to the best of our knowledge. However, some of the bioactivities of some components used in our study are known. This study is a preliminary research for animal study and a clinical trial on human volunteers to establish the efficacy of the preparations as antiageing creams. But before the preparations are tested on human subjects, animal toxicity studies and mechanism of action in animal models will have to be established.

CONCLUSION

Extracts from various parts of plants possess polyphenolic acids that are attractive antiaging ingredients in the cosmetic industry due to their beneficial biological properties. The implantation of antioxidants in creams is widely carried out today as it develops to be a convenient way to conserve the skin against oxidative stress attributable to a variety of sources. To sustain the antiaging effect of products, it is important to stabilize the physical properties as well as the antioxidant and enzymatic inhibitory properties of the final formulation. In line with a synergistic outcome, formulation of different combinations of natural polyphenolic sources is being progressively preferred to single-source formulations in the current practice. The present study demonstrated that all of the oleuropein-containing lotions exhibit a significant level of biological activates and can be a good candidate for cosmetic products formulations. It can be inferred that the addition of essential oils from natural sources are convenient not only as a fragrance but also for enhancing biological efficacy of the final formulation. The synergic antioxidant effects with oleuropein and *H. italicum* provide the most potent active agent source for skin care products. Furthermore, the oleuropein and kumquat combination is the best candidate as an active ingredient in cosmetics to protect skin from inflammation, pigmentation, and loss of collagen.

The results provide new information in terms of cosmeceutical use of the combination of oleuropein and two kinds of essential oils against skin aging conditions.

CONFLICT OF INTEREST DECLARATION

The authors state that there are no conflicts of interest to disclose.

REFERENCES

Acikara, O.B., Ilhan, M., Kurtul, E., Smejkal, K. and Akkol, E.K. (2019). Inhibitory activity of Podospermum canum and its active components on collagenase, elastase and hyaluronidase enzymes. *Bioorganic Chemistry* **93**:1–6. DOI: 10.1016/j.bioorg.2019.103330

Arancibia, C., Navarro-Lisboa, R., Zuniga, R.N. and Matiacevich, S. (2016). Application of CMC as thickener on nanoemulsions based on olive oil: Physical properties and stability. *International Journal of Polymer Science* **2016**:1–10. DOI: 10.1155/2016/6280581

Barreca, D., Bellocco, E., Lagana, G., Ginestra, G. and Bisignano, C. (2014). Biochemical and antimicrobial activity of phloretin and its glycosilated derivatives present in apple and kumquat. *Food Research International* **160**:292–297. DOI: 10.1016/j.foodchem.2014.03.118

Baumann, L. (2007). Skin ageing and its treatment. *Journal of Pathology* **211:**241–251. DOI:10.1002/path.2098

Becker, L.C., Bergfeld, W.F., Belsito, D.V., Hill, R.A., Klaassen, C.D., Liebler, D.C., Marks, J.G. Jr, Shank, R.C., Slaga, T.J., Snyder, P.W., Andersen, F.A. (2015). Safety assessment of synthetic fluorphlogopite as used in cosmetics. *International Journal of Toxicology* **34**:43S–52S. DOI: 10.1177/1091581815613513

Boismal, F., Serror, K., Dobos, G., Zuelgaray, E., Bensussan, A. and Michel, L. (2020). Skin aging: Pathophysiology and innovative therapies. *M S-Medecine Sciences* **36**:1163–1172. DOI: 10.1051/medsci/2020232

Buhren, B.A., Schrumpf, H., Gorges, K., Reiners, O., Bolke, E., Fischer, J.W., Homey, B. and Gerber, P.A. (2020). Dose- and time-dependent effects of hyaluronidase on structural cells and the extracellular matrix of the skin. *European Journal of Medical Research* **25**:1–12. DOI: 10.1186/s40001-020-00460-z

Cádiz-Gurrea, M.d.l.L., Pinto, D., Delerue-Matos, C. and Rodrigues, F. (2021). Olive fruit and leaf wastes as bioactive ingredients for cosmetics—A preliminary study. *Antioxidants* **10**:245. DOI: 10.3390/antiox10020245

Caruso, D., Colombo, R., Patelli, R., Giavarini, F. and Galli, G. (2000). Rapid evaluation of phenolic component profile and analysis of oleuropein aglycon in olive oil by atmospheric pressure chemical ionization-mass spectrometry (APCI-MS). *Journal of Agricultural and Food Chemistry* **48**:1182–1185. DOI: 10.1021/jf9905370

Ceylan, S., Cetin, S., Camadan, Y., Saral, O., Ozsen, O. and Tutus, A. (2019). Antibacterial and antioxidant activities of traditional medicinal plants from the

Erzurum region of Turkey. *Irish Journal of Medical Science* **188**:1303–1309. DOI: 10.1007/s11845-019-01993-x

Elmore, A.R. and Cosmetic Ingredient Review Expert Panel (2003). Final report on the safety assessment of aluminum silicate, calcium silicate, magnesium aluminum silicate, magnesium silicate, magnesium trisilicate, sodium magnesium silicate, zirconium silicate, attapulgite, bentonite, Fuller's earth, hectorite, kaolin, lithium magnesium silicate, lithium magnesium sodium silicate, montmorillonite, pyrophyllite, and zeolite. *International Journal of Toxicology* **22**:37–102.

Farajzadeh, N., Saglam, O., Akin, M., Saki, N. and Kocak, M.B. (2021). Investigation of tyrosinase enzyme (from mushroom) inhibitory activities and antioxidant properties of new fluorine-containing phthalocyanines. *Archiv Der Pharmazie* **354**:1–8. DOI: 10.1002/ardp.202000340

Fiume, M.M., Bergfeld, W.F., Belsito, D.V., Hill, R.A., Klaassen, C.D., Liebler, D.C., Marks, J.G. Jr, Shank, R.C., Slaga, T.J., Snyder, P.W. and Andersen, FA. (2015a). Safety assessment of alkyl ethylhexanoates as used in cosmetics. *International Journal of Toxicology* **34**:61S–73S. DOI: 10.1177/1091581815617794

Fiume, M.M., Bergfeld, W.F., Belsito, D.V., Hill, R.A., Klaassen, C.D., Liebler, D.C., Marks, J.G. Jr, Shank, R.C., Slaga, T.J., Snyder, P.W. and Andersen, FA. (2015b). Safety assessment of boron nitride as used in cosmetics. *International Journal of Toxicology* **34**:53S–60S. DOI: 10.1177/1091581815617793

Fiume, M.M., Bergfeld, W.F., Belsito, D.V., Hill, R.A., Klaassen, C.D., Liebler, D.C., Marks, J.G. Jr, Shank, R.C., Slaga, T.J., Snyder, P.W. and Andersen, FA. (2015c). Safety Assessment of alkyl esters as used in cosmetics. *International Journal of Toxicology* **34**:5S–69S. DOI: 10.1177/1091581815594027

Gismondi, A., Di Marco, G. and Canini, A. (2020). Helichrysum italicum (Roth) G. Don essential oil: Composition and potential antineoplastic effect. *South African Journal of Botany* **133**:222–226. DOI: 10.1016/j.sajb.2020.07.031

Goenka, S., Johnson, F. and Simon, S.R. (2021). Novel chemically modified curcumin (CMC) derivatives inhibit tyrosinase activity and melanin synthesis in B16F10 mouse melanoma cells. *Biomolecules* **11**:1–23. DOI: 10.3390/biom11050674

Greay, S.J. and Hammer, K.A. (2015). Recent developments in the bioactivity of mono- and diterpenes: Anticancer and antimicrobial activity. *Phytochemistry Reviews* **14**:1–6. DOI: 10.1007/s11101-011-9212-6

Gromkowska-Kepka, K.J., Puscion-Jakubik, A., Markiewicz-Zukowska, R. and Socha, K. (2021). The impact of ultraviolet radiation on skin photoaging – review of in vitro studies. *Journal of Cosmetic Dermatology* **2021**:1–5. DOI: 10.1111/jocd.14033

Hassen, I., Casabianca, H. and Hosni, K. (2015). Biological activities of the natural antioxidant oleuropein: Exceeding the expectation – A mini-review. *Journal of Functional Foods* **18**:926–940. DOI: 10.1016/j.jff.2014.09.001.

He, F., Bai, J.H., Chen, S.X. and Tan, X.B. (2019). Antifungal activity and mechanism of an essential oil from Eremothecium ashbyii. *Chemical Journal of Chinese Universities* **40**:272–278. DOI: 10.7503/cjcu20180477

Iozumi, K., Hoganson, G.E., Pennella, R., Everett, M.A. and Fuller, B.B. (1993). Role of tyrosinase as the determinant of pigmentation in cultured human melanocytes. *Journal of Investigate Dermatology* **100**:806–11. DOI: 10.1111/1523-1747.ep12476630

Juhász, M.LW., Levin, M.K. and Marmur, E.S. (2018). The use of natural ingredients in innovative Korean cosmeceuticals. *Journal of Cosmetic Dermatology* **2018**:1–8. DOI: 10.1111/jocd.12492

Jung, H. (2020). Response to letter: Comments on "Hyaluronidase: An overview of its properties, applications, and side effects". *Archives of Plastic Surgery* **47**:628–628. DOI: 10.5999/aps.2020.02047

Kandola, K., Bowman, A. and Birch-Machin, M.A. (2015). Oxidative stress – a key emerging impact factor in health, ageing, lifestyle and aesthetics. *International Journal of Cosmetic Science* **37**:1–8. DOI: 10.1111/ics.12287

Kang, S.M., Heo, S.J., Kim, K.N., Lee, S.H., Yang, H.M., Kim, A.D. and Jeon, Y.J. (2012). Molecular docking studies of a phlorotannin, dieckol isolated from Ecklonia cava with tyrosinase inhibitory activity. *Bioorganic & Medicinal Chemistry* **20**:311–316. DOI: 10.1016/j.bmc.2011.10.078

Lacatusu, I., Istrati, D., Bordei, N., Popescu, M., Seciu, A.M., Panteli, L.M. and Badea, N. (2020). Synergism of plant extract and vegetable oils-based lipid nanocarriers: Emerging trends in development of advanced cosmetic prototype products. *Materials Science & Engineering: C* **108**:1–15. DOI: 10.1016/j.msec.2019.110412

Lin, X.C., Cao, S., Sun, J.Y., Lu, D.L., Zhong, B.L. and Chun, J. (2021). The chemical compositions, and antibacterial and antioxidant activities of four types of citrus essential oils. *Molecules* **26**:1–12. DOI: 10.3390/molecules26113412

Lohan, S.B., Ivanov, D., Schuler, N., Berger, B., Zastrow, L., Lademann, J. and Meinke, M.C. (2021). Switching from healthy to unhealthy oxidative stress – does the radical type can be used as an indicator? *Free Radical Biology and Medicine* **162**: 401–411. DOI: 10.1016/j.freeradbiomed.2020.10.319

Lohani, A., Mishra, A.K. and Verma, A. (2018). Cosmeceutical potential of geranium and calendula essential oil: Determination of antioxidant activity and in vitro sun protection factor. *Journal of Cosmetic Dermatology* **2018**:1–8. DOI: 10.1111/jocd.12789

Lou, S.N. and Ho, C.T. (2017). Phenolic compounds and biological activities of small-size citrus: Kumquat and calamondin. *Journal of Food and Drug Analysis* **25**: 162–175. DOI: 10.1016/j.jfda.2016.10.024

Mari, A., Napolitano, A., Masullo, M., Pizza, C. and Piacente, S. (2014). Identification and quantitative determination of the polar constituents in Helichrysum italicum flowers and derived food supplements. *Journal of Pharmaceutical and Biomedical Analysis* **96**:249–255. DOI: 10.1016/j.jpba.2014.04.005

Masson, F. (2010). Acide hyaluronique et hydratation cutanée. *Annales de Dermatologie* **137**:523–525. DOI:10.1016/S0151-9638(10)70005-3

Merecz-Sadowska, A., Sitarek, P., Kucharska, E., Kowalczyk, T., Zajdel, K., Ceglinski, T. and Zajdel, R. (2021). Antioxidant properties of plant-derived phenolic compounds and their effect on skin fibroblast cells. *Antioxidants* **10**:1–24. DOI: 10.3390/antiox10050726

Mohammed, A.H., Blebil, A., Dujaili, J. and Hassan, B.A.R. (2021). Perception and attitude of adults toward cosmetic products amid COVID-19 pandemic in Malaysia. *Journal of Cosmetic Dermatology* **20**:1992–2000. DOI: 10.1111/jocd.14147

Musa, A., Shady, N.H., Ahmed, S.R., Alnusaire, T.S., Sayed, A.M., Alowaiesh, B.F., Sabouni, I., Al-Sanea, M.M., Mostafa, E.M., Youssif, K.A., Abu-Baih, D.H., Elrehany, M.A. and Abdelmohsen, U.R. (2021). Antiulcer potential of Olea europea L. cv. arbequina leaf extract supported by metabolic profiling and molecular docking. *Antioxidants* **10**:1–17. DOI: 10.3390/antiox10050644

Nakagawa, S., Ohmura, R., Toshima, S., Park, H., Narasako, Y., Hirano, T., Otani, M. and Kunitake, H. (2021). Changes in polyphenols, anthocyanins, and DPPH radical-scavenging activities in sweetpotato (Ipomoea batatas L.) during tuber growth. *Scientia Horticulturae* **284**:1–10. DOI: 10.1016/j.scienta.2021.110100

Namngam, C. and Pinsirodom, P. (2017). Antioxidant properties, selected enzyme inhibition capacities, and a cosmetic cream formulation of Thai mango seed kernel extracts. *Tropical Journal of Pharmaceutical Research* **16**:9–16. DOI: 10.4314/tjpr.v16i1.3

Nikolakis, G., Makrantonaki, E. and Zouboulis, C.C. (2013). Skin mirrors human aging. *Hormone Molecular Biology and Clinical Investigation* **16**:13–28. DOI:10.1515/hmbci-2013-0018

Nincevic, T., Grdisa, M., Satovic, Z. and Jug-Dujakovic, M. (2019). Helichrysum italicum (Roth) G. Don: Taxonomy, biological activity, biochemical and genetic diversity. *Industrial Crops and Products*. **138**:1–10. DOI: 10.1016/j.indcrop.2019.111487

Nohl, H., Gille, L. and Staniek, K. (2005). Intracellular generation of reactive oxygen species by mitochondria. *Biochemical Pharmacology* **69:**719–723. DOI:10.1016/j.bcp.2004.12.002

Nunes, A., Gonçalves, L., Marto, J., Martins, A.M., Silva, A.N., Pinto, P., Martins, M., Fraga, C. and Ribeiro, H.M. (2021). Investigations of olive oil industry by-products extracts with potential skin benefits in topical formulations. *Pharmaceutics* **13**:465. DOI: 10.3390/pharmaceutics13040465

Percoco, G., Patatian, A., Eudier, F., Grisel, M., Bader, T., Lati, E., Savary, G., Picard, C. and Benech, P. (2021). Impact of cigarette smoke on physical-chemical and molecular proprieties of human skin in an ex vivo model. *Experimental Dermatology* **2021**:1–9. DOI: 10.1111/exd.14192

Pljevljakušić, D., Bigović, D., Janković, T., Jelačić, S. and Šavikin, K. (2018). Sandy everlasting (Helichrysum arenarium (L.) Moench): Botanical, chemical and biological properties. *Frontiers in Plant Science* **9**: Article No.:1123. DOI: 10.3389/fpls.2018.01123

Poli, F., Muzzoli, M., Sacchetti, G., Tassinato, G., Lazzarin, R. and Bruni, A. (2003). Antioxidant activity of supercritical CO_2 extracts of Helichrysum italicum. *Pharmaceutical Biology* **41**:379–383. DOI: 10.1076/phbi.41.5.379.15934

Poljšak, B. and Dahmane, R. (2012). Free radicals and extrinsic skin aging. *Dermatology Research and Practice* **2012**:1–4. DOI: 10.1155/2012/135206

Rhodes, L.E., Gledhill, K., Masoodi, M., Haylett, A.K., Brownrigg, M., Thody, A.J., Tobin, D.J. and Nicolaou, A. (2009). The sunburn response in human skin is characterized by sequential eicosanoid profiles that may mediate its early and late phases. *Faseb Journal* **23**:3947–3956. DOI: 10.1096/fj.09-136077

Rinaldi, F., Pinto, D., Trink, A., Giuliani, G. and Sparavigna, A. (2021). In vitro and in vivo evaluation on the safety and efficacy of a brand-new intracutaneous filler with α1-R-collagen. *Clinical Cosmetic and Investigational Dermatology* **14**: 501–512. DOI:10.2147/CCID.S295618

Rodrigues, F., Gaspar, C., Palmeira-de-Oliveira, A., Sarmento, B., Amaral, M.H. and Oliveira, M. (2016). Application of coffee silverskin in cosmetic formulations: Physical/antioxidant stability studies and cytotoxicity effects. *Drug Development and Industrial Pharmacy* **42**:99–106. DOI: 10.3109/03639045.2015.1035279

Romes, N.B., Wahab, R.A. and Hamid, M.A. (2021). The role of bioactive phytoconstituents-loaded nanoemulsions for skin improvement: A review. *Biotechnology & Biotechnological Equipment* **35**:711–729. DOI: 10.1080/13102818.2021.1915869

Ross, G. (2006). A perspective on the safety of cosmetic products: A position paper of the American Council on Science and Health. *International Journal of Toxicology* **25**:269–77. DOI: 10.1080/10915810600746049

Sala, A., Recio, M.D., Giner, R.M., Manez, S., Tournier, H., Schinella, G. and Rios, J.L. (2002). Anti-inflammatory and antioxidant properties of Helichrysum italicum. *Journal of Pharmacy and Pharmacology* **54**:365–371. DOI: 10.1211/0022357021778600

Sano, K., Gotoh, M., Dodo, K., Tajima, N., Shimizu, Y. and Murakami-Murofushi, K. (2018). Age-related changes in cyclic phosphatidic acid-induced hyaluronic acid synthesis in human fibroblasts. *Human Cell* **31:**72–77. DOI: 10.1007/s13577-017-0185-7

Sharmeen, J.B., Mahomoodally, F.M., Zengin, G. and Maggi, F. (2021). Essential oils as natural sources of fragrance compounds for cosmetics and cosmeceuticals. *Molecules* **26**:1–24. DOI: 10.3390/molecules26030666

Soobrattee, M.A., Neergheen, V.S., Luximon-Ramma, A., Aruoma, O.I. and Bahorun, T. (2005). Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. *Mutation Research* **579:**200–213. DOI: 10.1016/j.mrfmmm.2005.03.023

Tarbiat, S. (2018). In vitro antioxidant, anti-inflammatory and lipid lowering activities of Artocarpus lakoocha fruit extract and its implication on treatment of dyslipidemia. *International Journal of Pharmaceutical Sciences Review and Research* **52**:75–82.

Thiagarajan, S., Yeng, C. and Sreenivasan, S. (2020) Oxidative stress and aging and medicinal plants as antiaging agents. *Journal of Complementary Medicine Research* **11:**1–5. DOI: 10.5455/jcmr.2020.11.03.01

Thomsen, P.S., Jensen, T.M., Hammer, K.A., Carson, C.F., Mølgaard, P. and Riley, T.V. (2011). Survey of the antimicrobial activity of commercially available Australian tea tree (Melaleuca alternifolia) essential oil products in vitro. *Journal of Alternative and Complementary Medicine* **17**:835–841. DOI: 10.1089/acm.2010.0508

Trojahn, C., Dobos, G., Lichterfeld, A., Blume-Peytavi, U. and Kottner, J. (2015). Characterizing facial skin ageing in humans: Disentangling extrinsic from intrinsic biological phenomena. *Biomed Research International* **2015**:1–9. DOI: 10.1155/2015/318586

Wei, A. and Shibamoto, T. (2010). Antioxidant/lipoxygenase inhibitory activities and chemical compositions of selected essential oils. *Journal of Agriculture and Food Chemistry* **58**:7218–7225. DOI: 10.1021/jf101077s

Zaazaa, L., Mrabti, H.N., Ed-Dra, A., Bendahbia, K., Hami, H., Soulaymani, A. and Ibriz, M. (2021). Determination of mineral composition and phenolic content and investigation of antioxidant, antidiabetic, and antibacterial activities of Crocus sativus L. Aqueous stigmas extracts. *Advances in Pharmacological and Pharmaceutical Sciences* **2021**:1–7. DOI: 10.1155/2021/7533938

Zota, A.R. and Shamasunder, B. (2017). The environmental injustice of beauty: Framing chemical exposures from beauty products as a health disparities concern. *Amercian Journal of Obstetrics and Gynecology* **217**:418.e1–418.e6. DOI: 10.1016/j.ajog.2017.07.020