



Cosmeceutical Benefit of *Abelmoschus esculentus* L. Seed Extract

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Authors' contributions

This work was carried out in collaboration between both authors. Author SM performed the research and statistical analysis. Author JK contributed on the study's conception and design, analysis and interpretation of data and drafting the article critically for important intellectual content. Both authors read and approved the final manuscript.

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ABSTRACT

Background: *Abelmoschus esculentus* L. or okra is a vegetable of Malvaceae family.

Aim: Okra seeds were dried at 60°C, ground, and extracted by using absolute ethanol. The acquired extract was investigated for chemical constituents, cytotoxicity, anti-tyrosinase activity, and anti-oxidant property.

Results: Total phenolic content was equal to 4.6 mg gallic acid/g dry weight, as constituted by tannins, flavonoids, and polyphenols. Saponins and terpenoids were also existent. Based on molecular ion (M⁺) peaks of GC-MS, long chain fatty acids and their esters (~59%), as well as sterols and their derivatives (~40%) were identified as major components. Glutathione was of 2.5 mg/g dry weight. The anti-tyrosinase activity was equivalent to 15 mg ascorbic acid/g dry weight. The extract was nontoxic to cells of normal fibroblast L929, and exhibited the DPPH radical scavenging activity as well as the ferric reducing antioxidant power, which respectively equivalent to 350 µmol trolox and 170 µmol Fe²⁺ per g dry weight. Lotions (o/w) containing 1-7% w/w extract

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were physically and chemically stable over a 6-heating/cooling cycles-period of the stability test. Transdermal penetration of the lotion excipients and active compounds of the extract was not detected according to the Franz-type skin permeation study.

Conclusion: Cosmeceuticals containing *Abelmoschus esculentus* L. seed extract might be useful for whitening effect and to delay skin aging.

Keywords: *Abelmoschus esculentus* L.; okra seed; anti-oxidation; anti-tyrosinase; okra lotion; accelerated stability testing; skin permeation study; franz-type diffusion cell.

1. INTRODUCTION

Skin aging caused by internal factors is inevitable since it is determined by our biological age. When getting older, the delivery of nutrients and oxygen to the skin is impeded due to a poorer blood supply, and the skin becomes duller and irradiant. Certainly, skin aging is accelerated by external factors. Prolonged and everyday exposure to the sun's rays is responsible for uneven pigmentation and skin aging via oxidative stress. Pollution can trigger the release of free radicals from damaged skin and worsens the effects of sun exposure. Chemicals contained in cigarettes are responsible for an upsurge of free radicals in the skin, leading to oxidative stress. Under normal circumstances, the produced free radicals are caught and neutralized by various antioxidants present in the skin, including α -tocopherol, ascorbic acid, ubiquinone and glutathione [1]. The poorly cared skin will age earlier. Effective sun protection is a key to prevent premature skin aging and wrinkle formation. Regular application of skin care products targeted to individual concern can improve skin appearance to a great extent. Eating lots of antioxidant-rich fruits and vegetables is an important part for caring our skin. Overall, a holistic approach to lifestyle and skin care can help prevent premature skin aging and reduce the visible signs of aged skin [2].

Under regular physiology, skin pigmentation or melanogenesis is beneficial by protecting the skin from harmful UV rays. However, as challenged by oxidative stress, an uneven production and accumulation of melanin in melanocytes may be existent, resulting in dermatological problems, such as freckles and aged spots. Tyrosinase enzyme functions in controlling the process of melanin synthesis [3]. Accordingly, tyrosinase inhibitors, such as arbutin, kojic acid and hydroquinone, are widely used to lighten the skin [4]. Ideally, tyrosinase inhibitors should be specifically active against tyrosinase in melanocytes without affecting other

cells. Unfortunately, many adverse effects of using them have been reported. For example, an imbalance stimulation of mono- and di-phenolase by arbutin may result in blackened skin [5]. The compound is rather unstable, and its degraded product is potentially toxic to bone marrow [6]. Furthermore, kojic acid is not approved by US-FDA for sale as skin whitening agent in over-the-counter pharmaceutical products, although it is extensively used in Japan. Its use at a concentration of 1.0% in skin care formulations may pose a risk by interfering thyroid functions and sensitizing the skin [7]. Instead, hydroquinones are not only mutagenic to mammalian cells [8], but also can induce numerous adverse reactions, such as contact dermatitis, erythema, pricking sensation, hypochromia, etc. [9]. Consequently, it remains necessary to search for new tyrosinase inhibitors that are stable and slightly or non-toxic.

Glutathione is a tripeptide formed by glutamate, cysteine, and glycine, and is one of the most active antioxidant systems in human physiology [10]. Fresh fruits, nuts, and vegetables, such as tomatoes, avocados, oranges, walnuts and asparagus are the most common foodstuffs that help increase glutathione levels in the body [11]. Inhibition of tyrosinase by glutathione has been demonstrated thereby making the substance very attractive in applications as skin whitening agent [12]. Topical formulations containing glutathione are commercially available. They are claimed for improvement of melasma, hyperpigmentation and skin ageing although evidence of efficacy is not demonstrated [13]. Meanwhile, okra plants have found to respond to non-hostile environments by increased synthesis of sulfhydryl compounds, which exhibit antioxidant activity [14]. Xia *et al.* subsequently proved that such antioxidant activity was concerned with constituents in okra seeds [15]. Hence, this study is aimed to clarify whether seeds of *A. esculentus* possess glutathione or substances beneficial for lightening the skin and anti-aging.

2. MATERIALS AND METHODS

2.1 Materials

L929 mouse fibroblast cell line (ATCC[®] CRL-6364[™]) was kindly obtained from Dr. Teerapol Srichana, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand. Dulbecco's modified eagle's medium (DMEM), fetal bovine serum (FBS), penicillin/streptomycin, Dulbecco's phosphate buffered saline (DPBS) and trypsin ethylenediamine tetraacetic acid (EDTA) were purchased from Gibco[™]. Tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was from Hi Media Labs (Mumbai, India). Folin-Ciocalteu reagent, gallic acid, 2,2-diphenylpicrylhydrazyl (DPPH), ascorbic acid, L-dopa, and mushroom tyrosinase were obtained from Sigma Aldrich (Darmstadt, Germany). Absolute ethanol was purchased from Loba Chemie (Mumbai, India). Other chemicals were of analytical grade and bought from Merck Co., Ltd. (NJ, USA).

2.2 Preparation of Okra Seed Extract

Mature okra pods were purchased from local markets in Songkhla province, Thailand. Fresh seeds inside the pods were picked out and oven dried at 60°C for 72 h. After that they were ground into fine powder with diameter range of 0.1-0.5 µm using a ball mill and kept at -20°C until use. Fifty grams of the seed powder were shaken in 100 mL of absolute ethanol for 48 h. The supernatant was separated by centrifugation at 1000 g for 20 min and evaporated at 60°C on a water bath to obtain a viscous extract. Then, it was diluted to a final volume of 25.0 mL by absolute ethanol, resulting in a sample stock solution of 2 g/mL. The solution was stored in a well-closed container at 4°C and immediately diluted to a desired concentration by absolute ethanol for colorimetric analyses or by phosphate buffer saline pH 7.4 (PBS) for cell culture study before use.

2.3 Assay of Total Phenolic Content (TPC)

The TPC of the extract was assayed by using Folin-Ciocalteu method [16]. In brief, a 50-µL sample was added in a tube containing 375 µL of ten-fold diluted Folin-Ciocalteu reagent and thoroughly mixed for 5 min. Then, 375 µL of 6% w/v sodium carbonate solution was added and completely mixed. After incubated at room

temperature for 90 min, the OD₇₂₅ was measured by using a microplate reader. Gallic acid was used as a standard. Its stock solution was prepared in absolute ethanol to a concentration of 0.5 mg/mL. In plotting the calibration curve, a concentration range of 0.01-0.10 mg/mL gallic acid was utilized. Each experiment was performed in triplicate. The TPC was calculated and expressed as mg gallic acid equivalent per g dry weight.

2.4 Identification of Phytochemicals by Chemical Tests

Procedures and reagents used for identification of phytochemicals were in accord with the previous studies [17,18].

2.4.1 Tannins

About 0.5 g sample was boiled in 20 ml of water and then filtered. A few drops of 0.1% ferric chloride were added, and a brownish green or blue-black coloration indicates the presence of tannins.

2.4.2 Saponins

Five grams of the sample powder were added to 40 ml of distilled water in a conical flask, boiled for 5 min, and filtered when still hot. To a test tube containing 5 ml of distilled water, an equal volume of the cooled filtrate was added. The test tube was shaken vigorously for 30 seconds and allowed to stand for 30 min. Formation of honey comb froth indicates the presence of saponins.

2.4.3 Flavonoids

Five millilitres of dilute ammonia solution were added to a portion of the filtrate followed by adding of concentrated H₂SO₄. Formation of yellow color indicates the presence of flavonoids.

2.4.4 Terpenoids

Five millilitres of a sample were mixed with 2 ml of chloroform. Concentrated H₂SO₄ (3 ml) was carefully added to form a layer, indicating the presence of terpenoids.

2.4.5 Phenols

Two millilitres of ferric chloride were added to 2 ml sample. A deep bluish-green solution indicates the presence of phenols.

2.4.6 Alkaloids

Three millilitres of concentrated H₂SO₄ were added to 1 ml sample, followed by adding of potassium dichromate crystals. An olive-green color indicates the presence of alkaloids.

2.5 Determination of Phytochemicals by GC-MS Technique

Experiments using GC technique were carried out on Agilent 7800B GC system equipped with 5977A MSD, a HP-5ms Ultra (Santa Clara, California, USA), and a capillary column (30m in length, 0.25 mm in diameter, and internally coated with a 0.25-µm thick film composing 95% dimethyl polysiloxane). Helium was the carrier gas operated at a flow rate of 1 mL/min. The injection volume was 1 µL. The inlet temperature was maintained at 250°C. The oven temperature was initially programmed at 110°C for 4 min, then increased to 240°C, and next to 280°C at a rate of 10°C/min. The total running time was 60 min. The MS transfer line was maintained at 200°C, and the source temperature was maintained at 180°C. For compound identification, the Mass Hunter GC/MS Acquisition B.07.04.2260 was used by comparing with known spectral database stored in the instrument library.

2.6 Assay of DPPH Radical Scavenging Activity

To determine DPPH radical scavenging activity of samples, the method of Lu *et al.* was applied [19]. In brief, a 40-µL sample was mixed with 160 µL of 0.1 µmol/L DPPH solution in a well of a 96-wells plate. After incubation in the dark at room temperature for 30 min, the OD₅₁₅ was measured using a microplate reader. Trolox was used as a standard. It was dissolved in methanol to a concentration range of 0–500 µmol/L for preparing the calibration curve. The DPPH radical scavenging activity of the sample was expressed as µmol trolox equivalent per g dry weight.

2.7 Analysis of Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay was carried out as previously described [20]. Solutions of 300 mmol/L acetate buffer pH 3.6, 10 mmol/L of 2,4,6-tripyridyl-striazine (TPTZ) in 40 mmol/L HCl, and 20 mmol/L of FeCl₃ were separately prepared. The FRAP reagent was prepared by mixing 25 mL of

acetate buffer solution, 2.5 mL of TPTZ solution and 2.5 mL of FeCl₃ solution immediately before use. To a tube containing 750 µL of the FRAP reagent, a 25-µL sample was added, mixed, and incubated in the dark for 10 min. Then, the OD₅₉₃ was measured using a microplate reader. The solutions of FeCl₂ at varying concentrations were used for preparing the calibration curve. Results were expressed as µmol Fe²⁺ equivalent per g dry weight.

2.8 Assay of Total Glutathione

Two forms of glutathione, namely oxidized (GSSG) and reduced (GSH) glutathione, function in catabolism of xenobiotics and are catalysed by glutathione S-transferases. To assay total glutathione, a reagent consisting of 0.5 U/mL glutathione reductase, 0.3 mmol/L 5-5'-dithiobis-2-nitrobenzoic acid (DTNB), and 4.4 mmol/L EDTA in 100 mmol/L phosphate buffer pH 7 was prepared. Samples to be tested were diluted in 2% w/v sulfosalicylic acid (SSA) containing 1 mmol/L EDTA and 2% w/v polyvinylpyrrolidone. To a tube containing 150 µL of the prepared reagent, a 10-µL sample was added, mixed and incubated for 5 min. After that 50 µL of 0.16 mg/mL NADPH solution was added. Changes of the OD₄₁₂ were monitored at 2 min intervals over 30 min using a microplate reader. Glutathione standard solution of 10 ng/µL was prepared in the SSA solution as previously described and used to substitute the sample. The sample-free mixture was used as the blank control. Calculation of total glutathione was achieved by the equations below.

Total glutathione

$$= (\text{OD}_{\text{sample}} - \text{OD}_{\text{blank}}) / \text{Slope}_{\text{STD curve}} \quad \text{or} \\ = (\text{Slope}_{\text{sample}} - \text{Slope}_{\text{blank}}) / \text{Slope}_{\text{STD curve}}$$

2.9 Determination of Anti-Tyrosinase Activity

Anti-tyrosinase activity was determined according to the previously described method [21] with some modifications. Briefly, 20 µl of mushroom tyrosinase (1000 U/mL), 20 µl of 0.1 M phosphate buffer (pH 6.8) and 100 µl of a sample were mixed. In parallel, mixed reactants deficient of the enzyme or the sample were separately prepared. Ascorbic acid was a positive control and used in plotting the calibration curve. Twenty microliters of 0.85 mM L-dopa were separately added to each tube, mixed, and incubated at room temperature for 10

min. Then, the OD₄₇₅ was measured using a microplate reader. The % tyrosinase inhibition was calculated by the following equation.

$$\% \text{ Tyrosinase inhibition} = (A - B) - (C - D) \times 100 / (A - B)$$

Where:

A = absorbance of blank solution with enzyme
B = absorbance of blank solution without enzyme
C = absorbance of sample solution with enzyme
D = absorbance of sample solution without enzyme

2.10 Cytotoxicity Test

L929 cells were routinely cultured with DMEM supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin in a 5% CO₂ incubator at 37°C. The cell monolayer of about 80–90% confluence was starved in serum-free DMEM for 2 h before testing cytotoxicity.

Cytotoxicity test was performed by using MTT method as previously described [22]. Briefly, a 100-µl MTT solution (5mg/ml in DMEM) was added to the cell monolayer in a well of 96-wells plate, and the plate was incubated in a 5% CO₂ incubator at 37°C for 4 h. Then, excess MTT solution was discarded. The formed formazan crystals were dissolved with 100 µl of dimethyl sulfoxide and measured for the OD₅₇₀ by using a microplate reader. The cells routinely grown in DMEM without any treatments were considered 100% viability.

2.11 Preparation and Characterization of Lotions Containing Okra Seed Extract

Ingredients for preparing an O/W lotion were listed in Table 1. Those of water phase and oil phase were separately mixed and warmed to 60°C. The oil phase was poured into the water phase while rapidly stirring to result in a lotion. It was continually agitated and cooled to 45°C before okra seed extract and Glydant™ were added and homogeneously mixed. The finished product was organoleptically characterized by evaluating its color, odor, texture, and appearance. The pH was determined using MColorpHast™ pH-indicator strips (Merck, Darmstadt, Germany). The viscosity was measured using a Model LVDV III Ultra Brookfield viscometer (Middleboro, MA, USA) at 25°C±2 and reported in centipoises (cP).

Table 1. Components for preparing 100 g of lotion base or 100 g of lotion containing different amounts of okra seed extract

Ingredient (g)	Lotion base	Okra seed lotion
Oil phase		
Olive oil	2	2
Sunflower oil	4	4
Cetyl alcohol	2.5	2.5
Glycerol monostearate-self emulsifier	3	3
Span 60	1	1
Tween 60	1	1
Water phase		
Glycerin	2	2
EDTA	0.02	0.02
Water	qs 100	qs 100
Additive		
Glydant™	0.5	0.5
Okra seed extract (2 g/mL)	–	1 to 7

2.11.1 Stability test

The stability test was performed in accord with the heating and cooling method. Basically, a lotion under investigated is kept at 4°C for 24 h, followed by at 45°C for 24 h to complete a cycle. A good confidence in the product stability is accepted if it passes the 3-cycles test. Nevertheless, a really stable product is indicated if it passes the 5-cycles test. At ending time point, changes in physical and chemical integrity of lotions, such as color, odor, pH, viscosity, flow, texture, and signs of separation were evaluated.

2.11.2 *In vitro* permeation study

The skin permeation property of lotion components was determined using Franz diffusion cell system (PermeGear, Inc., Bethlehem, PA, USA) that reveals the sample exposing area of 1.77 cm² and the receiver volume of 12.0 ml filled with PBS. Frozen pig abdominal skin was commercially available. It was thawed for 30 min and visually examined for punctures or defects. The skin was stripped using adhesive tape to remove stratum corneum disjunctum and mounted in the Franz cell. After completely equilibrated at 37°C, 1 g of a lotion was applied onto the mounted skin and its excess was occluded using Parafilm™. One millilitre of PBS in the receiver was withdrawn at 30 min, 1, 2, 4, 6, 8, and 12 h after application, while an equal volume of fresh PBS was added

to replace at each sampling. The collected sample was centrifuged at 13,000 rpm for 10 min and analyzed for the TPC and DPPH radical scavenging activity as described previously. Next, the exposed skin was taken out from the Franz cell, washed with 50% ethanol, and left to air dry for 3 h. This skin was trimmed using a circular aperture of 1.4 cm diameter, minced using a dissection blade, and homogenized in 10 ml methanol. After centrifugation at 1,500 rpm for 10 min, the supernatant was collected for analysis of the TPC and DPPH radical scavenging activity.

2.12 Statistical Analysis

Data were presented as means \pm SD with $n=3$. They were analyzed by using Student's *t*-test on Statistics Package for Social Science (SPSS) software and considered statistically different at $p < 0.05$.

3. RESULTS AND DISCUSSION

Plant antioxidants such as vitamin C and E, flavonoids, and polyphenols are recognized to play a role in fighting against free radicals. These structurally diverse secondary metabolites are considerable in herbal crude extracts and proven to be effective for improvement of adverse skin reactions caused by oxidative stress. Indeed,

single isolated plant compounds are potential [23]. In this work, an ethanol crude extract from okra seeds was investigated to contain a range of compounds, including glutathione, tannins, saponins, flavonoids, terpenoids and polyphenols (Table 2). Long chain fatty acids, sterols and their derivatives were mainly detected by GC-MS method, the GC chromatogram of which displayed repeatable and satisfactory separation of the compounds (Fig. 1). The TPC was equivalent to 4.6 ± 0.3 mg gallic acid/g of the seed powder. This amount of TPC is likely found in strawberry, apple, spinach and onion [24]. The magnitude of antioxidant activity for samples was assayed by two different methods, i.e., the DPPH radical scavenging activity and the ferric reducing antioxidant power, respectively. The former method is based on the reduction of DPPH• free radical into DPPH₂ by a sample [25], while the latter is to evaluate the ability of samples in reducing Fe³⁺ to Fe²⁺ [26]. The extract exhibited DPPH radical scavenging activity of 350 ± 25 μ mol trolox equivalent/g, which is considerably higher than that of strawberry and red plum, but slightly lower than that of raspberry. The FRAP value of 170 ± 31 μ mol Fe²⁺/g was apparent, being similar to that of raspberry and red plum, but significantly lower than that of strawberry [24]. Accordingly, an attenuation of skin aging as induced by oxidative stress can be achieved by using okra seed extract.

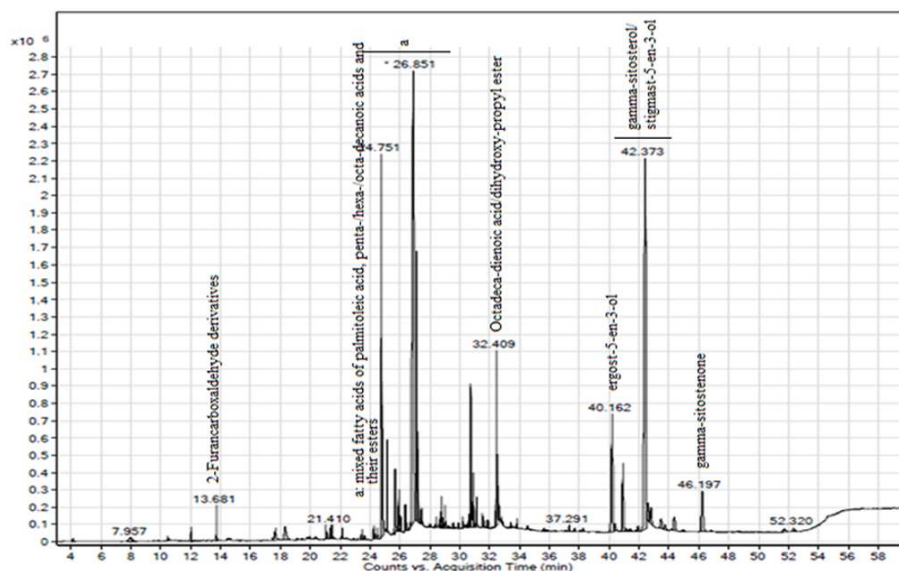


Fig. 1. The GC-MS spectrum of compounds present in okra seed extract: 1, Glycerin; 2, 2-Furancarboxaldehyde, 5-(Hydroxymethyl); 3, n-Hexadecanoic acid; 4, 9,12-Octadecadienoic acid (z,z); 5, (z,z)-9,12-Octadeca dienoic acid, 2,3- dihydroxy-propyl ester; 6, (3methyl,24R)-Ergost-5-en-3-ol; 7, γ -Sitosterol; 8, γ -Sitostenone

Table 2. Constituents in okra seed extract, identified by chemical tests

Constituent	Observation	Result
Glutathione	change of the OD ₄₁₂	+
Tannins	blue-black coloration	+
Saponins	Formation of honey comb froth	+
Flavonoids	Formation of yellow color	+
Terpenoids	Form a layer	+
Alkaloids	Not changed	-
Phenols	A deep bluish-green solution	+

+ means present; - means not present

Glutathione can be found in many plants, such as avocado, asparagus, broccoli, grapefruit, potato, strawberry, orange, tomato, peach and spinach. It functions as an antioxidant by converting peroxides and ROS to non-reactive species, and plays important roles in reduction/oxidation balance [27]. Thus, dietary intake of fruits and vegetables containing glutathione is significant for aged peoples who have reduced amounts of the body glutathione. In this work, the contents of GSSG and GSH were determined to be of 1.24 ± 0.04 and 1.27 ± 0.05 mg/g, respectively. Although information of glutathione contents in plants is not available at present, it is promising for the extract in its ability to eradicate cellular oxidative insults. Furthermore, an anti-tyrosinase activity of the extract was demonstrated, being equivalent to 14.96 ± 8.86 mg ascorbic acid/g. The cytotoxicity of okra seed extract was determined using normal fibroblast cell line, L929. The cells were incubated with the extract for 2-3 days, followed by the investigation of cell viability using MTT assay. Results of Fig. 2 showed that more

than 80% of the tested cells were viable following in contact with ≤ 2.5 mg/ml extract for 3 days. About 65-70% of the cells remained alive after challenged by the extract of 3 mg/ml for 2 days. Indeed, the determination of IC₅₀ was not achieved concerning the concentration range tested. Thus, the extract seemed to be non-toxic. It is significant to include okra seed extract in formulations, such as creams and lotions, followed by testing for their stability and skin permeation property.

In regard to the extract's concentrations able to maintain the viability of L929 cells above 80%, the extract of 2 mg/ml was utilized to prepare lotions by using ingredients listed in Table 1. Results based on organoleptic characterizations showed that the texture of lotions was soft, smooth and homogeneous (Table 3). Their pH was ~ 5 , thereby expected to be compatible with the skin. Changes in color, odor, pH, and transparency were not observed regarding the heating-cooling stability test (6 cycles). This observation was applied to all of the prepared

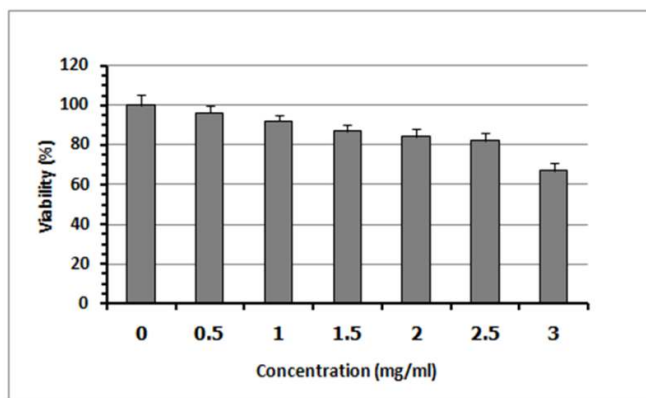


Fig. 2. Results of cytotoxicity testing for the extract on L929 fibroblast cells; the cells were incubated with the extract of concentrations ranging between 0.5 and 3 mg/ml for 2-3 days, followed by the determination of cell viability (%) using MTT assay

products that contained different concentrations of the extracts ranging between 1 and 7% (data not shown). In contrast, the viscosity was increased from ~30,000 cP for the finished product to ~45,000 cP after ending the 1st cycle and to ~52,000 cP after completing the 2nd cycle, but being stabilized thereafter. Such former changes might be contributed by the dispersion and/or flocculation of particles that affected the structure and thickness of the emulsion system, whereas the persistent phase was achieved when the system was in equilibrium [28]. The TPC and DPPH radical scavenging activity of all products were monitored to judge whether active compounds therein were adequately stable. In Table 4, the quantified TPC and the radical scavenging activity were proportional to the amount of the extract added in the lotions, and these were not decreased by changing

temperature of the stability test (data not shown). Consequently, the formulated lotions might help protection of active substances in the extract from degradation induced by heat. Such measurements were performed for samples in donor and receiver chambers of the Franz diffusion apparatus as well. The TPC and the radical scavenging activity determined in the donor chamber at 30 min, 1, 2, 4, 6, 8, and 12 h after operation were shown in Table 5. Instead, those of the receiver chamber were not detected. Hence, there was very low amount of antioxidant compounds (~1.5 %) in the lotions capable to permeate the skin membrane. Such compounds might be held or surrounded by other structurally liked substances of the lotion base, thereby hindering the skin permeation of the formers. In addition, ingredients of the base might have higher fluxes than actives, thus impeding the skin

Table 3. Characteristics of lotions containing 1 or 7% w/w okra seed extract before (C₀) and after subjected to the stability test (C₁...C₆)

Property	Control (C ₀)	Cycle no.					
		C ₁	C ₂	C ₃	C ₄	C ₅	C ₆
pH	5.0			not changed			
Texture	soft, smooth, homogeneous			not changed			
Color	white for 1% extract pale yellow for 7% extract			not changed			

Table 4. The TPC and % DPPH inhibition for the finished products loaded with 1, 3, 5 or 7%w/w okra seed extract

Test	The concentration of okra seed extract				
	Lotion base	1%	3%	5%	7%
% DPPH Inhibition	4.82±0.26	33.78±1.18	61.26±0.34	78.30±1.56	86.74±0.13
TPC (mg GAE/g DW)	0.293±0.009	0.989±0.000	2.021±0.018	2.660±0.009	3.702±0.016

Table 5. The TPC and %DPPH inhibition for the finished preparations and samples collected from the donor chamber, the membrane, and the receptor chamber of Franz diffusion system

Details	Base lotion		7% lotion	
Finished product				
TPC (mg GAE/g DW)	0.29 ± 0.01		3.70 ± 0.02	
% DPPH Inhibition	4.81 ± 0.26		86.74 ± 0.13	
Franz Diffusion System	Donor chamber	Membrane	Donor chamber	Membrane
TPC (mg GAE/g DW)	0.06 ± 0.01	0.22 ± 0.01	1.44 ± 0.01	0.28 ± 0.01
% Permeation (TPC)	20.69 ± 0.03	75.86 ± 0.03	38.92 ± 0.27	7.57 ± 0.27
% DPPH Inhibition	3.85 ± 1.10	0.67 ± 0.46	72.74 ± 5.19	1.33 ± 0.39
% Permeation (DPPH inhibitory activity)	80.04 ± 22.87	13.92 ± 9.56	83.84 ± 5.98	1.53 ± 0.45

permeability of the latter [28]. That the mentioned antioxidants might be sensitive to heat of the Franz diffusion system where samples were lasted for 12 h at 37°C could not be ignored. Their degraded products were deficient in phenolic structure, thereby decreasing the OD₇₂₅ of the TPC analysis. It can be expressed that actives and ingredients of the prepared lotions were impermeable to the skin. Then, systemic side effects of using them are unexpected.

4. CONCLUSION

The ethanol extract of okra seeds contains constituents able to inhibit oxidation reactions through different mechanisms, such as free radical scavenging activity, iron reducing power, and glutathione-associating antioxidant effect. The former two actions can be attributed by the existing long chain fatty acids, their ester analogues [29], sterols [30], and phenolic compounds [31]. However, these chemicals may not be the only compounds responsible for the bioactivities investigated. Other compounds not identified by the GC/MS method may exhibit a more significant role in eradicating oxidative stress.

Excipients that enhance skin penetration, such as glycerol and Tween/Span 60 are used to prepare an o/w lotion by adding 1-7% w/w extract. These concentrations are kept below 10% w/w, which is the maximum level of plant ethanolic extracts permitted to add in topical products without measurable toxicity [32]. The lotions with and without the extract are stable under 6 cycles of the heating/cooling stability test. Phase separation and changes in texture, color, odor, and pH are not apparent. The product reproducibility is indicated by the consistent viscosity data. Active compounds therein are not percutaneously absorbed, thus systemic side effects in using the products can be avoided. Our results provide scientific basis and demonstrate a feasible approach in developing a topical product effective for moisturizing the skin and improving skin beauty by incorporation of okra seed extract. However, formulating a product that possesses optimal efficacy and expected sensory quality can be of challenges.

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CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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