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Quantitative Estimation of the Hydroquinone, Mercury and Total Plate Count in Skin-Lightening Creams

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Abstract: Generally white color of the skin is regarded as a feature of superiority and prettiness around the world. Both the males and females in Pakistan apply skin-lightening creams (SLC) but they do not know about the side-effects of their constituents. Skin-lightening products include SLC and related ointments. The SLC are made by mixing fates and water in standard procedure. Here, 20 SLC specimens were obtained and subjected to mercury, hydroquinone and the total plate count (TPC). The hydroquinone in SLC was determined using HPLC, mercury level was assessed by ICP OES and finally TPC were computing by utilizing nutrient media (Agar). The hydroquinone in SLC ranged from 0 to 7.14 ± 0.18% with a median value of 0.33%. In 25% of the studied samples, hydroquinone was not detected, 70% of the samples showed values within the limit and 5% of the samples (1 sample) had a hydroquinone concentration above the permissible limit defined by Pakistan (5%). The mercury ranged 0-7.7 ppm, with a median value of 2.5 ppm. Mercury was detected in 95% of the samples; thus, only 5% of the samples had no mercury. In turn, 20% had mercury within the limit value while 75% of the samples had concentration above the Pakistan standard limit (1 ppm). Moreover, TPC obtained in this study was less than the allowable value set according to European Union (EU). Hence, the SLC samples showed high concentration of toxic constituents which could cause deleterious skin diseases. Government must monitor such kind of cosmetic products regularly in order to reduce the danger.

Keywords: hydroquinone; mercury; skin-lightening creams; total plate count

1. Introduction

Currently, the skin-lightener or skin-whitener market is flourishing worldwide. The white color of individuals is regarded as a feature of social status and grace around the world [1]. Skin-lightening products include skin-whitening creams and ointments. Skin-lightening creams (SLC) are cosmetic products which are the mixture of fats and water and used on face in order to treat dermal issues such as dark and/or aging spots, hyperpigmentation and discoloration. Pigmentation in humans is owing to the presence of a pigment (melanin) in skin. If the melanin level increases in human body, this can lead to serious skin diseases for example hyperpigmentation, melisma and dark complexion.

Individuals dislike dark color of skin and wish to possess white complexion, which makes them happy. Due to this perception, both women and men are intensively using different skin-whitening creams without any awareness about their toxic ingredients [2]. Ingredients used in skin-whitening creams are mercury, arbutin, hydroquinone, kojic acid, paraben, azelaic acid, vitamin C, niacinamide and glutathione which are dangerous to health [3].

Hydroquinone has been largely included previously as one of the major constituent in many SLC products. It inhibits the synthesis of melanin and restricts the production of skin-color-changing substances. The permissible limit for hydroquinone is 2% according to US FDA and WHO. Pakistan has also set a standard (PS3228/2006) for hydroquinone, which is 5%. Hydroquinone is considered to cause respiratory disorders and skin irritation as well as carcinogenic effects and is banned in many countries, such as Australia and in Europe, Asia and Africa [4]. Mercury is ranked among the top ten chemicals or group of chemicals which are considered serious health concerns to the people according to the World Health Organization (WHO) [5]. Mercury occurs in an elemental (or metallic) form as well as organic and inorganic forms [6,7]. It is a neurotoxicant that is strictly banned in various European and African countries [8]. The SLC containing toxic mercury has been applied widely by the dark-skinned individuals in various countries of Asia and Africa [9–11]. Mercury is added in skin-lightning creams as a skin-bleaching agent since it reduces the production of melanin. It is also used as a strong preservative in body- or hand-care creams/lotions and others cosmetic products, despite not justifying its use in these products [12]. The maximum permissible limit of mercury in cosmetics is 1 $\mu g\ g^{-1}$, established in 1992 by the US FDA and WHO. Mercury has been reported to induce lethal effects on human skin such as rashes, scarring and discoloration [4,6]. Al-Saleh et al. [13] found mercury in skin-lightening creams and examined the chronic effects of mercury on mice. They observed maximum mercury accumulation in the ovaries of mice based on the frequency of mercury-containing creams application. It was also concluded that mercury accumulation could disrupt reproductive behavior, leading to ovarian failures. Hence, mercury exposure via creams causes a serious danger to the public health.

The bacterial growth occurring in skin whitening creams could deteriorate the quality of creams and this could happen due to various reasons. The components of creams are the major reason behind the occurrence of bacterial growth [14–16]. Among them, water is the main component of skin-whitening creams and inclusion of contaminated water results in the bacterial growth as it provides medium for the growth of bacteria in creams. Use of contaminated creams on the skin results in various kinds of skin diseases [15]. Thus, it is imperative to determine the microbial count of skin-whitening creams.

According to Asian culture, white color of the skin shows richness while and a dark/black skin color is linked to poorness. Furthermore the people are motivated to have white color because in many movies the hero or heroine is sleeted on the basis of white color showing positivity while villains are casted with dark skin color representing negativity. A large number of SLC and bleaching products are found in local markets of Asia. Moreover, many skin whitening treatments and procedures are also offered by the dermatologists as well as beauticians in beauty salons in Asian countries. Pakistani people are also greatly impressed with the SLC creams as white color of the skin adds value is marriage market [17–19]. People in Pakistan are also completely unaware of the toxic effects caused by constitutes in skin-whitening creams and using these creams extensively.

Despite many studies conducted worldwide on the determination of the mercury, hydroquinone and total plate count in SLC and cosmetics, to our knowledge, no data are available in the literature on the quantification of the abovementioned parameters in Pakistani local brands of skin-lightening creams. Thus, being first of its kind, the objectives of the current study are to (1) determine the concentration of hydroquinone and mercury in different skin-lightening creams of local brands available in Pakistan; (2) compare the hydroquinone and mercury concentrations in skin-whitening creams with the Pakistan standard limits; and (3) determine the risk posed to the Pakistani population. Moreover, the bacterial growth (TPC) was also examined in the present study.

2. Materials and Methods

The samples (Twenty) of SLC were obtained from local market in Lahore, Pakistan. These SLC were selected as they were inexpensive, famous and bought abundantly (Figure S1; Supplementary Materials). The date of production was demonstrated clearly and SLCs had no ointment in them. The major analyses were done in the labs of Pakistan Council of Scientific and Industrial Research (PCSIR) while TPC was examined at the lab of CEES, University of the Punjab, Lahore, Pakistan (Table 1).

Table 1. Different brands of skin-lightening creams available in Pakistan.

Obs. No.	Brand Name	Manufacturing Date	Manufactured By	Ingredients Mentioned on Packaging
1.	SLC_1	4 January 2016	NA †	Bee wax, essential oils, petrolatum, preservatives, herbal extract, fragrance.
2.	SLC_2	February 2016	Mashyam Enterprise International (Pvt) Ltd.	Carnuba wax, bees wax, herbal extract, sun screen agents, emulsifiers, DM water, vitamin E, softener, preservatives and FD&C colors.
3.	SLC_3	December 2015	NA	Petrolatum, bees wax, blackberry extract, titanium dioxide, bismuth subnitrate, methyl paraben, Vitamin A, E, fragrance.
4.	SLC_4	March 2016	NA	Petrolatum, bees wax, titanium dioxide, bismuth subnitrate, methyl paraben, Vitamin A, E, fragrance.
5.	SLC_5	30 April 2015	NA	Not mentioned
6.	SLC_6	22 October 2015	Shaheen Cosmetics Pakistan, Lahore, Pakistan	Alfha arbutine, giga white, kojic acid, licorice extract, niacinamide, mulberry extract, lactic acid, emblica extract, vitamin K, ceramide, zinc oxide, mica powder, petrolatum and fragrance.
7.	SLC_7	December 2015	NA	Kojic acid dipalmitate, Carnauba wax, bees wax, sun screen chemcials, emulsifier, herbal extract, FD&C colors and preservatives
8.	SLC_8	15 October 2015	NA	talcum, Petrolatum, bees wax, zinc oxide, zinc oxide, squalane, candelilla wax, stearic acid, titanium dioxide, paraffin, glyceryl monostearate, calcium carbonate, bismuth subnitrate, BHT, triclosan, methyl paraben, propyl paraben, ascorbic acid tocopheryl acetate (vitamin E), fragrance.
9.	SLC_9	13 March 2016	Poonia Brother (Pak), Gujranwala, Pakistan	Deionized water, vitamin A, cetyl alcohol, vitamin E, vitamin B, citric acid, vitamin B3, propyl paraben, methyle paraben, natural wax, zinc oxide, white oil, petroleum jelly, natural colors, kojic acid, fragrance.
10.	SLC_{10}	6 May 2014	Unilever Pakistan Limited, Karachi, Pakistan	Stearic acid, demineralized aqua, glycerin, perfume, butyl methoxy dibenzoyl, prophyl paraben, methylparaben, cetyl alcohol, titanium dioxide, hydrolyzed milk protein.
11.	SLC_{11}	November 2013	The Stillman's beauty Karachi, Pakistan	Mineral oil, emollient petrolatum, C12-C18 wax, zincum, liquorice extract, retinyl palmitate, fragrance, methylparaben.
12.	SLC_{12}	February, 2016	NA	Petrolatum, mineral oil, propylene glycol, bee wax, candelilla wax, zinc oxide, talc, titanium dioxide, allantoin, salicylic acid, kojic acid, bismuth subnitrate, aloe vera extract, tocopheryl acetate, perfume
13.	SLC_{13}	12 March 2016	NA	Arbutin beeswax, carnuba wax, titanium, herbal extract, sun screen agents, emulsifiers, preservatives and FD&C color.
14.	SLC_{14}	May 2016	Kohinoor Chemical Co. (Pvt) Ltd.	Water, palmitic acid, stearic acid, perfume, potassium stearate, cetyl alcohol, methyl paraben.

Table 1. *Cont.*

Obs. No.	Brand Name	Manufacturing Date	Manufactured By	Ingredients Mentioned on Packaging
15.	SLC_{15}	July 2015	Thailand	Petrolatum, mineral oil, bees wax, *Euphorbia cerifera* (candelilla) wax, bismuth subnitrate, paraffin, arbutin CAS No. 497-76-7, kojic acid, CAS No. 501-30-4, allantoin, zinc oxide, titanium dioxide, triclosan, BHT, tocopheryl acetate (vitamin E), propyl paraben, iron oxide red Cl No. 77491, iron oxide black Cl No. 77499, fragrance (rose).
16.	SLC_{16}	January 2015	L.P. inter-cosmetics Co., Ltd., Bangkok, Thailand	Vitamin A, C, D, E, B2, B6, pyridoxine pipalmitate, biosol, allantion.
17.	SLC_{17}	April 2016	NA	Not mentioned.
18.	SLC_{18}	April 2013	H & Sons Enterprises Lahore, Punjab, Pakistan	Bee wax, natural skin tonic.
19.	SLC_{19}	July 2015	HCL London, London, UK	Talcum, Petrolatum, bees wax, zinc oxide, squalene, titanium dioxide, kojic acid, stearic acid, panax ginseng extract, glyceryl monostearate, BHT, bismuth subnitrate, methyl paraben, triclosan, tocopheryl propyl paraben, vitamin E, FD&C color and fragrance.
20.	SLC_{20}	February 2015	Enaura Cosmetics, New York City, USA	Not mentioned.

[†] SLC = skin-lightening cream.

2.1. Estimation of pH

The pH of the samples was measured by using a pH meter (HANNA Instrument, HI 2211, pH/ORP Meter, Woonsocket, RI, USA). An appropriate quantity of the sample solution was taken in a 100 mL beaker to immerse the tips of the electrodes. The electrodes of the pH meter were rinsed with distilled water and then with the sample solution. The temperature and pH of the samples were recorded.

2.2. HPLC Analysis

2.2.1. Preparation of Cream Sample

Samples of cream (2 g each) was added in a beakeralong with mobile phase (25 mL) (water:methanol, 40:60) [20,21]. The solution was thoroughly mixed using the water bath (model: HWS 26, Shanghai, China) for 45 min at optimum temperature of 60 °C. Afterwards cooling was done by putting the mixture in refrigerator which resulted in separation of waxes and fates. The solution was carefully filtered and stored in Teflon vials.

2.2.2. Preparation of the Reference Solution

A total of 0.1 g of hydroquinone was added into a 100 mL flask and mobile phase (very little amount) was added to make it soluble. Finally the remaining volume was filled with deionized water (DW). After that, about 5 mL was taken from this standard stock, and the volume was filled up to 50 mL using the mobile phase.

2.2.3. Prepartion of Mobile Phase

A mixture (methanol:water mixture 40:60) was prepared by the addition of about 100 mL methanol solution in about 150 mL of DW following heating and then for 20 min, the solution was sonicated using the ultrasonic bath (Elma, Wetzikon, Switzerland, model: JP-04OST) to degas the mobile phase.

2.2.4. Procedure for HPLC

Almost 20 µL of each sample was injected in the HPLC (Shemadzu, model: LC-9, Kyoto, Japan) and the chromatograms were recorded (Figure 1). The presence of hydroquinone was indicated by analytical determination. The area of the peak obtained for each cream sample was analyzed and then it was compared carefully with standard reference solution. The retention time determined for the hydroquinone standard reference solution against the cream sample solution was employed to confirm the presence of hydroquinone. The percentage of hydroquinone was computed using the following Equation 1 [20]:

$$\%\text{age of hydroquinone} = bi/pi \times w_{ref}/w_{spl} \times d \times 100 \quad (1)$$

where, bi and pi are the peak areas of hydroquinone in sample solution and standard solution, receptively while W_{spl} and W_{ref} show the weight of sample solution and standard solution, respectively; d is dilution factor which is 0.1.

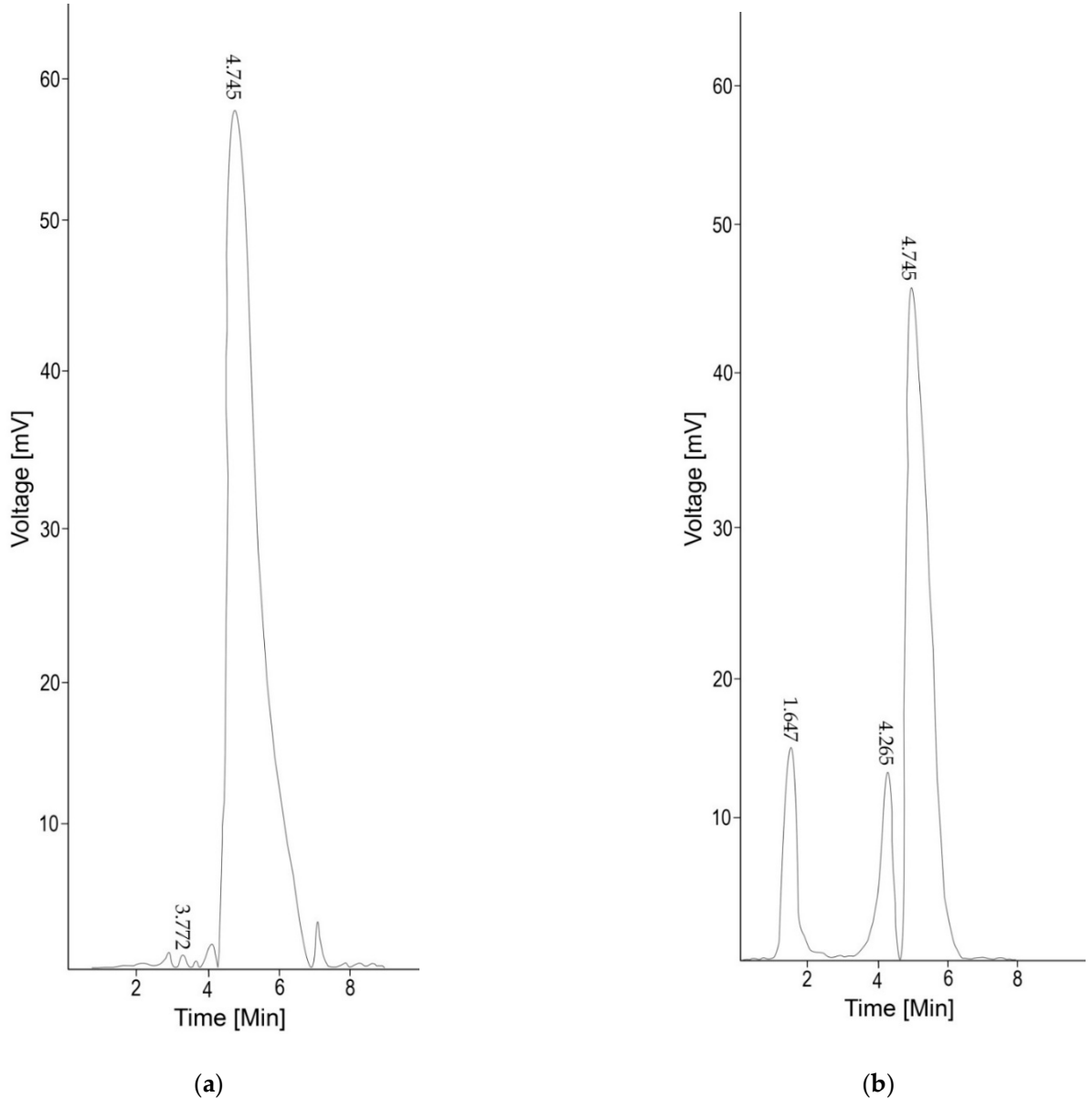

Figure 1. (**a**) Chromatogram of the standard solution of hydroquinone; (**b**) chromatogram of the sample solution (SLC_2) of hydroquinone.

2.2.5. HPLC Conditions

Isocratic HPLC system;
Temperature: 30 °C;
Wavelength: 295 nm;
Column: 250 mmL, 4.6 mm ID, C-18, (Octadecyl-silica) ODS, reverse phase;
Flow rate: 1 mL/minute;
Mobile phase: methanol:water mixture (40:60);
Detector: UV (ultraviolet)—Vis (visible), with range 190–700 nm;
Extraction: Liquid/liquid extraction;
Pump: Reciprocating pump.

2.3. ICP OES Analysis

2.3.1. Preparation of Sample

A cream sample (1 g) was weighed in 100 mL of beaker and then 25 mL of nitric acid solution (10%) was put in all the prepared samples (each). The solution was subjected to heating at 120 120 °C using hotplate (A&E Lab, model: 106 MA, London, UK) to for digestion of cream samples and after that the mixture became clear. The solution was filtered (Whatman filter paper 41) and stored in a refrigerator.

2.3.2. Preparation of the Reference Solution

To analyze mercury in collected creams, a mercury reference standard solution of 1000 mg L^{-1} was employed and analyses were done by using the Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Optima DV 5300 by Perkin Elmer, Waltham, MA, USA) technique. The standard solution of mercury was diluted up to make 1 mg L^{-1} of substock solution using the following Equation:

$$M_1V_1 = M_2V_2$$

where, M_1 and M_2 are stock solution and diluted solution concentrations (moles/L) while V_1 and V_2 are volume of stock and diluted solutions.

2.3.3. Procedure for ICP-OES

All skin-whitening cream samples were estimated by using ICP-OES containing an auto sampler along with a quartz nebulizer. A cinnabar spray chamber (25 mL) was used to reduce the memory effect. A mercury sampler and skimmer cones were also attached with ICP-OES. An auto sampler was used for the injection of samples while suction was done using quartz nebulizer.

2.3.4. ICP OES Operating Conditions

RF power: 1300 W;
RF generator: 40 MHz;
Air flow rate: 18 L/min;
Argon flow: 0.6 L/min;
Sample flow rate: 1.5 mL/min;
View: Dual view;
Nebulizer: 0.8 L/min;
Pump: Peristaltic pump;
Detector: Segmented-array Charge Coupled Device (SCD).

2.4. TPC Analysis

2.4.1. Media Preparation and Sterilization

Media was prepared by adding 28 g of nutrient agar (beef extracts + yeast extracts + peptone + sodium chloride) in 1 L of deionized water and it was heated/boiled

to dissolve thoroughly. The nutrient media and other apparatus were carefully sterilized using autoclave (Biobas, model: LE-75D, Shandong, China) at 121 °C and 15 psi for 15 min.

2.4.2. Procedure for TPC

The air blower and UV lamp attached to laminar flow (Esko Company, model: LC-2A, Shanghai, China) were warmed up approximately 30 min before experiments. About 25 mL of agar media was added into each of the petri dish. The samples of the creams were thoroughly spreadin the petri dishes containing nutrient agar. The petri dishes were agitated manually clockwise for 4–5 times. The Petri dishes were placed in an incubator (Memmert, model: In-50, Büchenbach, Germany) at for 72 h time at 37 °C temperature and finally colonies of microorganisms were detected and they were counted by using a colony counter.

2.5. Statistical Tools

The microsft word® software was sed for basic statistical analyses. The skewness in the obtained data was computed using statistical software SPSS 16.0 to observe whether data is skewed or normal [22].

3. Results and Discussion

The pH was observed to be acidic, slightly acidic, neutral and slightly basic in 10, 20, 6 and 5% of the samples, respectively (Table 2). The HPLC analysis confirmed the existence of hydroquinone in the sampled skin-whitening creams at different concentrations (Table 3). The hydroquinone concentration ranged 0 to 7.1404 ± 0.1823% in creams samples. The skewness value was noted as 4.086 (Table 4) for the hydroquinone, that is above standard value (+1 to −1) [11], showing a normal distribution of the data. Moreover, hydroquinone was not detected in 25% of the samples, including SLC_5, SLC_8, SLC_{12}, SLC_{16} and SLC_{17}. Only one sample (SLC_{11}, 7.1404 ± 0.1823%) showed a hydroquinone concentration above the permissible limit of Pakistan. This sample cream was manufactured in Pakistan and it was very economical. The hydroquinone level was within the permissible limit in 95% of the samples, ranging from 0.04 to 1.26.

Table 2. The pH level in the skin-lightening creams available in Pakistan.

Obs. No.	Product Name	Temperature	pH
1	SLC_1 †	28.3	7.00
2	SLC_2	28	7.17
3	SLC_3	28	7.09
4	SLC_4	27.7	6.26
5	SLC_5	28.2	7.10
6	SLC_6	28.6	7.04
7	SLC_7	28	6.34
8	SLC_8	28	8.00
9	SLC_9	28	4.97
10	SLC_{10}	28.2	6.90
11	SLC_{11}	28.3	4.92
12	SLC_{12}	28	7.00
13	SLC_{13}	28	7.00
14	SLC_{14}	30	7.05
15	SLC_{15}	28	7.00
16	SLC_{16}	28.3	6.56

Table 2. *Cont.*

Obs. No.	Product Name	Temperature	pH
17	SLC_{17}	28.5	7.12
18	SLC_{18}	28	7.01
19	SLC_{19}	28.5	7.03
20	SLC_{20}	27.3	7.23

† SLC = skin-lightening cream. Minimum value of pH = 4.92. Minimum value of temperature = 27.3. Maximum value of pH = 8.00. Maximum value of temperature = 28.6. Average value of pH = 6.43. Average value of temperature = 28.19.

The analyses of hydroquinone in different SLC have been reported previously n the United Kingdom [23]. It was reported that about 10 creams showed having hydroquinone, which were subjected to a chromatographic test in the Plateau state, Nigeria [24]. The researchers demonstrated positive results regarding hydroquinone; however, hydroquinone concentration was less than the allowable limit, i.e., 2% in seven cream samples, 2 to 5% for two samples and 5% for one cream sample [24]. In another study, the hydroquinone level ranged from 0.0002 to 0.0350%, showing low values reported in our study [25]. Long-term application of SLC with hydroquinone may lead to exogenous ochronosis, which is an uneven hyper pigmentation showing brown and yellow pigment deposition on skin. The long-term impacts of hydroquinone also include cancer in humans [26].

Table 3. Hydroquinone content of the skin-lightening cream samples available in Pakistan.

Serial #	Name	Hydroquinone (%)	Peak Area (PA) §	Retention Time (RT)
I	SLC_1 †	0.06 ± 0.02 ‡	9291	4.75
II	SLC_2	0.24 ± 0.09	23135	4.74
III	SLC_3	0.65 ± 0.06	108933	4.73
IV	SLC_4	1.26 ± 0.03	276284	4.74
V	SLC_5	0 ± 0	0	0
VI	SLC_6	0.93 ± 0.0003	221421	4.74
VII	SLC_7	0.04 ± 0.02	9021	4.74
VIII	SLC_8	0 ± 0	0	0
IX	SLC_9	0.33 ± 0.08	48451	4.73
X	SLC_{10}	0.98 ± 0.07	205611	4.45
XI	SLC_{11}	7.14 ± 0.08	1509163	4.73
XII	SLC_{12}	0 ± 0	0	0
XIII	SLC_{13}	0.22 ± 0.02	47241	4.74
XIV	SLC_{14}	0.43 ± 0.08	55200	4.74
XV	SLC_{15}	0.23 ± 0.06	41319	4.73
XVI	SLC_{16}	0 ± 0	0	0
XVII	SLC_{17}	0 ± 0	0	0
XVIII	SLC_{18}	0.33 ± 0.003	49351	4.74
XIX	SLC_{19}	0.14 ± 0.02	30162	4.75
XX	SLC_{20}	0.46 ± 0.01	55761	4.73

Note: Pakistan standard limit = 5% [24]. † SLC = skin-lightening cream. ‡ Abovementioned readings are mean ± SD data. § PA for quantification. RT is retention time of the standard solution = 4.74. Minimum value of hydroquinone = 0 ± 0. Maximum value off hydroquinone = 7.14 ± 0.08. Average value of hydroquinone = 0.07 ± 0.03.

Table 4. Skewness value for various ingredients.

Obs. No.	Ingredients	Skewness Values
1.	Hydroquinone	4.086
2.	Mercury	0.996
3.	Total plate count	1.044

Similarly, the mercury concentration ranged between 0 and 7.7 ± 0.2 ppm, with a median value of 2.5 ppm (Table 5). The skewness calculated for mercury was 0.996 (Table 4), which was close to the range defined (+1 to −1) and showing a normal data distribution. Mercury was detected in 95% of the samples; thus, only 5% of the samples showed a zero mercury concentration (SLC_8). Mercury was within the permissible limit in 20% of the samples, i.e., SLC_1, SLC_{10}, SLC_{14} and SLC_{19}. However, 75% of the samples had a concentration above the standard limit (1 ppm). Moreover, the manufacturing country was not mentioned in most of these samples. The maximum concentration of mercury found in this study was 7.7 ± 0.2 ppm. The sample cream with the highest mercury concentration was manufactured in India and was the most popular skin-whitening cream among the population of Pakistan.

Table 5. Mercury content of the cream samples available in Pakistan.

Serial #	Name	Concentration (mg/kg)
I	SLC_1 †	0.8 ± 0.3 ‡
II	SLC_2	1.9 ± 0.2
III	SLC_3	2.6 ± 0.3
IV	SLC_4	5.5 ± 0.5
V	SLC_5	2.6 ± 0.1
VI	SLC_6	3.1 ± 0.1
VII	SLC_7	2.3 ± 0.1
VIII	SLC_8	0 ± 0
IX	SLC_9	7.7 ± 0.2
X	SLC_{10}	0.3 ± 0.05
XI	SLC_{11}	3.5 ± 0.05
XII	SLC_{12}	2.5 ± 0.05
XIII	SLC_{13}	2.5 ± 0.1
XIV	SLC_{14}	0.1 ± 0.05
XV	SLC_{15}	3.5 ± 0.1
XVI	SLC_{16}	2.4 ± 0.1
XVII	SLC_{17}	2.5 ± 0.2
XVIII	SLC_{18}	1.6 ± 0.05
XIX	SLC_{19}	0.4 ± 0.2
XX	SLC_{20}	5.5 ± 0.2

Note: Standard limit (Pakistan's standard) = 1 mg/kg [25]. † SLC = skin-lightening cream. ‡ Abovementioned readings are mean ± SD data. Minimum value of mercury = 0 ± 0. Maximum value of mercury = 7.7 ± 0.2. Average value of mercury = 2.56 ± 0.1.

A higher concentration of mercury in SLC obtained from Middle East Asia and Saudi Arabia and was also reported in another study [15]. A high concentration (0.16 to 25.30 mg/kg) of mercury (>standard limit) was estimated in skin-lightening cream samples obtained from the Dar es Salaam market of Tanzania [27]. The cumulative impact of the continuous/repetitive use of even a low level of mercury-containing creams can lead to nephritic syndrome [28]. A study confirmed that application of of SLC containing inorganic mercury resulted in accumulation and absorption of mercury in the body [29].

Moreover, Table 6 shows that the microbial count ranged from 0 to 7.0 × 10^2 ± 1 CFU/g in this study. Samples SLC_{12} and SLC_{19} showed no colony count while the skewness value (1.044) for data was also close to the standard range (+1 to −1) (Table 6), which is set for a normal distribution. The results showed that all SLC samples contained relatively less

microbial count as compared to US FDA and ASEAN limits. In a study conducted on microbial contamination in Europe, approximately 24 cosmetic samples were observed to contain higher number of different types of microbes [30]. In another experiment TPC ranged from 1.7×10^2 to 5.4×10^2 CFU/g [31].

Table 6. Total plate count of the SLC samples available in Pakistan.

Serial #	Name	Colony-Forming Unit (CFU/g)
1	SLC_1 †	80 ± 1 ‡
2	SLC_2	$2.8 \times 10^2 \pm 2$
3	SLC_3	$1.4 \times 10^2 \pm 1$
4	SLC_4	$1.2 \times 10^2 \pm 1$
5	SLC_5	60 ± 2
6	SLC_6	40 ± 1
7	SLC_7	$3.6 \times 10^2 \pm 2$
8	SLC_8	$2.6 \times 10^2 \pm 2$
9	SLC_9	$4.0 \times 10^2 \pm 1$
10	SLC_{10}	$1.0 \times 10^2 \pm 2$
11	SLC_{11}	$1.8 \times 10^2 \pm 2$
12	SLC_{12}	0 ± 0
13	SLC_{13}	$6.6 \times 10^2 \pm 3$
14	SLC_{14}	$1.4 \times 10^2 \pm 2$
15	SLC_{15}	$2.4 \times 10^2 \pm 2$
16	SLC_{16}	$2.2 \times 10^2 \pm 1$
17	SLC_{17}	$2.8 \times 10^2 \pm 2$
18	SLC_{18}	$7.0 \times 10^2 \pm 1$
19	SLC_{19}	0 ± 0
20	SLC_{20}	$5.8 \times 10^2 \pm 2$

Note: US DA, ASEAN and EU limit = 1000 CFU/g [26]. † SLC = skin-lightening cream. ‡ Abovementioned readings are mean ± SD data. Minimum value of TPC = 0 ± 0. Maximum TPC value = $7.0 \times 10^2 \pm 1$. Average value of TPC = $2.42 \times 10^2 \pm 1.5$.

The occurrence of bacterial contamination in skin-whitening creams is expected very often because bacteria can grow even at a neutral pH, which is the dominant pH range in most of the cosmetics. The occurrence various pathogenic bacteria in different cosmetic products has been observed [32]. In a study, TPC in 13 out of 15 skin creams was 0.24×10^3 to 0.56×10^3 CFU/g [33]. In this study, TPC was less in SLC samples as compared to the standard value hence there is a low risk of bacterial growth and resulting skin diseases. However, the possible skin diseases with the prolonged use of skin-lightening creams containing high bacterial contamination include erythema, edema, inflammation, sensitization, photosensitization and itching [14]. Microbial presence in skin beauty creams could cause deterioration and wastage of these creams, posing a serious risk to the health of consumers [34].

Mercury concentration found in the current study was compared with various studies conducted in different countries. The concentration of mercury in skin-lightening creams of different countries is described in Table 7. It can be seen that the concentration of mercury was less in the skin-lightening creams that originated from Armenia, Nepal and India compared to Pakistan. In contrast, the mercury level was higher in the skin-lightening creams produced in the Philippines, Mexico, China, Caribbean, Cambodia, Norway, Denmark, Austria, USA, Bangladesh and Saudi Arabia compared to Pakistan. Overall, the mercury level in the skin-lightening creams was above the permissible limit in most of the countries compared here. The maximum concentration of mercury was observed in the skin-lightening creams that originated from Mexico.

Table 7. Comparison of the mercury levels of the skin-lightening creams in Pakistan with various countries.

Sr. No.	Source of Product	Concentration of Mercury (ppm)
1.	Philippines	62,200 [35]
2.	Mexico	210,000 [36]
3.	China	42,875 [37]
4.	Caribbean	490.75 [38]
5.	Cambodia	12,590 [39]
6.	Norway	24,000 [35]
7.	Denmark	40,000 [35]
8.	Austria	38,800 [35]
9.	USA	41,600 [35]
10.	Armenia	0.08860 [35]
11.	Bangladesh	4643 [35]
12.	Saudi Arabia	5650 [35]
13.	Nepal	0.52 [35]
14.	India	0.009 [35]
15.	Pakistan (This study)	7.7

4. Conclusions

The current study confirmed that that concentration of hydroquinone and mercury in the samples investigated were more than the permissible limit defined by the US FDA and WHO. The total plate count (TPC) analyzed in the present study was found to be below the standard limit set. Various skin-lightening creams did not mention hydroquinone and mercury on their ingredients list but were found to contain high levels of these two. The high concentrations of mercury and hydroquinone in skin-lightening creams may lead to serious health impacts, such as erythema, edema, inflammation, sensitization, photosensitization and itching. Although the TPC in all cream samples was lower as compared to the permissible limits, repetitive application of hydroquinone/mercury-containing skin-whitening creams should be avoided. Moreover, in future research work, analyses of both pathogens and molds/yeasts should be done in skin-lightening creams for better understanding of the effects caused by their use. The labeling of the mercury and hydroquinone concentration/content in skin-lightening creams by production brands as well as strict monitoring and control by the Pakistani government are required to minimize/avoid their presence in skincare products. This will also reduce the health risk of mercury toxicity to people using skin-lightening creams.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/su13168786/s1, Figure S1: Photographs of the skin-whitening creams used in the current study.

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