

RESEARCH ARTICLE

EVALUATION OF THE LEVELS OF HYDROQUINONE AND HEALTH RISK ASSESSMENT OF TOXIC METALS IN SKIN-WHITENING CREAMS

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Abstract

This study assessed the level of toxic metals and hydroquinone in skin-lightening-creams commonly use in Ogun State, Nigeria. The pH of all the cream samples was determined and metals quantification was carried out using ICP-OES after acid digestion while that of hydroquinone was done using HPLC. The health risk of dermal exposure to heavy metals via the regular application was done by evaluating the carcinogenic and non-carcinogenic risk. The pH of all the samples are acidic and it follows this trend; Sample 3 > Sample 1 > Sample 4 > Sample 2. All the metals considered in this study were detected in all the analyzed samples except Cd, Co and Cr in sample 1, As, Ba and Pb in sample 2, Ag, As and Hg in sample 3 and Ba in sample 4. Highest levels of Al, Co, Pb and Zn were recorded in Sample 3 (Organic cream). Though the concentrations of most of the toxic metals (Al, Zn and Mn) are above the permissible levels, they pose no non-carcinogenic health risk based on the calculated HQ and HI. The carcinogenic risk of metals in the sampled creams were all within the acceptable limit except that of Cd. Hydroquinone was detected in all the four samples analyzed ranging between 2.58 and 4.17 % which exceeded the permissible limit by WHO. This finding show that long-term use of skin-whitening-creams may pose serious health hazards so, public should be continually enlightening on the hazardous health effects of the use.

Keywords: Skin-whitening, Hydroquinone, Toxic metals, Health risk, Carcinogenic risk.

1. Introduction

Skin-whitening has been a typical cosmetics practice for decades among women and men all over the world, particularly in Africa and Asia [1-3]. Despite the debilitating consequences of skin bleaching, many African women still indulge in its abuse as they believe light-skinned women are more attractive, beautiful and effectively achieve their goals in life than dark-skinned ones [4]. Alghamdi [5] postulates that people associate bleaching to higher social status and economic mobility.

Many skin-lightening-creams contain harmful substances such as hydroquinone, Kojic acid, and metals (Al, Cu, Fe, Mn, Ni, Ag, As, Ba, Cd, Co, Cr, Pb, Hg, Zn, Th, V). Despite the fact that European Union and United States have banned the inclusion of these substances in lotions [6,7], many manufacturers still add them as part of the constituents of creams because of their effectiveness in skin-whitening [2,6]. These substances are highly hazardous to human skins: they infringe diseases and cause severe damages to the human skin, particularly, if they stay longer [8].

Long term use of cosmetics may lead to accumulation of toxic elements in the soft tissues causing deleterious diseases in human. The most hazardous heavy metals to human health are As, Cd, Pb, Hg and Sb [9]. Increasing levels of Pb and Cd in the human body can lead to skeletal muscle damage, cancer cell activation, reproductive deficiencies, brain impairment and kidney impairment [10]. Hg affects the brain and central nervous system [11], can cause cardiovascular and neurologic disorders and hepatotoxicity in human [12,13].

The presence of toxic metals has been confirmed in cosmetic products across the globe and their main sources have been linked to raw materials, additives and mineral pigments added as colorants [14-16]. UkoNaku et al., [17] reported the presence of Cr, Mn, Ni and Pb in most common cosmetics in Nigeria. Wang *et al.*, [18] detected the presence of As, Cd, Co, Cr, Cu, Ni, Pb, and Zn in face paints from China with Cr having the highest carcinogenic risk. Some metals are usually added to cosmetics as parabens and preservatives as they are purported to possess antibacterial and antifungal properties but, these have been reported to be endocrine

disruptors [16]. Arsenic induces hematological disturbance, dermal disruption, gastrointestinal disorder, respiratory changes, neurological abnormalities, cardiovascular weakness, genotoxic effects and reproductive abnormalities [19].

Hydroquinone is considered an essential ingredient in bleaching cream for restraining melanin production and has been found to be hepatotoxic and carcinogenic agent [20]. Hydroquinone when present in the body replaces tyrosine and metabolizes into quinones and free radicals which can attack melanocyte membranes exerting a cytotoxic effect [21]. Hydroquinone has been found to cause diseases such as thyroid disorder, leukemia, and liver damage [22]. The health risk assessment study of toxic metals in skin-whitening creams have been evaluated in terms of carcinogenic and non-carcinogenic risk. The non-carcinogenic risk of the metallic constituents were determined by calculating Hazard Quotient (HQ) and Hazard Index (HI) while that of carcinogenic risk was done by calculating the carcinogenic or cancer risk (CR) [17,18,23].

The high incidences and popularity of the use of skin lightening or bleaching creams and the potential consequences necessitated this study. Though skin care products are regulated for health and safety, there are concerns regarding the presence and effect of harmful chemicals, especially heavy metals, in these products. Also, the fact that the levels of these harmful contaminants are not usually listed on the cream label by their manufacturers and in order to ensure the safety of the users, it is pertinent to regularly monitor their levels and evaluate their health risk. This study evaluates the levels of hydroquinone and assesses the health risk of toxic metals in whitening creams commonly used in Ogun State, Nigeria.

2. Materials and methods

2.1. Sample collection

This study adopts purposive sample design and sample selection was done based on their high demand and preference. Selected samples were randomly purchased from stores in Ago-iwoye market, Ogun State. Table 1

contains the description of the collected samples in terms of brand name, manufacturing date and ingredients is presented in Table 1. Collected samples were later transported to the laboratory for proper analysis.

2.2. Determination of pH

The pH of the collected samples were measured using pH meter IONIX PC50 Multimeter. The pH electrode was thoroughly washed with distilled water to prevent contamination and the electrode was carefully cleaned with laboratory cleaning tissue to remove excess water. The meter was set at pH measurement mode before use and was calibrated with buffer solution of 4 and 9. The electrode was later dipped into the sample and the result was recorded.

2.3. Extraction and Analysis of Hydroquinone

Analytical grade Hydroquinone standard (purity, 98% Sigma-Aldrich, Germany) and HPLC grade reagents (methanol, acetonitrile and Orthophosphoric acid) were used for this study. Sample preparation was done using the method of Arshad *et al.*, [24] and quantification of Hydroquinone in the prepared samples was carried out using AGILENT HPLC 1100 series. 25.5 mg of Hydroquinone standard (equivalent to 25mg Hydroquinone) was dissolved with 20mL of diluents (Methanol: Water 1:1) in a 25 mL volumetric flask. The solution was made to mark with the diluents to get a stock solution of 1mg/mL (1000ppm) HYQ which was used for instrument calibration. The calibration curve is presented in Figure 1 with R² value of 0.999.

50 ml of diluents was added to 0.5 g of each sample in a 100 ml volumetric flask. The flask was placed in ultrasonic bath to sonicate for 10 mins at 60 °C. After cooling in an ice bath and made up to 100 ml mark with diluents, the solution was filtered using a Whatman filter paper and the filtrate was transferred into HPLC instrument for Hydroquinone quantification.

Table 1: Description of the collected samples of skin lightening creams

Sample No.	Brand Name	Manufactured By	Ingredients
Sample 1	Caro white	Dream Cosmetics, Cote d'Ivoire.	Petrolatum, mineral oil, stearic acid, cetearyl alcohol, isopropyl myristate, glycerin, hydroquinone (Max 2%), Fragrance, methyl and propyl paraben, carrot oil, Vitamin E, sodium metabisulfite.
Sample 2	Paw paw	Dream Cosmetics, Cote d'Ivoire.	Water, Stearic acid, petroleum jelly, white oil, glycerin, propylene glycol, kojic acid, AHA, Sodium metabisulfite, citric acid, fragrance, papaya extract.
Sample 3	Honey e	Local (organic)	N/A
Sample 4	White essence	Blueworld cosmetics Ltd. Cote d'Ivoire.	Aqua, Petroleum jelly, silicon oil, carrot oil, jojoba oil, arbutin, Shea butter, IPM oil, Vitamin C&E, polyvalent K, fragrance.

2.4. Instrumentation and Operating Conditions

The concentration of hydroquinone in the extracted samples was determined using AGILENT HPLC 1100 series with a reversed-phase column. The mobile phase A consists of a mixture of 0.01M Phosphoric Acid in water (92% v/v) and the mobile phase B is HPLC grade Acetonitrile (8% v/v). The stationary phase is Waters Xbridge C18 (150 × 4.6 mm, 5 μm). The column temperature was set at 40°C with a flow rate of 1.0 mL/min, a wavelength of 295 nm and injection volume of 10 μL.

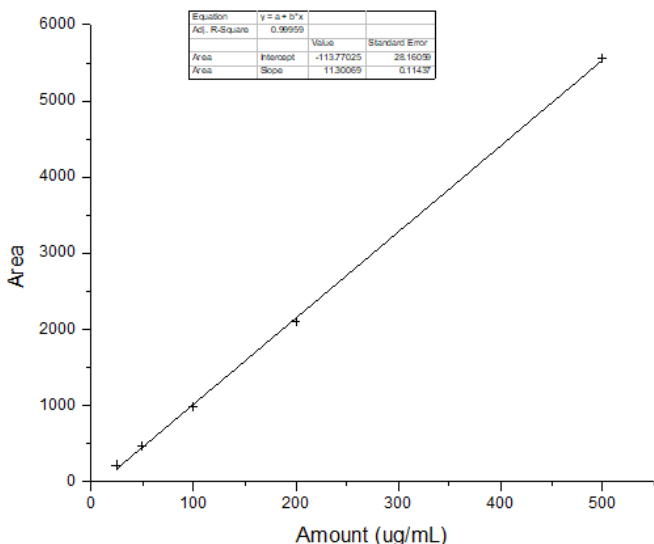


Fig.1: Calibration curve of hydroquinone standard

2.5. Recovery Study

Recovery study was used for result validation. This was performed by adding standard hydroquinone solutions to each of the cream samples and was taken through similar extraction procedures as the real cream samples. The percentage recovery for hydroquinone using HPLC was found to be 99.7 % which is within the acceptable range of 90-110%.

2.6. Sample Digestion and Heavy Metals Analysis

25 mL of nitric acid (10%) was added to accurately weighed 1 g ± 0.05 g cream sample in a 100 mL Pyrex flask and was heated on a heating mantle at 120 °C in a fume cupboard until a clear solution is formed. After cooling, 20 ml of distilled water was added and filtered through Whatman 42 filter paper and made to mark in a 50 ml volumetric flask [24].

Concentration of metals in each of the digest was determined using Agilent 720 ICP-OES with megapixel CCD detector (Agilent Technologies, USA). Standard solutions used for instrument calibration were prepared from Accustandard QCSTD-27 multi-element solution.

2.7. Risk Assessment study

The health risk of dermal exposure to heavy metals via the regular application of skin whitening cream was

carried out by evaluating the carcinogenic and non-carcinogenic risk. The non carcinogenic risk was estimated by calculating Hazard Quotient (HQ) and Hazard Index (HI) while carcinogenic health risk was done by calculating CR. HQ was calculated using equation 1.

$$HQ = \frac{CS \times SA \times AF \times ABS \times EF \times ED \times CF}{AT \times BW \times RfD} \quad \text{Equation 1}$$

CS indicates metal concentration in the sample (mg/kg), SA: exposed skin area (0.53 m²/day), AF: adherence factor (0.07 mg/cm²), ABS: dermal absorption fraction 0.001 (all metals); EF: exposure frequency (730 days/year), ED: exposure duration (30 years), CF: unit conversion factor (10⁻⁶ mg/kg), AT: averaging time for non-carcinogens (365 days/year ED); BW: body weight (70 kg for adult); and *RfD_{derm}*, is the absorbed reference dose (for dermal exposure, mg/ (kg·day) [25,26,27]. *RfD_{derm}*, is given as (10, 3, 3, 300, 20, 20, 3, and 360 μg/kg for Cd, Pb, Cr, Zn, Cu, Ni, Cr, and Fe, HQ <1 means no potential health risks are expected from exposure, while HQ > 1 means there are potential health risks [26,27]. HI was calculated to estimate the overall additive non-carcinogenic risk of dermal exposure to mixture of toxic metals in skin whitening cream. This is the sum of the hazardous quotient (HQ) of all heavy metals as described in equation 2.

$$HI = \sum [HQAs + HQCd + HQCo + HQCr + HQCu + HQFe + HQMn + HQNi + HQPb + HQZn] \quad \text{Equation 2}$$

HI > 1, means there is possibility of adverse effects of additive non-carcinogenic risk of dermal exposure on human health.

The carcinogenic or cancer risk (CR) is the incremental probability of developing cancer over a lifetime as a result of exposure to a given daily amount of a carcinogenic element [27]. CR is calculated using equation 3.

$$CR = \frac{CS \times SA \times AF \times ABS \times EF \times ED \times CF}{AT \times BW} \times CSF \quad \text{Equation 3}$$

CR is the incremental probability of a person developing cancer over a lifetime as a result of twenty-four hours per day exposure to a given daily amount of a carcinogenic element for seventy years [26]. The body weight (BW) of adult group is 70 kg and CSF is the cancer slope factor for Pb is 0.0085, for Cr is 0.5, for Ni is 0.91 and for Cd is 6.7. For a carcinogenic element, the allowable or tolerable limits are 0.0001 to 0.000001 [27].

3. Result and Discussion

3.1 pH

The pH of all the samples are acidic, it follows this trend; Sample 3 > Sample 1 > Sample 4 > Sample 2 (Table 2) and all falls below the permissible standard set by WHO

except sample 3, the organic locally produced cream. The pH of human skin is acidic and ranges from 4.5 to 6. So, application of acidic cream on skin may cause irritation and this can expose the skin to dermal diseases [28]. The pH recorded for creams in this study falls within the range reported by [29,30] Ekpunobi *et al.*, [29], and Oyedeji *et al.*, [30] but, higher than the results of Mulooji *et al.*, [28]. and Saleh *et al.*, [31].

Table 2: The pH of the skin-lightening creams

Sample No.	pH
Sample 1 (S ₁)	4.16 ± 0.02
Sample 2 (S ₂)	4.07 ± 0.01
Sample 3 (S ₃)	5.34 ± 0.02
Sample 4 (S ₄)	4.14 ± 0.01
WHO permissible limits	4.6 - 5.50

3.2 Result of metal analysis

All the metals considered in this study were detected in all the analyzed samples except Cd, Co and Cr in sample 1, As, Ba and Pb in sample 2, Ag, As and Hg in sample 3 and Ba in sample 4.

Highest levels of Al, Co, Pb and Zn were recorded in Sample 3 (Organic cream). The concentration of Mn in all the samples were above the permissible limits, same for the concentration of Al in all the samples except sample 2. The concentrations of Ag, As, Ba, Cd, Cr, Pb, Hg recorded in all the samples were below the permissible limit. Highest concentration of Ni was recorded in sample 2 and this is far above the permissible limit and the least in sample 3. Ni has been considered to cause allergic contact dermatitis [26].

Highest concentration of Cd, Co, Al and Zn were recorded in the local organic cream (sample 3), while Hg was below the detection limit of the instrument in sample 3. Hg was detected in all the imported samples (1, 2 and 4) though all are below the permissible limit. The possible

source may be from the formulation process because it is as an active bleaching agent.

Mercury is toxic to the body and has the potential to affect endothelial and cardiovascular system [32]. The concentration of Zn recorded in this study is significantly higher than the permissible level except for sample 1 with sample 3 having the highest concentration.

The major route of human exposure to Al is the skin [33], Al has no physiological role in metabolic processes in the body [34], but toxicity can occur when Al tolerant level in the body is exceeded [35]. The normal body burden of Al in healthy humans is approximately 30–50 mg/kg body weight and typical levels of Al in serum are approximately 1–3 µg/L [36]. Toxic actions of Al includes; oxidative stress, immunologic alterations, genotoxicity and pro-inflammatory effect. Al toxicosis has been associated with desquamative interstitial pneumonia, granulomatosis and fibrosis, toxic myocarditis, ischemic stroke, anemia, Alzheimer's disease, dementia, sclerosis, autism and infertility, breast cancer and cyst [33,37]. The level of Al in all the samples exceed the permissible limit and the level reported by [27] and their continuous use may lead to Al toxicosis.

The level of Pb recorded in this study for all the samples is below the permissible standard with sample 3 having the highest. This is lower than the level reported in cream samples (mg/kg) by Iwegbue *et al.*, [6] (≤ 4.5), Jose and Ray, [38] (≤ 23.3) and Arshad [16] (≤ 6.37) but higher than the level reported by Sani *et al.*, [2] (≤ 0.14). Lead poisoning can occur even at low concentration and may cause neurological damage and adversely affects the body systems [6]. Due to the health hazard of Pb in human, its inclusion in cosmetics have been banned by regulatory bodies, yet detectable levels are still found in some skin-whitening creams [28]. The action of melanogenesis a catalyst in melanin production is inhibited Hg, thus affect pigment formation in skin [32]. It should be noted that none of these metals (Hg, Pb, As), were listed in the labels of all the sampled creams.

Table 3.0: The results of metal analysis in skin whitening creams

ELEMENT	Concentration (mg/kg)				Permissible limit (WHO)
	Sample 1	Sample 2	Sample 3	Sample 4	
Ag	0.59	0.25	BDL	0.26	15.0
Al	101.5	2.62	191.7	93.43	10.0
As	0.11	BDL	BDL	0.59	10.0
Ba	0.16	BDL	0.06	BDL	2.0
Cd	BDL	0.33	0.78	0.09	3.0
Co	BDL	0.38	7.38	0.02	-
Cr	BDL	0.22	0.04	0.63	1.0
Cu	0.33	6.67	2.29	39.38	-
Fe	78.1	142.9	50.9	132.8	-

Mn	0.3	0.15	0.66	5.41	0.02
Ni	0.74	16.9	0.37	0.57	0.67
Pb	0.11	BDL	0.86	0.09	10.0
Hg	0.01	0.74	BDL	0.78	1.0
Zn	1 1.73	150.98	278.03	158.81	99.4

*BDL- Below Detection Limit

3.3. Hydroquinone

All the analyzed cream samples contain appreciable concentration of hydroquinone in the range of 2.58–4.17 % as presented in Figure 5. Sample 2 has the highest level of hydroquinone while Sample 4 has the lowest and it follows the trend of Sample 2 > Sample 1 > Sample 3 > Sample 4. The concentration of hydroquinone in all the samples were above the permissible level set by WHO which is 2%. The presence of hydroquinone was only indicated in the label of sample 1 but the level recorded in the sample in this study is greater than the level coated on the label. Drugs containing 2-4% hydroquinone are usually prescribed for the treatment of melasma, freckles, and senile lentigines but, the application is for short-term (Arshad *et al.*, 2021).

Hydroquinone inhibits DNA and RNA synthesis and alters melanosome formation [Briganti *et al.*, 2003]. This exposes the skin to direct ultra violet radiations from sunlight thereby increasing the risk of carcinogenesis [24,28]. Long term application of skin whitening cream containing high level of hydroquinone can lead to exogenous ochronosis [24]. The level of hydroquinone recorded in this study is more than the result reported by Ekpunobi *et al.*, [29] ($\leq 2.35\%$), similar to that of Oyedeji *et al.*, [30] ($\leq 5.035\%$) and Saleh *et al.*, [31] ($\leq 4.533\%$), but less than that of Arshad *et al.*, [24] ($\leq 7.14\%$).

3.4. Health Risk Assessment

The non-carcinogenic; Hazard Quotient (HQ) and Hazard Index (HI) and carcinogenic risks were evaluated to determine the health risk of long term application of the sampled whitening creams. The result of Hazard Quotient (HQ) and Hazard Index (HI) are presented in Table 4 while that of carcinogenic is presented in Table 7. The HQ and HI values for all the metals are less than 1 and below the risk limit, indicating that no non-carcinogenic risk can occur on long term use. This is similar to the result of Shomar and Rashkeev, [25], Arsdad *et al.*, [24], Mansouri *et al.*, [26] and Khalili *et al.*, [27]. The carcinogenic risk (CR) assessment of As, Cd, Cr, Ni and Pb for adults was calculated and the result is presented in Table 5. The CR of all the metals considered is below the acceptable range ($1.0E-06$ to $1.0E-04$), so poses no carcinogenic risk except the CR for Cd in samples 2, 3, and 4. The CR of Cd in samples 2, 3, and 4 are higher than the acceptable range, similar to the report of Lim *et al.*, [39] and Surajo *et al.*, [40] for cosmetic products. So, overtime use of the cream may cause cancer due to Cd content in the cream. The CR value recorded by Arshad *et al.*, [16] was higher than the results of this study in all cosmetic products except lipsticks.

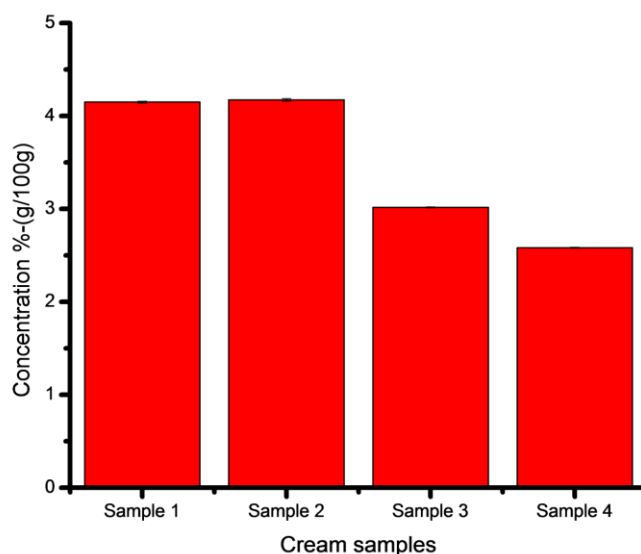


Fig. 2: Concentration of Hydroquinone in the sampled skin lightening creams

Table 4: Hazard Quotient (HQ) and Hazard Index (HI) of metals in the analyzed skin whitening cream

ELEMENT	1	2	3	4
Ag	2.22E-05	9.42E-06	BDL	9.79E-06
Al	0.002866	7.4E-05	0.005416	0.002639
As	4.14E-06	BDL	BDL	2.22E-05
Ba	1.29E-10	BDL	4.84E-11	BDL
Cd	BDL	0.000149	0.000353	4.07E-05
Co	BDL	1.43E-05	0.000278	7.53E-07
Cr	BDL	1.27E-07	2.32E-08	3.65E-07
Cu	9.32E-08	1.88E-06	6.47E-07	1.11E-05
Fe	1.26E-06	2.31E-06	8.22E-07	2.14E-06
Mn	2.42E-08	1.21E-08	5.33E-08	4.37E-07
Ni	4.18E-07	9.55E-06	2.09E-07	3.22E-07
Pb	3.11E-08	BDL	2.43E-07	2.54E-08
Hg	3.77E-07	2.79E-05	BDL	2.94E-05
V	BDL	7.3E-05	BDL	BDL
Zn	3.83E-06	5.69E-06	1.05E-05	5.98E-06
HI	0.0029	0.0004	0.0061	0.0028

Table 5: Carcinogenic Risk assessment of toxic metals in analyzed skin whitening cream

ELEMENT	As	Cd	Cr	Ni	Pb
1	6.22E-06	0.00	0.00	4E-07	2.64E-10
2	0.00	0.0010	6.37E-08	9E-06	0.00
3	0.00	0.0024	1.16E-08	2E-07	2.07E-09
4	3.33E-05	0.0003	1.83E-07	3E-07	2.16E-10

4. Conclusion

This study assessed the concentration and health risk of toxic metals and hydroquinone in skin whitening creams. The pH of most of the sampled creams is lower than the permissible limit which can cause skin irritation. Highest concentration of Cd, Co, Al and Zn were recorded in the locally made organic cream (sample 3) which can pose serious health issues. Though the concentrations of most of the toxic metals (Al, Zn and Mn) are above the permissible levels, they pose no non-carcinogenic health risk based on the calculated HQ and HI. The carcinogenic risk due to Cd content in most of the cream samples is higher than the acceptable limit; so long term use may lead to cancer. The concentration of hydroquinone in all the samples were above the permissible level as set by WHO which is 2%. Manufacturers of these beauty products should be strictly compel to include the amount of metallic contents and other toxic substances on the label of their products and also, public should be continually enlighten on the hazardous health effects of the use of skin-lightening-creams.

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تقييم مستويات الهيدروكينون وتقييم مخاطر للمعادن السامة في كريمات تبييض البشرة

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المُلخَص

حددت هذه الدراسة مستوى المعادن السامة والهيدروكينون في كريمات تفتيح البشرة التي يشيع استخدامها في ولاية أوجون بنيجيريا. تم تحديد الرقم الهيدروجيني لجميع عينات القشدة وتم إجراء تقدير كمية المعادن باستخدام ICP-OES بعد الهضم الحمضي بينما تم إجراء تقدير الهيدروكينون باستخدام جهاز HPLC. كما تم إجراء اختبار المخاطر الصحية للتعرض الجلدي للمعادن الثقيلة عبر الاستخدام المنتظم من خلال تقييم المخاطر المسببة للسرطان وغير السرطنة. كانت نتائج الدراسة كالتالي: الرقم الهيدروجيني لجميع العينات حمضي ويتبع هذا الاتجاه؛ العينة 3 < العينة 1 < العينة 4 < العينة 2. وتم اكتشاف جميع المعادن المذكورة في هذه الدراسة في جميع العينات التي تم تحليلها باستثناء الكاديوم والكروم في العينة 1، و As، و Ba و Pb في العينة 2، و Ag، و As، و Hg في العينة 3 و Ba في العينة 4. كما تم تسجيل أعلى مستويات من Al و Co و Pb و Zn في العينة 3 (كريم عضوية). وعلى الرغم من أن تراكيز معظم المعادن السامة (Al و Zn و Mn) أعلى من المستويات المسموح بها، إلا أنها لا تشكل أي مخاطر صحية غير مسرطنة بناءً على قيم HI و HQ. كانت مخاطر الإصابة بالسرطان للمعادن في الكريمات التي تم أخذ عينات منها ضمن الحد المقبول باستثناء مخاطر الكاديوم. تم الكشف عن الهيدروكينون في جميع العينات الأربعة التي تم تحليلها بنسبة تتراوح بين 2.58 و 4.17% والتي تجاوزت الحد المسموح به من قبل منظمة الصحة العالمية. تُظهر هذه النتيجة أن الاستخدام طويل الأمد لكريمات تبييض البشرة قد يشكل مخاطر صحية خطيرة، لذا يجب على الجمهور أن يطلع باستمرار على الآثار الصحية الخطرة للاستخدام.

الكلمات المفتاحية: تبييض البشرة، هيدروكينون، معادن سامة، مخاطر صحية، مخاطر مسرطنة.

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