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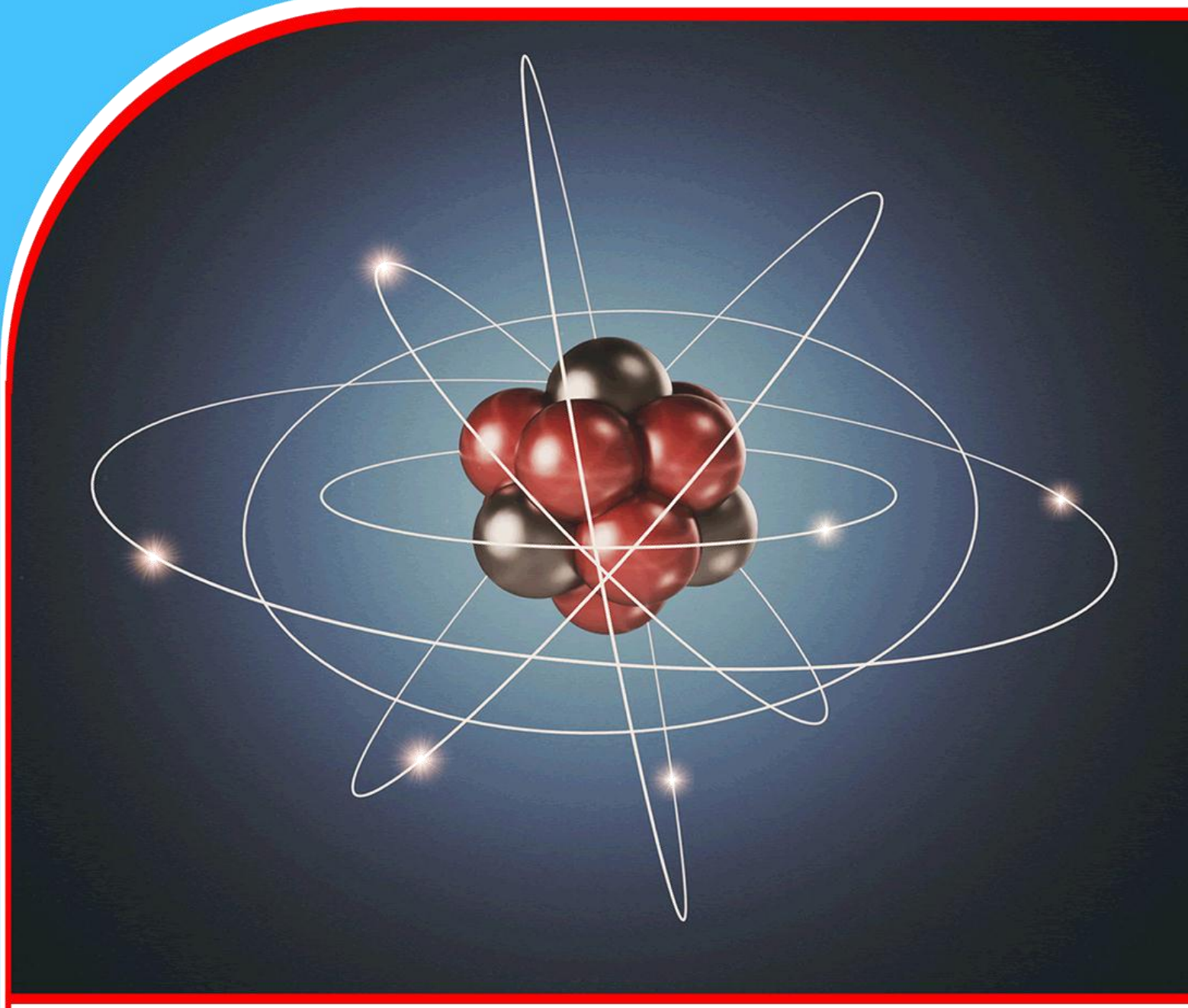

Levels of Hydroquinone, Selected Heavy Metals (Hg, Pb, As) and Chemical requirements in Some Skin Lightening Creams Sold in Mbarara Municipality.

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Levels of Hydroquinone, Selected Heavy Metals (Hg, Pb, As) and Chemical requirements in Some Skin Lightening Creams Sold in Mbarara Municipality.

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ABSTRACT

In this study, Skin-lightening creams commonly sold in Mbarara municipality were analyzed for chemical parameters (pH, thermal stability and fatty substance content), total hydroquinone, Lead, Mercury, and Arsenic contents. Total heavy metal content was determined by atomic absorption spectrophotometry. The levels of hydroquinone were determined using High Performance liquid chromatography (HPLC). All the creams showed detectable mean levels of mercury, ranging from 0.07±0.01ppm to 0.33±0.01ppm. Only 26.31% of the creams showed detectable levels of lead and 15.79% creams recorded detectable levels of arsenic. The mean levels of hydroquinone ranged from 0.54±0.02% to 4.47±0.02%. All the creams passed the thermal stability and fatty substance content tests. However, all the creams had very low pH values below the recommended 4.5- 8.5 pH ranges by Uganda National Bureau of Standards (UNBS). The levels of mercury, arsenic and lead in the samples were less than the UNBS, European Union and US Food and Drug Administration's acceptable limits. Only 84.2% of the cream samples analyzed contained hydroquinone levels higher than the recommended WHO limit of 2%. The use of such creams may lead to serious health hazards. While the low concentrations of heavy metals detected in the cream samples analyzed do not pose any potential risk to consumers, repeated application of these creams may cause a cumulative effect over prolonged exposure. The low pH values may cause skin irritations. Therefore, the community needs to be sensitized on the implications of using skin lightening creams and UNBS should conduct periodic analysis to ascertain the levels of hydroquinone, heavy metals, and chemical requirements of skin lightening creams sold in Uganda as well as encourage manufacturers to state the exact bleaching agents in their creams.

Key words: *Heavy metals, Chemical requirements, Hydroquinone, Atomic Absorption Spectrophotometry, High performance liquid chromatography.*

1.0 INTRODUCTION

In our societies, there seems to be a serious pressure on some men and women to fit into dominant beauty stereotypes. We are living in a society based on a fallacious belief that a fairer and lighter skin is associated with beauty and wealth. A dark skin is regarded as a marker of toiling in the sun whereas lighter skin signifies affluence [1]. In Uganda, one can't ignore the sight of some light-skinned women with dark feet, and elbows on the streets of urban towns. These are simply a representative of a section of population that has chosen to indulge in skin lightening using different skin lightening products. The different bleaching products that they apply contain a number of different bleaching agents which may be harmful and therefore exert significant effects on their health. Examples of chemicals in these products may include steroids, hydroquinone, Kojic acid, dipalmitate, Azleic acid, Arbutin, Bearberry, Vitamin C, Magnesium ascorbyl phosphate, Calcium ascorbate, L-ascorbic acid, mercury, lead and arsenic [2].

Previously, some of these chemicals like the heavy metals were used as ingredients of cosmetics. For example, thimerosal (mercury) and lead acetate were in the past added as preservatives in progressive hair dye while red cinnabar (mercuric sulfide) was used in a number of tattoo pigments [3]. This ultimately increased the levels of the constituent heavy metals in the cosmetics and pigments to which they were added [3]. The presence of these metals in cosmetics beyond permissible limits has since been banned in many countries[4,5]. The Uganda National Bureau of standards has tried to crack down on lightening cosmetics, but this effort has not been successful. The trend is actually going up as homemade cosmetic and dermatological products have become easily accessible.

The production of these skin-bleaching products is also on the rise and in high demand across the world [6]. Noticeable illegal application of these cosmetic products in many developing countries despite the health hazards associated with their use, has been reported [7]. There are also reports on the possible deliberate addition of these toxic chemicals, such as mercury, arsenic and lead containing compounds in the different cosmetics for skin whitening purposes, although some reports indicate that their presence is considered unavoidable as product impurities of manufacturing processes [8,9].

Unfortunately, acute and chronic exposure to these skin lightening products could result into a multiplicity of side effects in humans [10,11]. Chronic use of mercury-containing skin bleaching products has been reported to result in the accumulation of mercury in the body after absorption through the skin; especially in the kidney where it mainly accumulates in the tubular region, giving rise to the occurrence of severe reactions [12]. Long term chronic exposure to arsenic can lead to multisystem failure and various cancers [13] while the chronic exposure to lead is reported to have effects on brain, leading to convulsions and sometimes coma [14]. An enormous number of articles have since 1996, been published on the effects including carcinogenicity and leukomelanoderma of hydroquinone, benzene and related molecules [10,11,15,16]. These underlying effects of hydroquinone necessitated that its use in cosmetics be banned in the Netherlands since January 2001 [16] even though it had for decades been used as a skin lightening agent.

Notably, despite the various reported health side effects of these heavy metals and hydroquinone, the data regarding their levels in skin lightening creams sold on the Ugandan market is lacking, even though it is obvious that several of such products are seemingly

circulating in the market. More still, the information regarding the chemical characteristics of these creams is insufficient. This may indicate a deficit in the processes of formulation characterization. Stability testing of the cosmetic products ensures that the product meets the intended physical, chemical and microbiological quality standards as well as functionality and aesthetics when stored under appropriate conditions.

Uganda National Bureau of Standards recommends a maximum level of 2% hydroquinone content, 2.0 ppm of mercury and arsenic, and 20 ppm of lead. The United States Food and Drug Administration (US FDA) allows a maximum of two percent of hydroquinone and 1µg/g of mercury, a maximum level of 10 ppm for lead, and not more than 3 ppm of arsenic in skin care products. The Kenya Bureau of Standards banned some hydroquinone containing skin lightening creams because of fears of cancer risk [17]. Uganda National Bureau Standards recommends that all creams, lotions and gels for skin care should comply with the following chemical requirements: all creams must pass the thermal stability test, lie in a pH range of 3.5-8.5 and also possess a total fatty substance content of 5% by mass [5]. However, the results from periodic evaluation of these chemical requirements have not been reported anywhere. Therefore, there was a need to verify the quality and efficacy of these creams by subjecting them to tests for each of the specified chemical requirements.

This study was therefore intended to assess the thermal stability, pH, fatty substance content, total hydroquinone, and inorganic mercury, lead and arsenic contents in the commonly sold skin lightening creams in Mbarara municipality.

2.0 MATERIALS AND METHODS

2.1 Materials

All reagents used were of analytical grade. These included Nitric acid (65 % v/v, Sigma-Aldrich, Germany), Hydrochloric acid (30 % v/v, Sigma-Aldrich, Germany), nitric acid (65 % v/v, Sigma-Aldrich, Germany), potassium permanganate solution (95 % w/v, Merck, Germany), petroleum B.P, mercury(II) chloride, methyl orange indicator solution, deionised water, methanol (99.80% v/v, Shanghai Bojing chemical, China), sodium sulphate (99% v/v, Shanghai Bojing chemical, China), nitric acid-perchloric acid solution (1+1)-made by mixing 100 mL of perchloric acid (70 % v/v, Merck, Germany) into 100 mL of nitric acid, tin(II) chloride solution (10% v/v, Shanghai Bojing chemical, China), nitrogen gas (100 mL min^{1}) to expel any mercury from the solution, 5N sodium hydroxide solution (99.8 % v/v, Merck, Germany).

2.2 Sample Collection

A purposive sampling technique was employed. The main retail shops dealing in skin lightening creams around Mbarara municipality were selected. Questionnaire was designed and this was derived from UNBS' list of 50 prohibited skin lightening creams in Uganda. The questionnaire was hand delivered to the respondents after securing their consent. Using the results from the questionnaires, the most commonly purchased skin lightening creams were identified.

The most commonly sold creams were purchased and kept in dry cool cupboard but within their respective containers prior to analysis.

2.3 Instrumentation

Atomic absorption spectrophotometer (AAS) shimadzu 6300 model was used for quantitative determination of mercury and arsenic (Burner atomizer: Changeable laminar, 10 cm; optional 5cm N_2O; Autosampler with autodiluter and sample pretreatment for flame and graphite furnace; hybrid-vapour generator; Format: 1 channel, double beam, rotating beam combiner; Monochromator; Wavelength range: 185-900 nm; Resolution: 0.2-2.0 nm). Atomic absorption spectrophotometer (AAS) Agilent 280 FS 280Z model with specifications (Optics: True double optical design: Graphite Tube Atomizer : Monochromator; eight lamps; deuterium background corrector covering wavelength range 185–425 nm and correcting up to 2.5 background absorbance and 2 ms response; Automatic gas control gas flow within 30 ms for rapid regulation and stabilization of selected gas flow; a Typical performance (240/240FS/280FS AA) of >0.9 and Absorbance with precision of < 0.5% RSD) was used for quantitative determination of lead. High Performance liquid chromatograph with specifications (Oven model: CT020AC, Detector model: SPD- M20A, Communication gas model: CBM-20A, Degasser: DGU-20A5R, Pump: LC-20ABXR) was used for quantitative determination of hydroquinone; pH meter (Mettler Toledo type model S220) equipped with a glass electrode (reference no. 51340331) was used for pH measurements; thermostatically controlled Memmert oven (model number: DIN- 2880) was used for assessing the thermal stability. Ohaus weighing balance (EX 124) with a readability of 0.0001mg was used for the measurements; Hot plate - Capable of attaining a surface temperature of 250°C, 50-ml thick-walled volumetric flask made of Pyrex (150 mm total height, 13 mm inlet diameter), Volumetric flasks: 10, 100, and 1,000 mL, Measuring pipettes: 0.2, 0.5, 1.5, and 10 mL.

2.4 Sample preparation and digestion

2.4.1 Wet digestion for mercury analysis

Skin lightening creams were wet digested with a 4:1 mixture of nitric acid (65%) and perchloric acid (70%) on a hot plate in fuming hood near to dryness according to the method by Ayenimon, *et al.*,[18] by slowly increasing the temperature for 3 hours. The procedure was repeated through addition of the mixture of acid by slow and continuous heating until the evolution of white fumes (marking the end of the digestion process) and near to dryness [19]. The solutions were allowed to cool, filtered into a calibrated flask (100 mL), and diluted up to the mark with distilled water.

2.4.2 Sample preparation and Digestion for Lead and arsenic analysis

To a known weight of cream (between 0.205 – 3.03 g) in a porcelain crucible was added concentrated nitric acid (7 mL) followed by distilled water (2.0 mL). The resultant mixture was placed into a 50 mL decomposition pressure tube. The pressure vessel was closed and a pressure between 15 Nm^2 - 20 Nm^2 applied. The digestion took 2 hours and then the resultant was transferred to a 20 mL volumetric flask and made to the mark with distilled water [4].

2.4.3 Sample preparation and digestion for hydroquinone analysis

Each cream (1.2 g) was weighed accurately into a beaker followed by the mobile phase (25 mL) and then mixed until homogenous. The resultant solution was transferred into a 50 mL volumetric flask. The solution was vortexed for 2 minutes. The flask was placed in a water bath maintained at 60^{o}C for 15 minutes, and then cooled to room temperature. The mobile phase was added to the volume and mixed. The clear solution was filtered through a 0.45µm

membrane filter (roll filter paper manufacturer for lab analytical—model type: disc filter) into the flask and then diluted to the mark using a mobile phase [4].

2.5 Methods

2.5.1 Determination of hydroquinone levels

For each cream (1.2 g) was weighed accurately using an Ohaus weighing balance into a beaker followed by 25 mL of a mobile phase and then mixed until homogenous. The resultant solution was transferred into a 50 mL volumetric flask. The solution was vortexed for 2 minutes. The flask was placed in a water bath maintained at 60^{o}C for 15 minutes, and then cooled to room temperature. The mobile phase was added to the volume and mixed. The clear solution was filtered through a 0.45 µm membrane filter (roll filter paper manufacturer for lab analytical—model type: disc filter) and HPLC was conducted within about 24 hours [4].

Determination of the concentration of hydroquinone was carried out by a chromatographic method using a high-Performance liquid chromatograph (HPLC). Standard solutions of hydroquinone with concentrations of 0, 2, 4, 6, 8, and 10 ppm, were injected to obtain a calibration curve. The sample (20 µL) was injected into the sample loop and the flow rate of the mobile phase adjusted to 1.0 mL min^{-1} and the detector set to a wavelength of 295 nm. The resulting chromatogram was a plot of absorbance as a function of elution time. The concentration of hydroquinone in the sample was obtained by the expression;

$$\frac{Area\ of\ sample}{Area\ of\ standard\ \times Concentration\ of\ standard}$$ [4].

2.5.2 Determination of mercury levels

Determination of the level of mercury in all the digests was carried out by flameless atomic absorption spectrophotometry. In this method, the sample solution (5 mL) was introduced into the reaction vessel using a micropipette, stoppered tightly and tin (II) chloride (0.5 mL of 10% (w/v)) in HCl (2.0 mL of 1 M) added from a dispenser for the reduction reaction. During this time, air circulated through the four–way stop-cock to allow the mercury vapour to come to equilibrium and the acidic gases produced by the reaction also swept into the sodium hydroxide solution. After 30 seconds the four-way stop-cock automatically rotated through 90 degrees and the mercury vapour was swept into the absorption cell and the absorption measured at 253.7 nm. The aeration was then discontinued after the recorder pen had settled back to within a few chart divisions of the original baseline. Transient atomic absorption peak was obtained [4].

2.5.3 Determination of lead, and arsenic

The standard solutions for lead and arsenic were aspirated into the Air-acetylene flame in ascending order of concentration 0, 2, 4, 6, 8, and 10 ppm. The absorbance reading was taken for each concentration using AAS. All the measurements were run in triplicate for the samples and standard solutions. Deuterium lamp was used for background correction. A calibration curve of concentration of lead and arsenic in standard solution against the corresponding values of absorbance were obtained.

The sample solutions were aspirated into the flame and the absorbance was read from the AAS. The value was noted and used to read the corresponding value of concentration from the curve [4].

2.5.4 Assessment of the thermal stability of creams

Each cream (50 g) was scooped and placed effectively in a sealed glass tube or vial. The tube was then placed in a thermostatically controlled oven at 37^{o}C for 48 hours, and made sure that the sample was securely sealed [4].

2.5.5 Determination of the pH of the creams

To determine whether the cream was oil- in- water emulsion or water- in-oil emulsion, each of the creams (0.523 g) was placed on spot tile and sprinkled with a mixed indicator of oil orange and methylene blue. After 15 minutes, the predominant colour indicated whether the continuous phase was oil or water.

The pH meter was dipped in pH 7.0 disodium hydrogen phosphate buffer solution (50 mL). The meter was then rinsed with deionised water before being dipped into a pH 4.0 potassium hydrogen phthalate buffer solution (50 mL). The procedure was repeated using a pH of 9.0 disodium tetra borate buffer solution.

For oil- in- water emulsion: The cream (5.0 g) was weighed into a 100 mL beaker. 45 mL of water were added and the cream dispersed in it. The pH of the suspension at 25^{o}C was determined using a pH meter.

For water-in- oil emulsion: The cream (10.0 g) was weighed. Rectified spirit (90 mL) that was previously adjusted from pH 6.5 to 7.0 was added. The mixture was warmed to 45 ^{o}C and stirred thoroughly for 15 minutes. The alcoholic layer was filtered through a filter paper and pH measured at 25^{o}C using a pH meter [4].

2.5.6 Determination of the total Fatty substance content of the creams

For each cream, 2.0 g were accurately weighed into a conical flask. Dilute hydrochloric acid (1 M; 25 mL) was added, a reflux condenser fitted into the flask and the contents boiled until the solution was perfectly clear. The contents of the flask were poured into a 300 mL separation funnel and allowed to cool to 20^{o}C. The conical flask was rinsed with petroleum ether (50 mL) in portions of 10 mL. After each rinsing, the contents were poured into the separation funnel and shaken to leave the layers separate. The aqueous layer was separated and shaken with 50 mL of ether twice. All the ether extracts were combined and washed with water until they were free of the acid. Ether extracts were filtered through a filter paper containing sodium sulphate into a conical flask which had been previously dried at temperature of 60^{o}C and then weighed. The sodium sulphate on the filter was washed with ether and the washings combined with the filtrate. Ether and the dry material remaining in the flask were then distilled off at a temperature of 60^{o}C to a constant mass [4].

3.0 RESULTS AND DISCUSSION

A total of 19 different skin lightening creams were identified and considered in this study. The study population comprised of 15 respondents, who were shop attendants. The social demographics of the study population is as indicated in table 1 below.

Table 1: Social Demographic Characteristics (n = 15)

Variable		Number	Percentage
Age	Below 20 years	1	6.67
	21-30	2	13.3
	31- 40	7	46.67
	above 40 years	5	33.33
Gender	Male	2	13.33
	Female	13	86.67
Marital status	Single	7	46.67
	Married	8	53.33
Educational qualification	High school	5	33.33
	Degree/Diploma/Certificate	7	46.67
	PG	0	0
	None	3	20

3.1 Hydroquinone levels in skin lightening creams

The mean levels of hydroquinone in the creams ranged from 0.4±0.02% to 4.47±0.02%. The results are summarized in the fig.1 below.

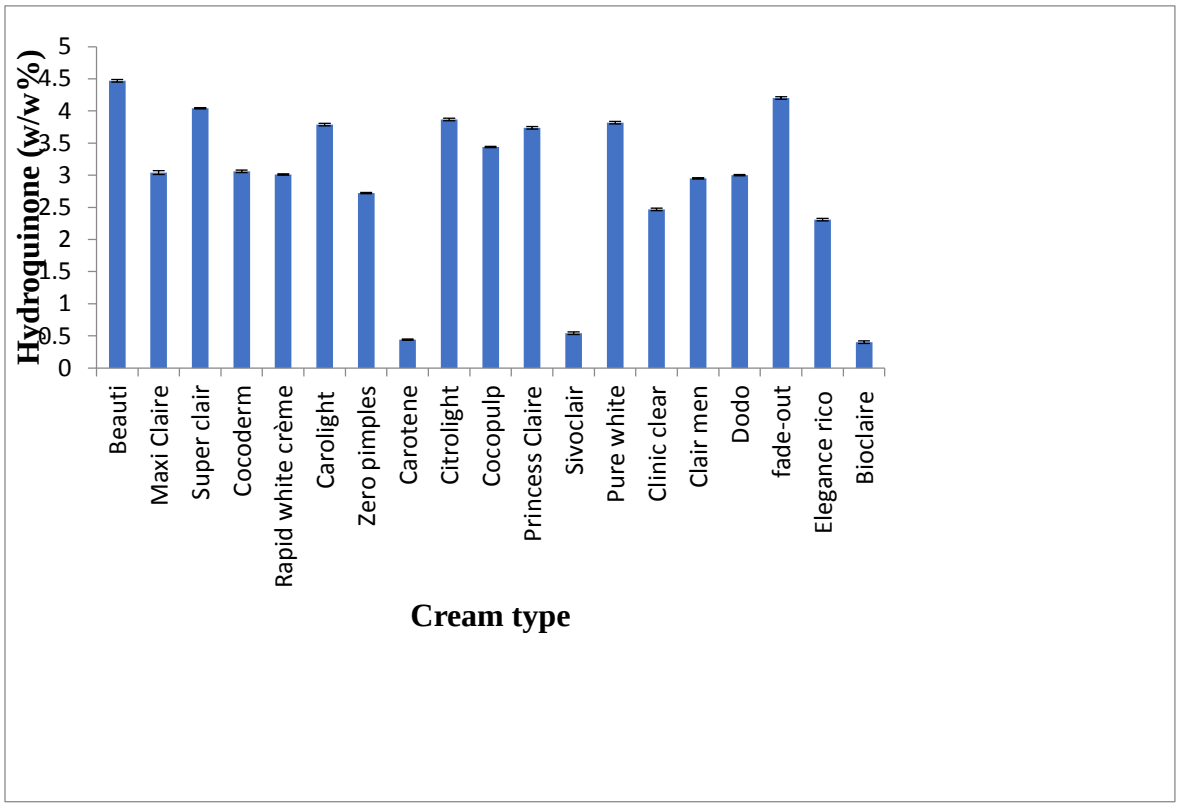

Fig. 1: **Variation of hydroquinone amount in different cream types.**

From Fig.1, the results of this study showed that all the 19 samples analyzed contain hydroquinone. Beauti recorded the highest mean level of hydroquinone of 4.47±0.02% while Bioclaire recorded the lowest mean level of hydroquinone of 0.54±0.02%. Only 15.8% of the analysed creams recorded levels below 2% hydroquinone which is the threshold limit recommended by UNBS and US FDA. These creams were Bioclaire, Sivoclair and Carotene. The rest of the creams analysed contained more than 2% hydroquinone which is above the threshold limit.

The results obtained compared well with a previous study conducted by Odumoso and Ekwe [20]. In their study on the creams obtained from the open market in Plateau state, Nigeria, all the ten sampled cosmetic creams that were subjected to chromatographic test for identification and quantification of hydroquinone, gave positive results. Seven creams had hydroquinone levels below two percent (2%), two creams had levels between 2 to 5% and only one had the levels above 5% [20].

High levels of hydroquinone pose a potential hydroquinone related health risk to the public. Carcinogenesis, hyper pigmentation with associated yellow deposits in the dermis (exogenous ochronosis) and vitiligo, have been reported as a possible long-term effect of continuous application of creams containing hydroquinone [21]. Hydroquinone exerts its lightening effect on the skin through melanocytotoxicity and inhibition of melanogenesis. As a result of its inhibition to tyrosinase activity, hydroquinone has a toxicity that is specific for melanocytes [22].

It inhibits DNA and RNA synthesis and alters melanosome formation [22, 23]. Hydroquinone competes with tyrosine as a substrate for tyrosinase, especially in the presence of L-dopa [24]. The inhibition of melanogenesis, exposes the skin to direct ultra violet radiations which increases the risk of carcinogenesis.

Therefore, with the risks associated with long-term and continuous topical application of hydroquinone containing creams, it is not worthy ignoring it. At least 84 % of the creams analysed in this study, pose a likelihood of serious health threat to the population applying them.

3.2 Heavy metal content in skin lightening creams

The levels of mercury, arsenic and lead in the different skin lightening creams were analysed. The results are summarized in fig. 2 below.

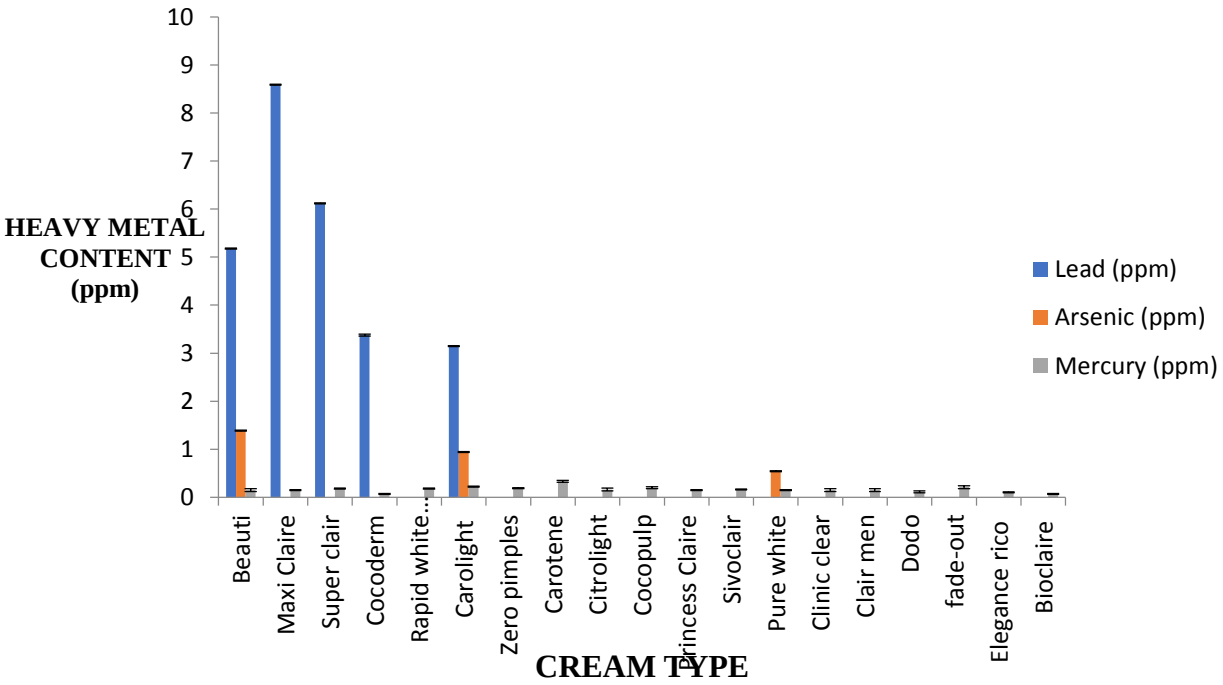

Fig. 2: Variation of heavy metal content in different cream type

3.2.1 Levels of mercury in skin lightening creams

The samples had the mean levels of mercury ranging from 0.07±0.01 to 0.33±0.02 ppm as shown in fig.2. The mercury levels are below the 2.0 ppm permissible limit recommended by Uganda National Bureau of Standards (UNBS) and the 1.0 ppm limit recommended by United States Food and Drug Authority (US FDA). Carotene recorded the highest mean level of 0.33±0.02 ppm while Cocoderm and Bioclaire recorded the lowest mean level of mercury at 0.07±0.01 ppm. Mercury's presence in all the creams, points to the possibility of intentional addition of metal containing compounds or its ions into the creams during the formulation process as an active bleaching agent. It could be present in the skin lightening creams in form of ammoniated mercury, mercury iodide,

mercurous chloride, mercurous oxide, or mercuric chloride [25]. Mercury inhibits melanogenesis in the epidermal melanocytes by inactivating the mercaptan enzymes, thereby inactivating tyrosinase enzyme, an important catalyst involved in melanin production [26,27].

The results of this study are in agreement with those of a similar study by Ramakant, Poornima, and Sapna conducted on creams for heavy metals in cosmetics sold in India. Out of the 32 samples analyzed in their study, 44% of the samples showed detectable mercury levels, with concentrations ranging from 0.10 ppm to 1.97 ppm [28]. Relatedly, Al-Saleh and Al-Doush [7] reported that the mean mercury concentration in the creams obtained from Saudi Arabian market, with origins of Asia and Middle East was 3.76μg/g. The mercury concentration in the creams, ranged from 0 to 5.65 μg, with most creams having levels excessively higher than the US FDA permissible limit and therefore capable of exerting mercury-related health side effects.

The toxic and health related effects of mercury are diverse [12]. However, the information regarding its effects arising from exposure to mercury containing skin creams particularly in the vast majority of African states has been inadequate [12, 29]. In a report published by Marzulli and Brown [31], it was indicated that the use of skin lightening creams containing inorganic mercury salts could result in substantial absorption and ultimate accumulation of mercury in the body tissues of those exposed to the metal. The bioaccumulation of the metal in the tissues of users could be attributed to the fact that the metal is readily absorbed through the fatty tissues of the skin and via the nostrils during inhalation [30]. Therefore, repeated application of these skin lightening creams, even at levels below the threshold limit, could possibly result into a cumulative effect of prolonged low-level mercury exposure. Among the various cumulative effects of mercury exposure, include nephritic syndrome [32]. In justification of Mercury- nephron interaction, Kahatano *et al.,* (1998) conducted a study which reported that even some women who were not active in artisanal gold mining could have up to 0.1 mg mercury per liter of urine [33]. It was therefore concluded that the mercury in the urine could have been derived from mercury containing bleaching soaps and creams that they had been applying [33]. In addition, Kuhnert, Kuhnert, & Ehrard [34] reported about the possibility of mercury being transferred from the mother to the fetus during pregnancy, which could result into foetal/ embryonic abnormalities.

Therefore, even though it can be concluded that there may be no potential mercury related health risk from the use of the skin lightening creams obtained from Mbarara Municipality Market, the repeated application of these creams may cause a cumulative effect over prolonged exposure.

3.2.2 Levels of lead in skin lightening creams

Maxiclaire recorded the highest mean levels of lead as shown in fig.2. Of the 19 samples analysed, five samples had detectable amount of lead levels. These creams re: - Maxiclaire (8.59±0.01 ppm), Superclair (6.12±0.01 ppm), Beauti (5.18±0.01 ppm), Cocoderm (3.38±0.02 ppm), Carolight (3.15±0.01 ppm). The rest of the creams had no detectable levels of lead. All the samples with detectable concentration of lead had their levels below the 20ppm limit recommended by Uganda Bureau of Standards (UNBS) and the 10.0 ppm limit recommended by the United States Food and Drug Administration (US FDA). On the other hand, The International Cooperation on Cosmetics Regulation and regions such as Canada and the European Union set a limit of 10 ppm for lead as

an impurity in cosmetics based on considerations of a reasonably achievable level, scientific risk assessment, good manufacturing practices, technical feasibility, and appropriate analytical methods [35]. Although the lead levels of all the creams analysed were below the threshold limit, current studies suggest that lead may have no identifiable safe exposure level, with even the lowest levels having shown to affect the foetus and central nervous system in the children [36,37].

A related analysis which was conducted by Ramakant, *et al.* [28] to assess heavy metal levels in cosmetics sold in India, showed no detectable levels of lead in lip-balm and anti-ageing creams. In another related study conducted by Iwegbue *et al.(2015)* on lead levels in lightening creams in Nigeria[38], lead levels were found to lie within a concentration range of 0.5ppm to 4.5 ppm. These levels were however more than those found in moisturizing creams analyzed within the same study.

The presence of lead in some cosmetics and the health-related side effects, have thus compelled regulatory bodies across the globe to ban its usage in cosmetics/ creams. The European Union (EU) law for cosmetics has since 1979 banned the usage of lead and lead containing compounds in cosmetics [39]. However, over the years, it has been noted that although the presence of lead could as a result of deliberate additions, the trace amount of lead present in creams may rather be as a result of unavoidable conditions even under good manufacturing practices [39].

3.2.3 Levels of arsenic in skin lightening creams

Only three of the analysed creams recorded detectable levels of arsenic. These were: Beauti (1.39±0.01 ppm), carolight (0.94±0.01 ppm) and pure white (0.54±0.01 ppm). Beauti recorded the highest levels of arsenic. The other 16 creams showed no detectable levels of the metal. The levels of arsenic in creams that showed detectable concentrations were less than 2.0 ppm and 3.0 ppm, the threshold limits recommended by UNBS and US FDA respectively.

The results are in agreement with a related study conducted by Alqadami, *et al.* [40] in which they assessed the levels of arsenic in thirty four skin whitening creams. In this study, they found that the arsenic concentrations in the creams ranged from as low as 0.34 ppm to 14.76 ppm, with most of them having levels exceedingly higher than US/FDA's permissible limit.

There are no sufficient reports linking arsenic metal to inhibition of melanin synthesis. This minimizes the likelihood of deliberate addition of arsenic metal in these creams. The existence of arsenic in these skin lightening creams can therefore be attributed to the metal's existence in substances used as raw materials in manufacturing of the products [41]. Centers for disease control and prevention [42] noted that arsenic contamination may also be as a result of unintentional migration of metal from wear and corrosion of metallic devices and machinery used in manufacturing process.

It should however, be noted that none of these metals (Hg, Pb, As) as discussed above, had been listed in the labels as a component of any of the products.

3.3 Chemical requirements of the skin lightening creams

Three chemical parameters of the different creams were assessed. These are pH, thermal stability and fatty substance content. The results of each of the parameters are shown in Fig. 3, Fig. 4 and Table 1 below;

3.4 pH of the skin lightening creams

All the creams showed low pH levels below UNBS' permissible limit. The results are as shown in the fig. 3 below;

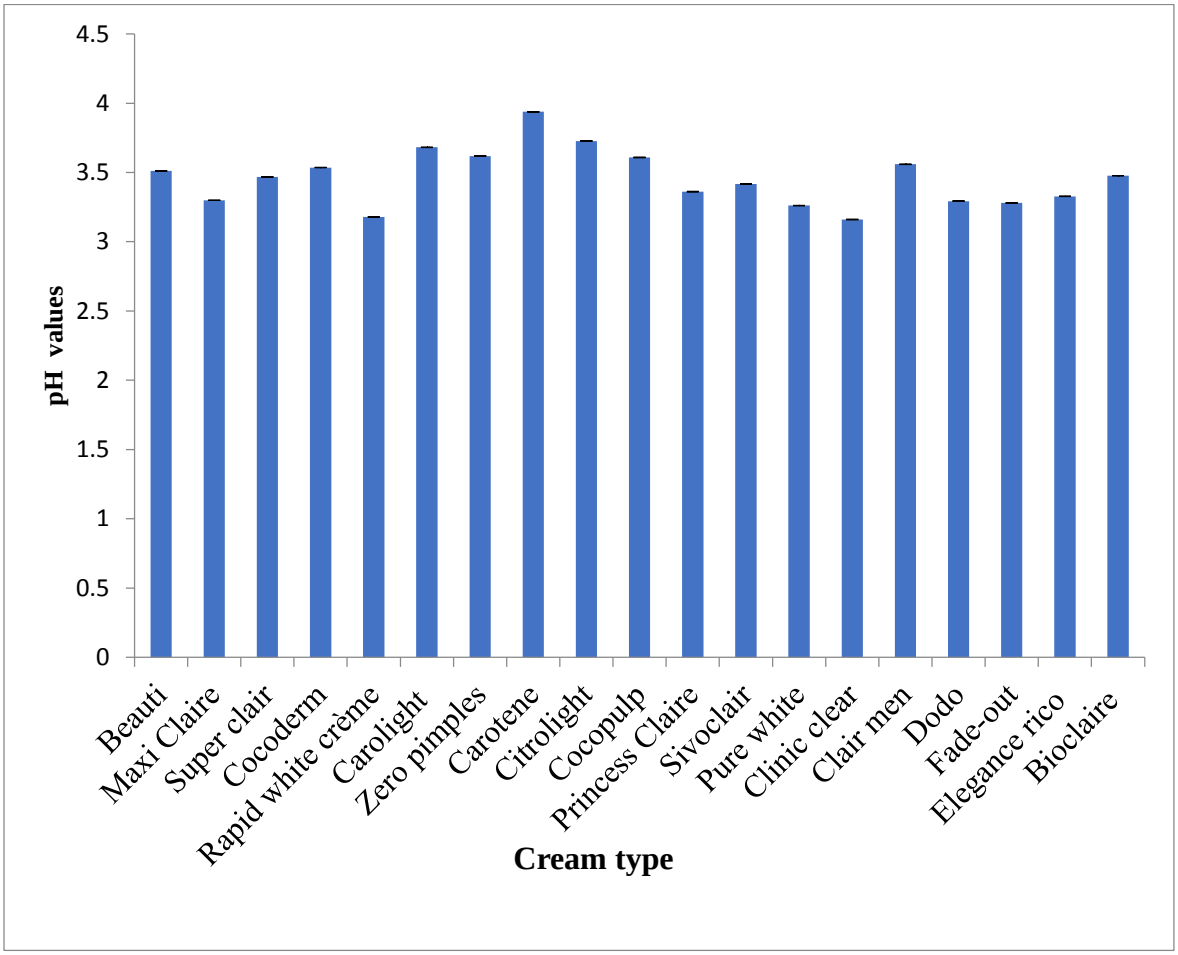

Fig. 3: Variation of pH range

The pH values ranged from the mean values of 3.160±0.002 to 3.938±0.001 as shown in fig.3. All the creams indicated low values of pH below the permissible range of 4.5- 8.5 as recommended by UNBS. The lowest pH value was recorded by clinic clear and the highest pH value was recorded by carotene.

The human skin pH normally ranges from 4.5 to 6 [43]. The acid pH of the skin surface, known as the "acid mantle" and the pH gradient [44] of the stratum corneum (SC), regulates at least three

epidermal functions: antimicrobial barrier, permeability barrier and barrier integrity/cohesion [45]. Fluhr *et al*.,[46] reported improved barrier integrity after exogenous acidification in neonatal skin. However, increased acidity increases the skin's pH gradient. All the creams have low (highly acidic) pH values which make them capable of exerting skin irritating properties. The decrease in pH levels can be attributed to the changes in storage conditions. Change in storage conditions like elevated temperatures can accelerate chemical reactions, thereby altering the activity of components and pH [47].

3.5 Fatty substance content in the skin lightening creams

The fatty substance content by mass ranged from 5.94±0.02% to 28.57±0.01%. Results are summarized in fig. 4 below;

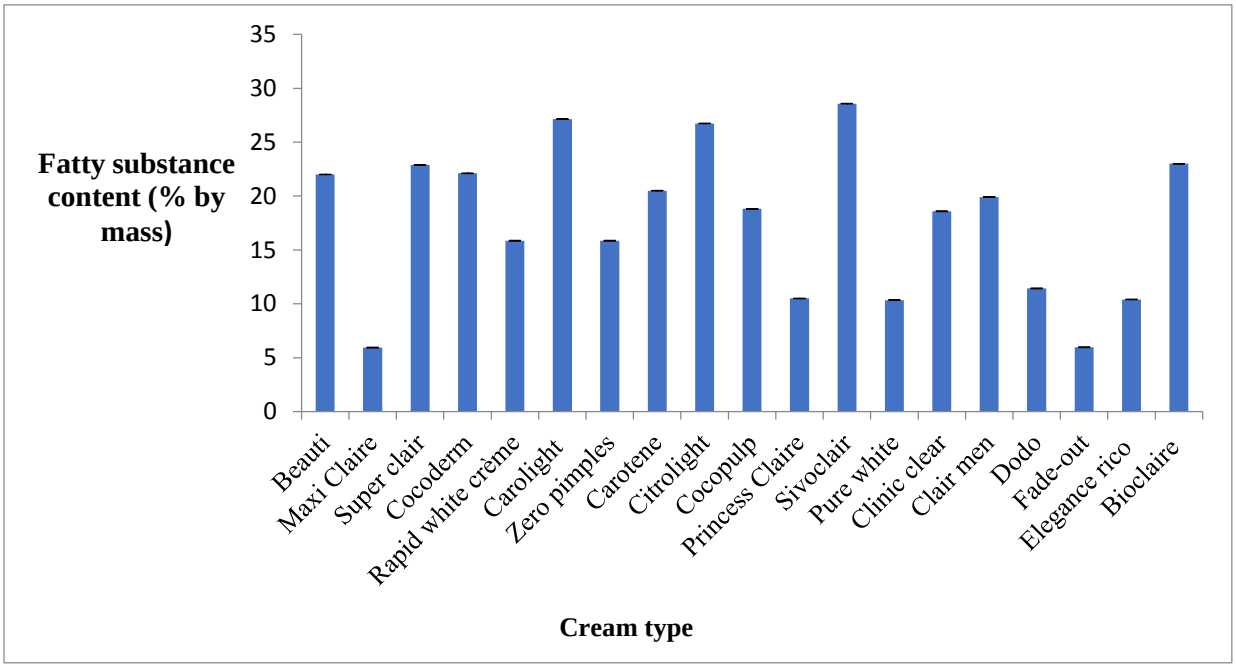

Fig. 4: **Variation of fatty substance content**.

The fatty substance content by mass ranged from the mean values of 5.94±0.02% to 28.57±0.01% as shown in Fig.4. All the creams have fatty substance content above the minimum threshold of 5% recommended by UNBS. The least fatty content was recorded by maxiclaire while the highest fatty content was recorded by sivoclair. All creams are recommended to have high fatty substance content to enable them counteract skin dryness in order to alleviate flaking, cracking, and roughness [43].

These observed differences in the total fatty substance are perhaps due to the difference in the manufacturing processes and methods. High fatty matter above 5% is suitable for dry skin. It makes the skin smooth, and additionally serves as a good lubricant throughout the day [48].

3.6 Thermal stability of the skin lightening creams

All creams passed the stability tests which were assessed basing on colour, smell, phase separation, granular formation and shrinkage due to evaporation. The results are as shown in Table 2

Table 2: Thermal stability results (n = 19)

	Parameters assessed				
Cream	No Colour change	No change of smell	No phase separation	No granular formation	No shrinkage due to evaporation
Beauti	√	√	√	√	√
Maxi Claire	√	√	√	√	√
Super clair	√	√	√	√	√
Cocoderm	√	√	√	√	√
Rapid white crème	√	√	√	√	√
Carolight	√	√	√	√	√
Zero pimples	√	√	√	√	√
Carotene	√	√	√	√	√
Citrolight	√	√	√	√	√
Cocopulp	√	√	√	√	√
Princess Claire	√	√	√	√	√
Sivoclair	√	√	√	√	√
Pure white	√	√	√	√	√
Clinic clear	√	√	√	√	√
Clair men	√	√	√	√	√
Dodo	√	√	√	√	√
Fade-out	√	√	√	√	√
Elegance rico	√	√	√	√	√
Bioclaire	√	√	√	√	√

Note: √ - passed the test

All the creams passed the stability tests as shown in Table 2. They showed colour consistency, exhibited no separation of phases, no changes in odour, no formation of granules or crystals and no shrinkage as a result of evaporation of water. Stability studies are essential for determination of product quality, efficacy and safety. Thus, these studies contribute to the development and improvement of formulations [49]. In a related study conducted by Deuschle *et al.*,(2015)[50] storage of the creams at different temperature conditions never changed their appearance, colour and odour.

4.0 CONCLUSIONS

The mean levels of mercury in the analyzed creams were below 2.0 ppm which is the recommended threshold limit by UNBS. The five samples that showed detectable amount of lead, had the mean lead levels below UNBS' recommended threshold limit of 20 ppm. The three creams that recorded detectable levels of arsenic, had arsenic levels below the threshold limit recommended by Uganda Bureau of Standards (UNBS) of 2.0 ppm. However, even when the levels of these heavy metals are low, continuous application of the creams containing them, can cause slow release of these metals in the body and result into detrimental health effects. The traces of metals present in creams can be attributed to either intentional addition during formulation or unintentional presence as impurities during the manufacturing process.

Since all the samples analyzed contained hydroquinone, with 84.2% of the samples recording levels above the threshold limit recommended by UNBS and US FDA (2%), it is very alarming, and consumers who apply any of these creams are at risk.

All the creams met the chemical requirements of thermal stability, fatty substance content as recommended by UNBS. However, none of the creams satisfied the pH requirement.

5.0 RECOMMENDATIONS

The community should be sensitized on the implications of using skin lightening creams.

UNBS should conduct periodic analysis to ascertain the levels of hydroquinone, heavy metals, and chemical requirements of skin lightening creams sold in Uganda.

Manufacturers should be encouraged to state the exact skin bleaching agents and their amount in the skin lightening creams.

Further studies can be conducted on heavy metals and hydroquinone in skin lightening soaps and lotions, effect of storage time on the creams' chemical characteristics, the specific gravity and sunscreen protection factor of the creams and the levels of organic species of the heavy metals in skin lightening creams.

References

1. Frost, P. (01/01/1990). Fair women,dark men:The forgotten roots of colour prejudice. *History of European ideas* , 669-679.
2. Balaguer, A., Salvador., A., & Chisvert, A. (2008). A rapid and reliable size-exclusion chromatographic method for determination of kojic dipalmitate in skin-whitening cosmetic products. *Talanta: PubMed* , 101-102.
3. Omolaoye., J. A., Uzairu, A., & Gimba, C. E. (2010, 5 2). Heavy metal assessment of some eyeshadow products imported to Nigeria from China, Arch. Appl. Sci. Res. pp. 76–84.
4. UNBS. (2013). *Final Draft Uganda Standard.* Arusha, Tanzania: East African Community.
5. *US FDA.* (2010,, October 28). Retrieved from Lipstick and Lead: Questions and Answers:http://www.fda.gov/Cosmetics/ProductandIngredientSafety/ProductInformation/ucm137224.htm
6. Perry. (2006). *Buying white beauty.* Cardozo J Law Gend 12:579–607.
7. Dadzie, O. E., & Petit, A. J. (2009). Skin bleaching: highligting the misuse of cutaneous depigmenting agents. *Eur Acad Dermatol Venerol* , 3(7): 741-750.
8. *Hcsc.* (2010, November 5). Retrieved July 25, 2018, from Draft Guidance on Heavy Metal Impurities in Cosmetics: http://www.hcsc.gc.ca/cps-spc/legislation/consultation/_cosmet/metal-metauxconsulteng.php.
9. Al-Saleh, I., & Al-Doush, I. (1997). Mercury content in skin-lightening creams and potential hazards to the health of Saudi women. *Journal of Toxicology and Environmental Health* , 51(2):123–130.
10. Badreshia-Bansal, S., & Draelos, Z. (2007). Insight into skin lightening cosmeceuticals for women of color. *J. Drugs Dermatol.*, 6, 32-39.
11. Jan, A. T., Ali, A., & Haq, Q. M. (2011). Glutathione as an antioxidant in inorganic mercury induced nephrotoxicity. *J. Postgrad. Med. . [PubMed]*, 57, 72–77.
12. Ladizinski, B., Mistry, N., & Kundu, R. V. (2011). Widespread use of toxic skin lightening compounds: medical and psychosocial aspects. Dermatologic Clinics.
13. Ratnaike, R. N. (2003). Acute and Chronic Arsenic toxicity. *Postgrad MedJ* , 79:391-6.
14. Ademuyiwa, O., Agarwal, R., Chandra, R., & Behari, J. R. (2009). Lead induced phospho lipidosis and cholesterogenesis in rat tissues. *chemico-Biol.interact* , 179: 314-320.
15. Ajose, F. O. (2005). Consequences of skin bleaching in Nigerian men and women-. *International Journal of Dermatology, doi:10.1111/j.13654632.2005.02812.x* , 44, 41-43.
16. Kooyers, T. J., & Westerhof, W. (2005). Toxicology and health risk of hydroquinone in skin lightening formulations. *Journal of the European academy of Dermatology and Venereology* , 20:777-780.

17. Aldrich, & Chemical, C. (1990). Aldrich catalog. Handbook of Fine Chemicals., *M Milwaukee, WI,Van Norstrad.co* , 235.

18. Ayenimon, J. G., Yusuf, A. M., Adekule, A. S., & Makinde, O. W. (2010). Bull. Environ. Contam. Toxicol. In *Heavy metal exposure from personal care product* (pp. 8-14).

19. Theresa, O. C., Onebunne, O. C., Dorcas, W. A., & Ajan, O. I. (2011). Potentially toxic metals exposure from body creams sold in Lagos Nigeria.

20. Odumoso, P. O., & Ekwe, T. O. (2010). Identification and spectrophotometric determination of hydroquinone levels in some creams. *African Journal of Pharmacy and Pharmacology* , 4: 231-234.

21. National Toxicology Program (NTP). (1989). *Toxicology and Carcinogenesis Studies of Hydroquinone (CAS No. 12331-9) in F344/N Rats and B6C3F (mice galvages).* Bethesda: U.S. Department of Health andHuman Services, Public Health Service, National Institutes of Health.

22. Penney, K. B., Smith, C. J., & Allen, J. C. (1984). Depigmenting action of hydroquinone depends on disruption of fundamental cell processes. *Journal of Investigative Dermatology*, 82, 308-310.

23. Briganti, S., Camera, ,. E., & Picardo, M. (2003). Chemical and instrumental approaches to treat hyperpigmentation. *Pigment Cell Research 16*, 101-110.

24. Gupta, A. K., Gover, M. D., Nouri, K., & Taylor, S. (2006). The treatment of melasma: a review of clinical trials. *Journal of the American Academy of Dermatology*, 55, 1048-1065.

25. Park, J. D., & Zheng, W. (2012). Human exposure and health effects of inorganic and elemental mercury. *Journal of Preventative Medicine and Public Health*, 45 (6): 344-52.

26. Engler, D. E. (2005). Letter to the editor: Mercury "bleaching" creams. *Journal of the American Academy of Dermatology*, ., 52(6):1113–1114.

27. Hamann, C. R., Boonchai, W., & Wen, L. (2014). Spectrometric analysis of mercury content in 549 skin-lightening products: Is mercury toxicity a hidden global health hazard? *Journal of the American Academy of Dermatology* , 70 (2): 281-7.e3.

28. Ramakant, S., Poornima, S., & Sapna, J. (January 2014). *Heavy Metals in Cosmetics.* New Delhi: Tughlakabad Institutional Area.

29. Bates, N. (2003). Metallic and inorganic mercury poisoning. *Emerg. Nurse [CrossRef] [PubMed* , 11, 25–31.

30. WHO. (1991). . *Environment health criteria 118. Inorganic Mercury.* World Health Organisation.

31. Marzulli, F. N., & Brown, D. W. (1972). Potential systemic hazards of topically applied mercurials. In *J Soc Cosmet Chem* (pp. 23: 875-886).

32. Enwon wu, C. O. (1987). Potential health hazard of the use of mercury in dentistry:Critical review of the Literature. *Environ Res* , 42: 257-274.

33. Kahatano, J. M., Mnali, S. R., & Akagi, H. (1998). A study of Mercury Levels in Fish andHumans in Mwakkitolyo Mine and Mwanza Town in the Lake Victoria Gold-Fields, Tanzania. *Br Med J* , 2: 543-545.

34. Kuhnert, P. M., Kuhnert, B. R., & Ehrard, P. (1981). Comparison of mercury levels in maternal blood, fetal cord blood and placental tissues. *Am J Obstet Gynecol* , 139: 209-213.

35. Regulation International Cooperation on Cosmetics. (2013, December). Retrieved from Considerations on Acceptable LeadLevelsin Cosmetic Products: http://iccrnet.org/topics.

36. Bellinger, D. C. (2008). very low lead exposures and children's neurodevelopment. *Curr Opin Pediatr*, 20:172-7.

37. Sanders, T., Liu, Y., Buchner, V., & Tchounwou, P. B. (2009). Neurotoxic effects and biomarkers of lead exposure: A review. *Rev. Environ Health*, 24:15-45.

38. Iwegbue, C. M., Bassey, F. I., Tesi, G. O., Onyeloni, S. O., Obi, G., & Marticingh, B. S. (2015). Safety evaluation of metal exposure from commonly used moisturizing and skin lightening creams in Nigeria. *Regulatory Toxicology and Pharmacology* , 71(3),484-490.

39. Amit, S. C., Rekha, B., & Atul, K. S. (2010). Determination of Lead and Cadmium in cosmetic products. *Journal of Chemical and Pharmaceutical Research* , 2(6):92-97.

40. Alqadami, A. A., Abdalla, M. A., Alothman, Z. A., & Omer, K. (2013). application of solid phase extraction on multiwalled nanotubes of some heavy metal ions to analysis of skin whitening cosmetics usin ICP-AES. *Int J Environ Res Public Health* , 10:361-74.

41. Naujokas, M. F., Anderson, B., & Ahsan, H. (2013). The broad scope of health effects from chronic arsenic exposure: update on a worldwide public health problem. *Environ Health Perspect* , 121:295.

42. Centers for disease control and prevention. (2016). *CDC-NBP-factsheet- Arsenic:CDC.* CDC.

43. Lucero, M. J., Vigo, J., & Lecin, M. J. (1994). A study of shear and compress deformations gels of tretinoin on hydrophilic . *International journal of pharmaceutics*, 106, 125-133.

44. Öhman, H., & Vahlquist, A. (1994). In Vivo Studies Concerning a pH Gradient in Human Stratum Corneum and Upper Epidermis. *Acta Dermato-Venereologica*, 375-379.

45. Elias, P. M. (2005). Stratum Corneum Defensive Functions: An Integrated View. *Journal of Investigative Dermatology* , 183-200.

46. Fluhr, J. W., Kao, J., Jain, S., Ahn, K., Feingold, R., & Elias, P. M. (2001). Generation of Free Fatty Acids from Phospholipids Regulates Stratum

47. Mariotti, D., & Frasson, A. P. (2011). Avaliado da estabilidade e atividade antioxidante de formulacoes cosmeticas contendo extrato etanolico dos frutos de Fragaravia vesca l (morango). *informa*, 3-4.

48. Mak-Mensah, E. E., & Firempong, C. K. (2011). Chemical characteristics of toilet soap prepared from neem (Azadirachta indica A. Juss) seed oil. *Asian J. Plant Sci. Res. 1(4)*, 1-7.

49. Deusche, V. C., Deusche, R. A., Bortoluzzi, M. R., & Athayde, M. L. (2015). Physical chemistry evaluation of stability, spreadability, in vitro anti oxidant, and photo protective capacities of topical formulations containing Calendula officinalis L.leaf extract viviane. *Journal pf pharmaceutical sciences* , 51(1) 63-75.

50. Deuschle, V. C., Deuschle, R. A., & Athayde, M. R. (2015). Physical chemistry evaluation of stability, spreadability, in vitro antioxidant, and photo-protective capacities of topical formualations containing Calendula officinalis L. leaf extract. *brazillian journal of pharmaceutical sciences vol.51*, 68-71.