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Simultaneous Determination of Hydroquinone, Kojic Acid and L-Ascorbic Acid in Some Cosmetic Emulsions

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Abstract: The presence of skin de-pigmenting agents in cosmetic products, have received considerable attention in Nigeria as a possible source of skin abuse. There has been a call by government for cosmetic products manufacturers to comply with regulatory limits of these additives in their products. This study contributes to the pool of data available on levels of skin de-pigmenting agents in cosmetic products. Fifty cosmetic emulsions for skin use were randomly purchased from some sales outlets in Ibadan, South West Nigeria with the aim of determining the level of hydroquinone, kojic acid and l-ascorbic acid in them. Manufacturer claims were determined by examining the cosmetic package labels. The three de-pigmenting additives were determined simultaneously and quantitatively using High Performance Liquid Chromatography (HPLC). Results show that 96% of the emulsions were manufactured in four different continents namely Africa (42%), North America (32%), Europe (20%), Asia (2%) and undetermined origin (4%) by 28 different manufacturers. 36% of the manufacturers indicated % hydroquinone content to be 2% while no manufacturer indicated kojic and ascorbic acid content. 52% of the emulsions were creams, 44% lotions and 4% milks. 34% of the samples did not contain any of the three de-pigmenting agents, while 50% contained just hydroquinone, 2% kojic acid alone and 2% only ascorbic acid. Both hydroquinone and kojic acid were in 8% of the samples and only 4% contained the three additives. Levels of occurrence of the additives were 0.1 – 4.9% hydroquinone, 0.3 – 1.3% kojic acid and ascorbic 0.01 – 3.6%. The emulsions which contained hydroquinone were mostly from Africa (28%) followed by North America (22%) followed by Europe (8%), while only 2% from Asia and unidentified continent. Samples from Cote de Voire contained higher concentrations of hydroquinone which were greater than 3% in all cases except one. In conclusion, this study showed that hydroquinone is the major skin de-pigmenting agent in cosmetics in Nigeria the Nigerian Market. 22% of the manufacturers used hydroquinone at levels higher than the regulatory limit (2%). 64% of these manufacturers (55% Cote de Voire + 9% others) are from Africa. Only one manufacturer from Nigeria included hydroquinone at a level above regulatory limit.

Keywords: Cosmetics, De-pigmenting agents, Hydroquinone, Kojic acid, L-ascorbic acid, Manufacturers

Introduction

The skin is the first point of contact of a person with the environment and a very important organ [1]. More attention is increasingly been paid to what is rubbed on the skin and various regulations have been put in place in order to prevent the abuse of human skin [2]; as the state of the skin is a good indication of the overall health status of an individual [3].

Cosmetic products are preparations intended for placement in contact with any external part of the human body [4]. Skin emulsions are mainly used in conditioning human skin and are usually applied on a daily basis. The purpose of a cosmetic emulsion for skin use is to improve the appearance of the skin by correction, cover up or removal of blemishes, improve skin elasticity, skin colour irregularities, reducing the effects of aging, perfume the skin and reduce sweating and odour[5].

Melanin is the pigment responsible for human skin colour and melanogenesis is the process by which melanin is formed in the skin [6]. Any major inhibition or malfunctioning of this process can lead to hyper or hypo pigmentation and may lead to uneven pigmentation of the skin. Skin hypo-pigmentation occurs as a result of inhibition of melanogenesis or destruction of melanocytes [7]. This can be as a result of the effect of diseases like psoriasis, leprosy and other immuno-deficiency

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processes or due to the use of skin de-pigmenting agents [8]. These agents are known to reduce hyper pigmentation when in proper use but can destroy the skin reducing its elasticity, inhibiting melanogenesis and destroying melanocytes [9] when improperly administered. This then results in the permission of harmful ultra violet rays into the dermis, eventually resulting in cancer of the skin [10, 11,12].

Cosmetic emulsions for skin use contain the basic mixture of oil and water and all kinds of additives [13] ranging from functional to non- useful. Cosmeceuticals are topical cosmetic – pharmaceutical hybrids that enhance beauty through constituents that provide additional health related benefits. On account of this, cosmeceuticals are added to skin emulsions so that they can influence the skin's biological function [14]. These additives include antioxidants, preservatives, sunscreens, emollients, vitamins and hypo-pigmenting agents such as hydroquinone, kojic acid, arbutin, L-ascorbic acid, green tea and so on which all work by interfering with the production of melanin in one way or the other [15, 16] resulting in improved skin appearance.

Hydroquinone is considered to be one of the effective inhibitors of melanogenesis [17]. It promotes the formation of reactive oxygen species, oxidative stress, promote tumor cell growth and suppress immune responses [18]. It causes a reversible inhibition of cellular metabolism by affecting both DNA and RNA synthesis. The cytotoxic effect of hydroquinone is not limited to melanocytes, but the dose required to inhibit cellular metabolism is much higher for non melanotic cells than for melanocytes. It is therefore considered to be a potent melanocyte cytotoxic agent [19]. Hydroquinone is commonly used at 2-4% levels [20], and clinical studies report well excellent induced responses by 2% hydroquinone. Effects of hydroquinone below3% are known to be transient and do not cause, skin injuries [21]. However concentrations above 5% could provoke local irritations [19] and chronic adverse effects like exogenous ochronosis (22), cataract, pigmented colloid millia, sclera, nail pigmentation, loss of elasticity of the skin, impaired wound healing and exuding an offensive fish odour [23]. This uncommon but yet important adverse effect of hydroquinone known as exogenous onchronosis causes the skin to progressively darken to a sooty colour when exposed to hydroquinone and is thus referred to as Coca cola skin by the local people. This effect is commonly observed among dark skinned people who have used high concentrations of hydroquinone for many years. This state of the skin is difficult to treat and is only reported as occasionally responding to topical application of steroids and chemical peeling [22].

Mequinol (4- hydroxyl anisole) is a derivative of hydroquinone that acts as a substrate for tyrosinase, inhibiting formation of melanin precursors [20]. The mechanism of action of this inhibition which results in de-pigmenting of the skin is unclear. Mequinol is marketed in the USA at 2% concentration in combination with 0.01% tretinoin. This combination can cause erythema, burning, pruritus, desquamation, skin irritation and halo- hypopigmentation, Combination with sunscreens is reported to reduce the incidence of adverse effects.

N-acetyl-4-S-cysteaniminyl phenol (NCAP) is a phenolic agent which inhibits tyrosinase by acting as an alternative substrate. It is a more stable compound and causes less irritation than hydroquinone. This compound is used at 4% level with clinical response after two weeks of treatment [24].

Kojic acid (5-hydroxy – 2- hydroxyl methy-4-pyrone a non- phenolic cosmeceutical is a naturally occurring hydrophilic fungal product derived from certain species Acetobacter, Aspergillus and Penicillium. It reduces hyperpigmentation by inhibiting the production of free tyrosinase and is *also* a potent antioxidant [25]. It is used from 1 - 4%. Its inhibition activity is carried out by the inhibition of catecholase activity of tyrosinase, which is the rate limiting essential enzyme in the considered to involve the biosynthesis of the pigment melanin [26]. Kojic acid although an effective de-pigmenting agent, has been reported to have a high sensitizing potential and thus may cause irritant contact dermatitis [27]. This Irritation can be decreased by combining it with a topical corticosteroid. The available human sensitization data supported the safety of Kojic acid use at a concentration of 2% in leave –on cosmetics [28].

Arbutin (Hydroquinone –β-δ- glucopyranoside) is a naturally occurring glycoside of hydroquinone found in various plant species. It is the most widely prescribed skin lightening and de- pigmenting agent. Arbutin inhibits tyrosinase activity competitively, but at a non-cytotoxic concentration in a dose dependent manner in cultured melanocytes [20].The de-pigmenting effect of arbutin has been reported to reduce cellular tyrosinase without changing the cell viability [29]. Arbutin also inhibits melanosomes maturation. It is less effective than kojic acid however for the treatment of hyperpigmentation. Deoxy arbutin is a synthesized topical derivative. Studies show enhanced sustained improvement in general skin lightening and safety profile comparable to that of hydroquinone [30]. The Taiwan FDA has placed the limit of arbutin in skin whitening ingredients in cosmetics at 7% [31]. Studies on arbutin show that it metabolises to hydroquinone [31] and in animals induce renal tube and liver adenomas,

as well as thyroid glands follicular cells hyperplasia [32].

Although many cosmetic formulations designed to protect and rejuvenate photo aged skin contain vitamin C (ascorbic acid) a natural occurring, potent water soluble antioxidant that occurs in many different forms each with its distinct properties [33]. Several of these forms have been shown to reduce melanin formation and produce a skin whitening effect when applied topically. These forms include l-ascorbic acid, magnesium ascorbyl phosphate and sodium ascorbyl phosphate. These forms when used individually or together can assist in slowing down hyperactive melanocytes resulting in fairer skin. L-ascorbic acid the biologically active and useful form of vitamin C [34] is soluble and functions in the aqueous compartments of the cell apart from the regeneration of the skin's vitamin E, improving synthesis of collagen [35] and offering antioxidant protection also act as a reducing agent blocking the oxidation chain reaction of melanin production at various points. This inhibition of melanin results in a lighter and brighter skin in just a few weeks. L-ascorbic acid is reported to have excellent safety and efficiency and is nontoxic with minimal irritation. However it has to be formulated at pH less than 3.5 to enter the skin and the maximum concentration for optimal per cutaneous absorption is reported to be 20% [36]. Magnesium- L- ascorbic-2-phosphate (MAP) is the most popular and stable form and is easily absorbed into the skin because it is lipophilic. It has a hydrating effect and decreases trans-epidermal water loss (TWL) [34]. It is a free radical scavenger that is photo-protective and increases collagen production under lab test conditions. It is known to suppress melanin formation when used at 10% levels in creams. It is also known to have a protective effect against skin damage induced by UVB radiation. 0.5 -25% by weight of MAP in skin lightening creams is reported to decrease skin irritation. Although many formulations contain vitamin C, very few are actually effective in topical application because of low concentration, compromised stability on opening of product and exposure to air and light and on account of the form in which the molecule exist which is not absorbed or metabolized. However when a stable formulation delivers a high concentration of the non-esterified, optimal isomer of the antioxidant; inhibition of acute UV, damage erythema, sunburn, tanning chronic UV ageing and skin cancer is inhibited [35]. Individually or combined these various vitamin C forms can slow down hyperactive melanocytes resulting in a fairer skin. Vitamin C however works best in formulations that include vitamin E.

Vitamin E most commonly known form is tocopherol. This additive causes depigmentation by interference with lipid peroxidation of melanocyte membranes, increase intracellular glutathione content and inhibits tyrosinase [37]. It is has no side effect and is normally used at 5% levels or lower in creams being more effective when used in combination with vitamin C[38] .

Niacinamide or Nicotinamide (3-pyridine-carboxamide) is the active form of vitamin B_3. It interferes with the interaction between keratinocytes and melanocytes thus inhibiting melanogenesis. It modulates protease activated receptor (PAR-2) which is involved in transfer of melanosomes to the surrounding keratinocytes from melanocytes. 2% formulations have been shown to reduce hyperpigmentation thus increasing skin fairness within four weeks [39].
Naturally derived extracts have also been used to initiate skin depigmentation. These include formulations with grape seed [40], orchid extract [41], aloe vera [42], marine algae leaves [43], green tea leaves [44] and so on.

Government has called for manufacturers of these products to comply with regulatory limits to guide against negative effects of de-pigmenting agents in particular hydroquinone included in cosmetic emulsion and therefore reduce unnecessary medical expenses especially in a poor economy like Nigeria.

This study has been undertaken to document the levels of hydroquinone and two other skin de-pigmenting additives in cosmetic emulsions in the Nigerian Market thereby contributing to data pool available on the levels of skin depigmenting constituents of cosmetic products.

Materials

Hydroquinone and l-ascorbic acid (HPLC grade) were obtained from Beauty Fair Industry Laboratory, Nigeria. Kojic acid, pyridoxine and methanol (HPLC grade) were purchased from Sigma Chemicals, from their Nigeria agent Zayo –Sigma Nigeria Limited in Jos, Nigeria, Phosphoric acid and Potassium dihydrogen phosphate (Analytical grade) were obtained from the Chemicals Store of the Department of Chemistry University of Ibadan, Nigeria. Cosmetic emulsions were purchased from a whole sale cosmetic products supermarket (Pinnacle) in Felele layout, Ibadan, Nigeria.

Instrumentation and Analysis Condition

HPLC Model 4200; Adept Series dual piston pump and UV/visible detector with C18 – RP 5µm (4.6mm ID x 250mm) chromatographic column was used. The mobile phase consisted of 0.05Moles/$dm^3$$KH_2PO_4$/ methanol at 95:5 with a flow rate of 0.967ml/min at 280nm. Injection volume= 20µl.

Methods

Manufacturer Claims

The packaging of each cosmetic emulsion was inspected and the country of manufacture, manufacturer, expiry date and additives included in each emulsion were recorded.

Preparation of Sample and Standard Solution for Equipment Calibration

1.5mg/ml of pyridoxine was prepared to serve as Internal Standard Stock Solution. 5.0mg/ml hydroquinone, 2.0 mg/ml Kojic acid and 10.0 mg/ml l-ascorbic acid Standard Stock Solutions were prepared. These solutions were serially diluted to produce a series of standard solutions for the calibration of the equipment.

1g each of the cosmetic samples were mixed with 5ml of the internal standard stock solution of pyridoxine and diluted with twenty fold of 0.05 moles/dm^3 KH_2PO_4 (pH 2.5). The homogenous suspension obtained by homogenizing for 30 minutes was filtered and the filtrate was analysed simultaneously for the three skin whitening components using the HPLC [45] at the University of Ibadan Central Laboratory. All analyses were carried out in triplicates and values obtained recorded as mean values of the triplicate measurements.

Results and Discussion

Manufacturer claims

The fifty (50) cosmetic emulsions analysed consisted of 52% creams, 44% lotions, and 4% of milks. The emulsions were manufactured by twenty-eight (28) manufacturers from four (4) continents of the world (Table 1); 42% from Africa, 32% from America, 20% from Europe, 2% from Asia and 4% from unidentified continent. 22% of the emulsions were made in Nigeria.

Table 1: Levels of De-pigmenting agents in cosmetic emulsion samples

Sample Code	Emulsion type	Manufacturer Code	Continent of Manufacture	%HQ	%KA	%LAA	Emulsion Function	Expiry date
G&G	Cream	NPG	Africa	4.04	0.26	ND	Fade	Indicated
BTM	Lotion	CCL	Africa	ND	ND	ND	Fade	Indicated
MAX	Cream	NPG	Africa	3.40	ND	ND	Fade	Indicated
LSM	Lotion	SBL	Africa	ND	ND	ND	Fade	NI
BWP	Cream	NPG	Africa	ND	ND	0.01	Fade	NI
MCL	Lotion	SIP	Africa	3.48	ND	ND	Fade	Indicated
SIV	Lotion	SIP	Africa	3.83	ND	ND	Fade	NI
MTE	Lotion	CCL	Africa	3.62	ND	ND	Fade	Indicated
LEM	Cream	BFL	Africa	1.90	ND	ND	Fade	Indicated
VMC	Cream	PZC	Africa	ND	ND	ND	Moisturising	Indicated
SCT	Lotion	SBL	Africa	1.57	ND	ND	Fade	Indicated
ODC	Cream	PAG	Africa	ND	ND	ND	Moisturising	NI
LGS	Cream	SBL	Africa	1.55	ND	ND	Fade	Indicated
BCL	Cream	NPG	Africa	0.06	1.28	0.57	Fade	Indicated
JHS	Lotion	JJL	Africa	ND	ND	ND	Moisturising	Indicated
EVE	Cream	SBL	Africa	1.75	ND	ND	Fade	Indicated
PEC	Cream	SIP	Africa	4.18	ND	ND	Fade	Indicated
CLL	Cream	SIP	Africa	4.69	ND	ND	Fade	Indicated
FAC	Cream	BFL	Africa	1.30	ND	ND	Fade	Indicated
VST	Cream	PZC	Africa	ND	ND	ND	Fade	Indicated
ARM	Lotion	SBL	Africa	1.68	ND	ND	Fade	Indicated
NNF	Cream	BED	Asia	0.05	ND	ND	Fade	Indicated
DBS	Cream	UNL	Europe	1.24	0.37	3.68	Moisturising	NI
PFW	Lotion	LAD	Europe	ND	ND	ND	Fade	Indicated
DH7	Lotion	RCC	Europe	1.47	ND	ND	Moisturising	Indicated
ECL	Lotion	ASP	Europe	1.34	ND	ND	Fade	Indicated
REP	Lotion	CBP	Europe	ND	ND	ND	Moisturising	Indicated
STI	Cream	BFL	Europe	2.00	ND	ND	Fade	Indicated
MCC	Lotion	SEP	Europe	ND	ND	ND	Fade	Indicated
NWM	Cream	ASP	Europe	ND	0.47	ND	Fade	Indicated
OTP	Lotion	KPL	Europe	ND	ND	ND	Moisturising	Indicated
HTP	Lotion	MMI	Europe	ND	ND	ND	Moisturising	Indicated
RMC	Cream	JMH	America	2.35	ND	ND	Moisturising	NI
NEA	Lotion	NEC	America	ND	ND	ND	Moisturising	NI

PWB	Lotion	UNL	USA	ND	ND	ND	Fade	NI
JSF	Lotion	JSC	USA	0.12	ND	ND	Moisturising	NI
VFC	Cream	VPC	USA	1.67	ND	ND	Fade	Indicated
MTF	Cream	CE	USA	2.80	ND	ND	Fade	Indicated
PSE	Lotion	EBD	USA	1.61	ND	ND	Fade	Indicated
CEM	Lotion	BFA	USA	2.57	ND	ND	Fade	Indicated
JWH	Lotion	JSC	USA	ND	ND	ND	Moisturising	NI
REN	Cream	J5P	USA	1.69	ND	ND	Moisturising	Indicated
PSF	Cream	EBD	USA	1.64	0.32	ND	Fade	NI
CDT	Lotion	AAI	USA	1.90	0.37	ND	Fade	Indicated
PCB	Cream	EBD	America	1.53	ND	ND	Moisturising	NI
TIM	Cream	AVN	America	ND	ND	ND	Moisturising	NI
FAH	Lotion	TAE	America	1.60	ND	ND	Moisturising	Indicated
SRS	Cream	NI	NI	ND	ND	ND	Moisturising	NI
SKL	Cream	NI	NI	4.87	ND	ND	Fade	Ni
NMC	Cream	AVN	America	ND	ND	ND	Moisturising	NI

64% of these emulsions were classified as skin toning /fade creams and 36% as moisturising. 55.6% of the fade creams were made in Africa and 56.3% of these from Nigeria, 22.0 from America, 15.6% Europe, 6.3% from unidentified continent and 3.1% from Asia. 65.6% of these toning emulsions were creams, while 34.4% were lotions. Most of the creams claim to contain 2% hydroquinone. 50% 0f the moisturising emulsions were made in America, 27.8% in Europe and 22.2% in Africa with only 5.6% of these made in Nigeria while none were made in Asia or the unidentified continent.

74% of the emulsions had expiry dates indicated on their packaging. 61.5% of those with no expiry date indicated were made in America.

Various additives were indicated as included in the emulsion formularies. These range from preservatives such as propyl paraben included in 92% of the emulsions, through waxes like dimethicone in 50% to de-pigmenting agents like hydroquinone in 36% and exotic components like serum albumin bovine in 2% and many others.

Quantitative Analysis

34% contain any of the three de-pigmenting agents, while 50% contained just hydroquinone, 2% kojic acid alone and 2% only l-ascorbic acid. Both hydroquinone and kojic acid were present in 8% of the samples, and only 4% contained the three additives. Levels of occurrence of the additives were 0.1 – 4,9% hydroquinone, 0.3 – 1.3 % kojic acid and 0.01 – 3.7% ascorbic acid.

Discussion

From the results of the analysis of the manufacturer claims, it can be seen that the cosmetic emulsions for skin use sold at the outlets sampled were obtained from a good spread of continents of the world. This is an indication of the desire of the management of the outlet to satisfy consumers from various countries of the world cutting across various social strata and consumer preferences. The expectation is that more of the emulsions will have been manufactured in Nigeria since the sampling was done in one of its cities. The departure from this norm may be an indication of the state of the cosmetic industry in the country and/or the desire of the populace for articles manufactured in other countries with the belief that they are of better quality which may not necessarily be the case. In terms of actual numbers however, there are more of the emulsions made in Nigeria than any other country of manufacture. The manufacturers indicated that they have included various additives in their products apart from the usual components like water, oil, colour and fragrance. The various additives included by the manufacturers may be an indication of the desire of the manufacturer's to increase the biological functions of the emulsions [14]. These additives were commonly included by manufacturers from the different countries at the usual levels in which they are found in cosmetic products [24].

The large percentage (74%) of the emulsion that had expiry date indicated showed that the most of the manufacturers understood the importance of expiry dates as an indication of the shelf life of these emulsions. The shelf life of any cosmetic emulsion is usually 30months. This is the length of time that the emulsion is expected to be stable for once produced. It also serves as a guide to how long the marketers and consumers alike can keep the emulsions in storage and use before discarding.

The indication of % additives added by some of the manufacturers may be taken as an indication of attempts to comply with regulations by standard organizations i.e. National Agency for Drugs Administration and Control (NAFDAC) and Standard Organization of Nigeria (SON) in Nigeria [47].

The quantitative analysis of the de-pigmenting agents showed that all the three skin de-pigmenting agents analysed for were used but not at level indicated by different manufacturers (Table 1). Hydroquinone appears to be more commonly used by these manufacturers (66%). This result agrees with reports by other researchers concerning the prevalent use of hydroquinone by cosmetic formulators [17]. This may also be attributed to the fact that this chemical has been known to have skin de-pigmenting properties for a longer period of time than the others; or as a result of the fact that it is known to have no permanent effect if used at the proper level [20] recommended by regulatory bodies. It could also be that the chemical was used as an antioxidant as it can act in such a capacity as well [46]. All creams containing ≥2% hydroquinone were already classified as toning/fade creams with the exception of one cream made in the United States of America (USA) which contained 2.4% and was classified moisturising . One of the emulsion made in Nigeria (BTM'10) classified as fade cream did not contain any of the depigmenting agent analysed for. It may have contained other depigmenting agents like arbutin, mequinol and so on. The emulsion that contained Hydroquinone were mostly from Africa (28%) followed by USA (22%) followed by Europe (8%) while only 2% from Asia and unidentified. Samples from Cote de' Voire contained higher concentrations of hydroquinone which were greater than 3% in all cases except one. The manufacturers did not indicate their use of kojic acid and l-ascorbic acid

In conclusion, this study showed that hydroquinone is the major skin depigmenting agent in cosmetics for skin conditioning in the Nigerian market. 22% of the manufacturers used this additive at levels higher than the regulatory limits of 2%. 64% of these manufacturers consisting of 55% Cote de'Voire +9% others are from Africa. Only one LSM'10) manufacturer from Nigeria included hydroquinone at level above regulatory limit.

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