HEAVY METAL CONTENT AND ANTIMICROBIAL ACTIVITIES OF SOME NATURALLY OCCURRING FACIAL COSMETICS IN NIGERIA

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ABSTRACT

Cosmetics are products, chemical or natural applied to the face or body to improve appearance. However, concern for our physical appearance must be balanced with finding conscientious means by which to care for our skin and our health in general. Naturally occurring facial cosmetic samples (calabash stone and black antimony) were assessed for their heavy metal contents and antibacterial activities. The samples were digested using aqua regia (HNO_3 and HCl). The digested samples were analyzed for lead, cadmium, chromium and zinc using atomic absorption spectrophotometer (AAS) Perkin Elmer Analyst 200. The cosmetics were screened against selected Gram positive and Gram negative bacteria using agar broth diffusion assay. Calabash chalk is relatively safer to use compared to black antimony which has higher concentrations of the metals investigated. The range of the geometric mean for the cosmetics samples were found to be: Pb, 3.74-171.14μg/g; Cd, 0.10-0.56 μg/g; Cr, 0.39-7.69 μg/g; and Zn, 0.07-251.60μg/g. Zinc and Lead were high in black antimony. Calabash chalk possessed moderate antibacterial activities against *Staphylococcus aureus, Staphylococcus epidermidis,* and *Streptococcus pyogene*s but showed no bioactivity against *Pseudomonas aeruginosa*. Black antimony, however, showed considerable antibacterial activity even against *P. aeruginosa*. These cosmetics when used continuously, entirely or as additives in cosmetic or personal care products will expose users to low level metal poisoning. *Bioccumulation* of the metals may lead to deleterious human health effects. The use of black antimony as cosmetics should be discouraged because of the high content of lead and cadmium. Further study to explore the potential antibacterial activity of the cosmetics materials is suggested.

Key words: Heavy Metals, Lead, Cadmium, Cosmetics, Antibacterial, Health Effects

INTRODUCTION

Cosmetics are products applied to the body for the purpose of cleansing, beautifying or improving appearance and enhancing attractive features (Singh, 2010). These products include cold cream, hand cream, eye cream, astringent, after shave lotion, talcum powder, body paints, shampoo, conditioner, hair mousse, styling gel. A subset of cosmetics that has to do with beautifying of the face is regarded as 'makeup' and examples of this are eye brow, eye pencil, mascara, eye shadow, eyeliner, rouge blusher, tone lipstick, lip gloss, loose powder and pancake (Claeyssens, 2009). The use of cosmetics dated back to around 10000BC (Price, 2001). In ancient times, the Romans, Greeks and Egyptians used various cosmetics which contain mercury and white lead, (Claeyssens, 2009). One of the Ancient Egyptians wide ranges of make-up tools is Kohl (Sormeh), which was used to outline the eyes. Kohl is made up of lead, copper, burned almonds, soot and other ingredients (Al-Hawi, 1986).

Cosmetics in ancient times symbolized status (Claeyssens, 2009). Rich people applied minerals to their faces, skin and used oiled-based perfumes in their bath. Scented oils and ointments were used to clean and soften the skin and mask body odor while dyes and natural paint were used to color the face, mainly for ceremonial and religious occasions (Price, 2001). It was also a common believe that eye makeup could ward off evil spirits and improve sight (Claeyssens, 2009). Today the use of cosmetics cuts across people of all spheres of life. Facial cosmetics are a necessity in the entertainment industry where hundreds of new recruits are daily added to the vast army of cosmetics users with a paucity of definite knowledge of the effects of cosmetics. Some of the cosmetics used contain varying components like amorphous carbon, zincite, cuprite goethite, elemental silicon or talc hematite, minium, organic compounds (Hardy *et al.*, 1998; 2004) and even heavy metals such as lead.

Black-antimony which was used as eyeliner in ancient Egypt (Badeeb *et al.*, 2008) is still being used by Northerners in Nigeria (Chukwuma, 1997). Black-Antimony (figure 1a) is known locally in Nigeria as "*Tiro, Otanjele* and *Buje*," (George *et al.,* 2006). Black-antimony has been reported to contain up to 81% lead and is mostly harvested from the Abakaliki lead and Zinc mine in the Eastern part of Nigeria. The mineral antimony (Sb) with atomic weight 121.75 is very white and more shining than silver (Biringuccio, 1990). In combination with sulfur in stibnite (Sb_2S_3), it is found within layers of rock where it is excavated. This natural material is usually ground into fine crystalline powder and poured into a metallic cone shaped container from where with the aid of thin stick is used to draw a straight line on the eyes. The local application to the eye is believed to serve as treatment of ophthalmologic infections and as an eye cleanser (Chukwuma, 1997). However, local eye liners have been reported as a suspected source of lead exposure to the occular system in adults and children (Gibbs, 2007).

Calabash chalk also known as Calabar stone (figure 1b) is composed of Aluminum silicate hydroxide from kaolin clay group with the basic formula - $Al_2Si_2O_5(OH)_4$ (Dean *et al.,* 2004, Ekosse and Jumbam, 2010). The natural cosmetic is made up of fossilized seashells, mixed with clay mud and other ingredients such as sand, wood ash and sometimes salt. It is usually sold in blocks, pellets and powder forms (Dean *et al.,* 2004). The calabash chalk is ground into a fine powder and applied to the face as facial powder and antiperspirant which makes the face to remain dry.

Evidence that some cosmetics contain varying chemical components including heavy metals and the report that local eye liners are a suspected source of lead exposure to the ocular system in adults and children (Gibbs, 2007) warranted the evaluation of the selected natural cosmetic for heavy metal contents. On the other hand, some naturally occurring facial cosmetic are believed to treat eye diseases (Al-Hawi, 1986) hence the samples were assessed for their antibacterial activities.

MATERIAL AND METHODS

Cosmetic Clay Sample Collection

The facial cosmetic clay samples were bought from a daily market in Umuoji in Idemili North Local Government Area in Anambra State, Nigeria. They were obtained in their natural state and sun dried in order to reduce the moisture content. The samples were transported to the laboratory where they were ground into powder and stored in ziploc transparent polythene bags at room temperature for further analyses.

Digestion Method

The samples were crushed in acid-washed mortar and pestle. One (1) gram of each processed sample was weighed into a beaker and digested using aqua regia (Sigma-Aldrich). After digestion and cooling, the solution was filtered through a Whatman 41 filter to remove insoluble particles and brought to a final volume of 50ml with de-ionized water. A blank sample was similarly prepared. Analysis of the filtrates using Atomic Absorption spectroscopy (Perkin Elmer Analyst 200) was carried out in the Chemistry laboratory of the University of Lagos, Nigeria. The corresponding wavelengths used for each metal was: Pb, 217.0 nm, Cd 228.9 nm and Zn, 213.86 nm (Baldwin and Marshall, 1999; Moor *et al.*, 2001; Adnan, 2003; Patnaik, 2004).

Microbiological Analysis

Antibacterial assessment of the samples against a range of Gram positive and Gram negative bacteria was carried out in the Medical Microbiology laboratory of Walter Sisulu University, South Africa. The isolates which include *Staphylococcus aureus, S. epidermidis, Streptococcus pyogenes* and *Pseudomonas aeruginosa* were obtained from the Microbiology unit of National Health Laboratory Services, Nelson Mandela Academy Hospital, Mthatha, South Africa. The basis for the selection was the skin as the habitat for *S. aureus* and *S. epidermidis* or the involvement in skin infections of *S. aureus, Strep. pyogenes, and P. aeruginosa.* The broth dilution method as previously described was used for the screening (FDA, 2001; Haydel, 2008). Isolates were cultured using Luria–Bertani (LB) broth and trypticase soy agar (TSA).

The samples already crushed with a mortar and pestle, were sterilized by autoclaving at 121°C for 1 h, before any susceptibility testing. Bacterial strains were grown overnight. The broth culture was standardized to McFarland 10^{-5} and diluted serially at 10^{-1} with fresh medium. One ml aliquots of each dilution of the bacteria culture was plated for total aerobic count on TSA. Fresh bacterial cultures were prepared and standardized to McFarland 10^{-5}. One gram each of the sterilized cosmetic samples was introduced into 9 ml of this broth culture and diluted serially with sterile fresh LB broth. After incubation at 37°C for 18 h to 24 h, 1 ml aliquots of each dilution of the bacteria–cosmetic mixtures were removed and placed into 9 ml of neutralizing LB broth. Cell viability was determined by the plate count method on TSA after 24 h incubation at 37 ºC. The sterility of the autoclaved cosmetic samples was performed by culturing separately in LB broth. Interpretation of growth inhibition was based on Koko test according to Siegert (2005). Culture plates were adjudged as free of growth (-), slight growth (+), moderate growth (++) or heavy growth (+++). Antibacterial activity was reported as a comparable reduction in bacterial count of corresponding concentration of broth culture only and bacteria–cosmetic mixtures.

Statistical Analysis

Statistical analysis of cosmetics parameters were calculated as means and standard deviation of means using Microsoft excel 2007.

RESULTS

The concentrations of heavy metals (μg/g) in the analyzed calabash stone samples are as shown (Table 1). Cadmium, chromium, zinc and lead were detected in all the clay samples in varying concentrations. The concentration of cadmium is generally low compared to other metals and was also not detected in calabash stone.

The level of lead in black antimony was 171.14 μg/g but very minimal (5.13 μg/g) in the calabash stone. Comparative amount of Cr and Zn were also found in the black antimony and calabash chalk while the levels of cadmium determined only for black antimony was much lower than 1 μg/g. The level of chromium was higher (7.69 μg/g) in calabash stone whereas the level of Zn was higher (251.60 μg/g) in black antimony.

The inhibitory activities of the clay materials are shown in Table 2. Inhibition is adjudged as free of growth (-) while slight growth (+), moderate growth (++), or heavy growth (+++) are indications of varying degrees of growth. Calabash chalk possessed moderate antibacterial activities against *Staphylococcus aureus, S. epidermidis, Streptococcus pyogenes* at a dilution of 10^{-1} but showed negligible or no bioactivity against *P. aeruginosa.* Black antimony, however, showed considerable antibacterial activity against all isolates and even against *P. aeruginosa.*

Table 1: Concentration of Heavy Metals (μg/g) in the Locally Used Facial Cosmetics

Metals analyzed	Cosmetic Clay Materials	
	Calabash Stone	Black antimony
Cr	7.69 ±4.41	5.75 ±3.33
Cd	ND	0.53 ±4.19
Zn	2.32 ±4.19	251.60 ±5.00
Pb	5.13 ±9.08	171.14 ±5.85

Table 2: Inhibition of Bacterial Isolates by Cosmetic Clay Extracts

Bacteria	Broth culture only (mg/ml)				Inhibitory activity							
					Calabash chalk culture (mg/ml)				Black antimony culture (mg/ml)			
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}
SA	+++	+++	+++	++	+	++	+++	+++	_	+	+++	+++
SE	+++	+++	+++	++	+	+	+++	+++	_	++	+++	+++
SP	+++	+++	++	++	+	+	+++	+++	_	_	++	+++
PA	+++	+++	+++	++	++	+++	+++	+++	_	++	+++	+++

Legend

Bacterial identity:

SA= *Staphylococcies aureus*SP= *Streptococcus pyogenes*

SE= *Staphylococcus epidermidis*PA= *Pseudomonas aeruginosa*

Assessments:

free of growth (-)moderate growth (++)

slight growth (+)heavy growth (+++).

Fig 1a. Black antimony Used to define the eyes and create a variety or aesthetic illusion

Fig 1b. Calabash chalk Used as facial powder, antiperspirant and sun block

Figures 1a and 1b: The Locally Used Facial Cosmetics of Interest

DISCUSSION AND CONCLUSION

The use of some of the calabash stone samples investigated in this study may pose a substantial risk of heavy metal poisoning especially those containing black antimony. The high concentration of Zn, 251.60 μg/g reported in black antimony is similar to an earlier study where Zn was found to be as high as 35.8% in ornamental lead which women used to adorn their eye lashes in Nigeria (Ajayi *et al*., 2002). Zinc is not basically of toxicological significance, since it is one of the essential nutrients or trace metals needed in the human body. Evidence has shown that Zn is necessary for oxygen metabolism and mitochondrial function. It enhances the molecular chaperone function and stability of Alpha-crystallin (Biswas and Das, 2008). Contrariwise, Cr has been described as a notable allergen (Kjuus *et al*., 2003). The regulations relating to cosmetic products give no limit values for heavy metals in cosmetic products except of 1 ppm for Hg (ACSB, 2007). The concentration of Cr was generally low, less than 8 μg/g while the highest level was found in Calabar stone. The low Cr levels may not likely pose a toxicological harm to the users of the facial cosmetic.

A high concentration of Pb 171.14 μg/g was noted in black antimony, making it grossly unacceptable for use as cosmetic. This was similar to that of the lead bare Kwali eye make-ups which has been reported to contain 60% lead (Funtua and Oyewale, 1997). Lead is readily inhaled or ingested in the body and can be found in the blood, in soft tissue and in bones, where it can remain for decades. The element disrupts calcium metabolism, development of chemical communication between neurons in the brain, and cellular activity (Gavaghan, 2002). In spite of the relatively low Pb concentration of 5.13 μg/g observed in Calabar stone, the fact that it is a geophagic substance eaten in large quantities especially by pregnant women and children (Bisi-Johnson *et al.,* 2010a) makes exposure to low level Pb poisoning a possibility (Hunter, 1973). Considering the fact that the Pb exposure is through direct ingestion, the health implications due to this geophagic tendencies for Calabar stone could be on the high side (Bergback *et al.,*1992). Gestational Pb poisoning is detrimental both to a pregnant woman and her developing fetus, invariably producing congenital lead poisoning (Nnorom *et al.,* 2005). Pb is a systemic toxicant with no known beneficial biological function and, for several endpoints, no identified threshold of toxicity (Healey, 2009).

Significantly higher levels of Cu, Pb and Cd have been observed in human lenses of people suffering from cataract. The use of eye cosmetics may be a major source of these metals in human lenses (Cekic, 1998). The applications of local preparations containing Pb in the treatment of opthalmogic infections, as an eye cleanser and in cosmetic have been identified as a major source of Pb in take in Africa (Chukwuma, 1997). The continuous use of these cosmetics may increase the absorption of Pb and Cd into the human body. A report by Centre for Diseases Control (CDC, 1991) suggests that even a blood level of 10 μg/dl of Pb can have harmful effects on children's learning and behavior. The determination of toxicity thresholds and an understanding of the mechanisms underlying toxicity would be required indices (Smith and Legal, 1995). The cosmetics, except for black antimony, may however be relatively safer to use when compared to most imported facial cosmetics commonly used presently in Nigeria (Omolaoye *et al.,* 2010).

Even though the chemical analysis of the cosmetic samples in this study showed the presence of detrimental heavy metals, this is not enough to condemn these natural products. It is noteworthy that some of these cosmetics exhibited considerable antibacterial activities. Based on the Koko test interpretation of Siegert (2005) inhibition of the growth of *S. aureus* was achieved at a concentration of 1g calabash chalk in 9 ml broth. However, at the same concentration, black antimony completely inhibited all the three Gram positive bacteria including the Gram negative, *P. aeruginosa.* While *S. epidermidis* and *S. pyogenes* were further inhibited at a much lower concentration in both cases; only the highest concentration of black antimony exhibited antibacterial effect against *P. aeruginosa.* French green clay materials have been reportedly used as cure for skin diseases such as Buruli ulcer described as a necrotizing fasciitis caused by *Mycobacterium ulcerans* (Williams *et al.,* 2004; 2011).

The probable mechanism by which these clay materials function as antibacterial has previously been described. According to Cohn *et al*. (2006), the presence of pyrite in some samples may be important for bactericidal action. Williams *et al.* (2011) observed that *E. coli* killed by aqueous leachates of antibacterial clay indicated elevated intracellular concentrations of Fe and P relative to controls. It was deduced that extracellular processes do not cause cell death; rather, Fe^{2+} overwhelms outer membrane regulatory proteins and is oxidized when it enters the cell, precipitating Fe^{3+} and producing lethal hydroxyl radicals. Apart from providing alternative sources of antimicrobials which may be harnessed to curb escalating problem of drug resistance (Bisi-Johnson *et al.,* 2010b), these natural mineral substances also function as detoxifiers or chelating agents. Clays and exfoliating scrubs are said to be used for cleansing the skin of external and internal sources of toxins (Medical Insider, n.d). Hence, there may be justifications for the use of these natural mineral substances as cosmetics.

The content of Pb determined in the facial cosmetics was greater in black antimony than Calabar stone. This study has revealed that the continuous use of black antimony for cosmetic purposes could result in an increase in the trace metal levels in human body beyond acceptable limits. An in-depth health implication of the metals may require monitoring the levels of these toxic metals in the blood and urine sample of the group engaged in the practice. Efforts should be made at enlightening the users and the general public on the dangers involved in the use of clay material contaminated with heavy metals. However, the local cosmetics could find use as antimicrobials owing to the antibacterial properties possessed; hence, efforts should be directed at research which could help render these natural products safer by way of incorporating substances that can bioutilize or bioremove the unwanted metals.

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