

Evaluation of cosmetic lipsticks for hazardous heavy metals and determination of antimicrobial potency

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ABSTRACT

Background: Lipstick, a cosmetic product containing pigment, wax materials, oils and emollient that apply color, is the most widely used cosmetic make-up to enhance the beauty of lips. Consciously or unconsciously, lipsticks have cast a spell over cultures for years and its possible health implications on the consistent wearer's remain a subject of controversy. This study evaluated commercial lipsticks purchased from selected beauticians' shops in Ibadan for antimicrobial potency and hazardous heavy metals.

Methods: One gram (1g) of representative lipsticks samples was weighed on analytical weighing balance and dissolve in 10 mL of acetone. A stock concentration of 100mg/mL was prepared using 50% acetone as diluents. Thereafter, 5mL of the stock was pipette in to 5mls of 5% acetone to make a concentration of 50mg/mL. A quantity of 0.8g of each representative samples were weighed and 8mL of HNO₃: HCl (1:3) were added to the samples in each beaker. The samples were heated and the preparation was allowed to cool and filtered to removed undissolved waxy materials, while the digested solutions were made up to mark 40 ml with sterile distilled water. The sample solutions were analyzed for Cr, Pb, Cd, Mn, Fe and Zn using Flame Atomic Absorption Spectrophotometer. Culture of *E. coli*, *S. aureus*, *K. pneumonia*, *Streptococcus sp.* and *Pseudomonas aeruginosa* seeded in molten Mueller Hinton agar were challenged with (100mg/mL and 50mg/mL) concentrations of selected acetone dissolved lipsticks samples using agar well diffusion technique.

Results: Lead was found in varied concentrations in all the 15 samples examined, while Cadmium, Magnesium, Zinc and Iron were found in 7 of the 15 samples, Chromium was found in 3 of the total samples examined. Thirteen (13) of the 15 samples of lipsticks examined exhibited antimicrobial property against *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella spp* at either 100 mg/mL and/or 50mg/mL.

Conclusion: The degree of heavy metals detected from the samples examined could be inimical to user's health coupled with the susceptibility of some of the lipsticks sample to bacteria of clinical potential. There is therefore a need for extensive testing to assess and assure the efficacy of lipsticks regularly before delivery to markets.

1. Introduction

Lipstick is a cosmetic that applies color, texture and protection to the lips. It is made of waxy fabric wall of different colors, varied composition, concentrations and additives packaged in cylindrical metal applicators tubes. Lipsticks, in particular, have been used by humans for over 500 years and was first introduced in France in 1869 as a cosmetic product made from animal fat and beeswax.¹ The rationale for wearing lipsticks varied from one users to the others, why some use it on some occasion, some wear it regularly on daily basis as a grooming rituals while some are due to the mentality of women's emulation of beauty images portrayed in mass media and its strong influence in how women want to look. As lipsticks enhance the beauty and attractiveness and confer social, psychological, and therapeutic benefit to the users, it can also pick up aerosolized microbes from the environment that can metabolize lipsticks ingredients or bacterial of gastrointestinal origins from voluntary tongue movement. Regular usage of synthetic lipsticks products that contains ingredients such as phthalates petrolatum and lead may cause harm to the users. Also, the dark damp tubing of lipstick is an ideal breeding ground for bacteria, even if it hasn't encounter people or surfaces that are infected. A recent survey discovered that one in five women keep their makeup well past its expiration date, which gives birth to bacteria that causes serious illness. Lipsticks are also used to lubricate lips².

The prices of lipsticks depend on consumers choice and those that are currently available in the markets includes; BeyondBeauty®, BeautylinebyDidi®, ClassicMakeup®, AvourCosmetics®, Zaron®, Nubanbeauty®, IMAN®, Romantic®, Beyond beauty®, First class®, House of Tara®, and LA Girl Matte®, The products are made in different colors and had longevity of use than each other's and some are fortified with herbs or vitamins. A brand of lipstick could contain four or more color separation, but to maintain a permanent pink lips Aloe Vera H3 lipstick is the choice which must be maintained with pink lips ointments.³

Lipsticks contain wide range of the ingredients made from natural sources, chemicals sources, and a mix of both. Nevertheless, synthetic base lipstick ingredients and natural base lipsticks

ingredients that had serious adverse reaction on human health are readily available in market. For example, the presence of lead in lipsticks and coloring ingredients is one of the most serious issues.⁴ It is difficult to keep track of safety level of every cosmetic products due to the

inestimable large volume of products released into the markets on monthly/yearly basis because some of these product may have been contaminated from production line, storage vaults, careless retailers, poor transportation and logistics failures and contaminants could be substances of carcinogenic potentials, microbial metabolites or inherent biochemistry of composition of the products.⁵

Though the medicated lipsticks are fortified with medicinal ingredients to provide protection against bacteria, bacteria still develop mechanisms to metabolize these ingredients for its survival. Metals are naturally occurring elements in the earth crust, their contents vary from one geographical zones to the others and hence their spatial variations of background concentrations. Metals that has atomic weight higher than 40.04(the atomic mass of CA) and a specific gravity of $>5\text{g/cm}^3$ are called heavy metals and of the 92 naturally occurring elements, 30 of such metals and metalloids are potentially toxic and harmful to humans once the safety level had been violated. The possibility of licking could not be prevented and as a result of this, lipstick could exposed its users to neurotoxic chemicals such as lead and other hazardous heavy metals which are harmful to the users and fetus in case of pregnancy. Therefore, consumers need to take precautions, as regular use of synthetic- based lipsticks might be irritating, allergic and threatening to health of the consumers because these products are not always be made available on regular basis to the regulatory agency for examination and approval, once they obtained a register numbers for a product, it could still be used to covered unregistered products.⁶ Therefore, this study was carried out to examine the antimicrobial potency and determine the hazardous metals components of some selected lipsticks obtained in selected markets in Ibadan.

2. Materials and Methods

2.1 Materials

Lipsticks brands; BeyondBeauty®, BeautylinebyDidi®, ClassicMakeup®, AvourCosmetics®, Zaron®, NubanBeauty®, Julia Rose®, IMAN®, Romantic®, First class®, Taos beauty®, House of Tara®, and LA Girl Matte®. Electronic weighing balance, beakers, Petri-plates, incubator, cork borer, spectrophotometer (210 VGP AAS, BUCK SCIENTIFIC, East Norwalk, USA) hot plate, test organisms and reagents; nitric acid, hydrochloric acid, and acetone.(Sigma Aldrich Germany)

2.2 Study Area and sample collection

The study was carried out in Ibadan situated in-between the

latitude 7.3964 and longitude 3.9167 co-ordinates in Oyo state of Nigeria and are predominantly occupied with Yoruba speaking people. Five (5) brands of unexpired lipsticks (Beyond Beauty, Julia Rose, Taos Beauty, IMAN and Romantic) of three different colors (pink, purple and red) were purchased from salon shops in Ibadan and transfer aseptically to the laboratory for chemical evaluation and microbial analysis

2.3 Digestion of the samples for hazardous metal ions

0.8g of each representative samples were weigh into varied beakers and 8mL of HNO_3 :HCl (1:3) were added to the samples in each beakers. The samples were placed on hot plate in a fume cupboard and were heated. The preparation was allowed to cool and filtered to removed undissolved waxy materials, while the digested solutions were made up to mark 40 ml with sterile distilled water. The sample solution was analyzed for Cr, Pb, Cd, Mn, Fe and Zn using Flame Atomic Absorption Spectrophotometer (210 VGP AAS, BUCK SCIENTIFIC, East Norwalk, USA). Biochemically confirmed bacteria isolates from clinical sample of skin infection was collected from routine laboratory bench from the department of pharmaceutical microbiology, Olabisi Onabanjo University. The isolates include *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella sp.* and *Streptococcus sp.*

2.4 Preparation of the samples (lipsticks) for microbial challenge test.

One (1g) of representative lipsticks samples was weighed on analytical weighing balance and dissolve in 10 mL of acetone. A stock concentration of 100mg/mL was prepared using 50% acetone as a diluent. Thereafter, 5mL of the stock was pipette in to 5mL of 5% acetone to make a concentration of 50mg/mL

2.5 Antimicrobial assay

The antimicrobial activity of selected lipsticks was carried out using agar diffusion technique. Molten Mueller Hinton agar prepared according to manufacturer specification was seeded with 0.2mL of 1:100 24 hours culture of each bacteria isolate. A sterile cork borer of 8mm wells were punched into the agar medium. The varied concentrations (100mg/mL and 50mg/mL) of selected lipsticks were introduced into the wells and were allowed to stand on the bench for 1 hour to allowed pre-diffusion before incubation at 37°C for 24 hours. The zones of growth inhibition were recorded as a measure of antimicrobial activity.

3. Results

A total of 15 lipsticks samples made up of 3 representative sticks in each brand in 3 different colors (purple, red and pink) namely BBL, JRL, TBL, IML and RML examined elicited varied concentration of heavy metal. Pb were detected in all the 15 samples with the minimum and maximum concentration range of 17.04 ± 0.02 ppm and 29.70 ± 0.03 ppm, which exceeded the permissible concentration 0.01ppm. Cadmium were detected in 7 of the 15 samples examined, with the minimum concentration of 0.02 ± 0.10 ppm and maximum concentration of 0.10 ± 0.02 ppm which exceeded the permissible concentration of 0.023ppm. Magnesium were detected in all the 15 samples with the minimum and maximum concentration of 161.86 ± 0.04 and 1189.73 ± 0.04 which was below the permissible concentration of 612ppm. Chromium were detected in 3 of the 15 samples within the minimum and maximum range of 6.82 ± 0.19 and 7.54 ± 0.08 ppm which exceeded the permissible limit of 1ppm while Zn and Fe were recorded in all the 15 samples examined with varied concentration of 1.28 ± 0.12 and 363.2 ± 0.16 ppm and 6.13 ± 0.05 and 97.63 ± 0.04 ppm which exceeded the permissible limit of 100ppm and 36.2ppm as showed in Table 1. The average of the minimum and maximum detectable concentrations results from flame absorption spectrophotometer of the examined metals (Pb, Cd, Mg, Cr, Zn and Fe) statistically summarized in Table 2. Pb, Mg, Zn, Fe were detected in all the 15 samples of lipsticks examined while Cd and Cr were detected in 7 and 3 of the samples of lipsticks examined. The average concentration of the 6 heavy metal examined were recorded ; Pb 24.175ppm, Cd 0.023ppm, Mg 612.0ppm, Cr 1.36, Zn 103.05 and Fe 43.39 as showed in Table 2. Figure 1 elicited the comparative graphical values of the standard permissible limit and average detectable limit of each of the 6 heavy metals obtained from lipsticks samples in ratios of; Pb 0.01ppm :24.175ppm, Cd 0.003ppm: 0.023ppm, Mg 595.3 ppm: 612.0ppm, Cr 1ppm: 1.36ppm, Zn 100ppm:103.5ppm and Fe 36.2ppm :43.39ppm. Different zones of growth inhibition of the isolates of bacteria , that were used to challenge 2 different concentrations (100mg/mL and 50mg/mL) of the acetone dissolved lipsticks samples examined indicated the antimicrobial efficacy of the tested lipsticks as showed in Table 3. Few of the lipsticks lack antimicrobial efficacy which were indicated by zero non inhibitory potential of the lipsticks.

Table 1: Concentrations of heavy metals from flame absorption spectrophotometry [ppm±SD]

Brands	Colours	Pb	Cd	Mg	Cr	Zn	Fe
BBL	Purple	17.04 ± 0.02	0.02 ± 0.10	275.42 ± 0.02	6.82 ± 0.14	12.8 ± 0.12	26.83 ± 0.10
BBL	Red	18.56 ± 0.05	0.00 ± 0.00	742.66 ± 0.42	0.00 ± 0.00	58.56 ± 0.02	29.12 ± 0.02
BBL	Pink	19.31 ± 0.06	0.00 ± 0.00	816.58 ± 0.05	0.00 ± 0.00	50.56 ± 0.02	19.25 ± 0.06
JRL	Purple	21.62 ± 0.05	0.00 ± 0.00	613.34 ± 0.18	0.00 ± 0.00	51.84 ± 0.05	70.24 ± 0.05
JRL	Red	23.60 ± 0.02	0.00 ± 0.00	1189.73 ± 0.04	7.54 ± 0.08	79.04 ± 0.13	55.46 ± 0.15
JRL	Pink	24.13 ± 0.03	0.00 ± 0.00	393.70 ± 0.09	0.00 ± 0.00	294.08 ± 0.04	46.77 ± 0.18
TBL	Purple	25.46 ± 0.02	0.00 ± 0.00	307.87 ± 0.05	0.00 ± 0.00	49.36 ± 0.02	56.46 ± 0.00
TBL	Red	25.34 ± 0.03	0.00 ± 0.00	1088.45 ± 0.05	0.00 ± 0.00	43.52 ± 0.01	48.21 ± 0.07
TBL	Pink	24.18 ± 0.03	0.00 ± 0.00	861.02 ± 0.02	0.00 ± 0.00	199.36 ± 0.06	39.06 ± 0.14
IML	Purple	26.30 ± 0.01	0.02 ± 0.03	806.79 ± 0.23	0.00 ± 0.00	141.12 ± 0.06	34.61 ± 0.02
IML	Red	26.34 ± 0.03	0.08 ± 0.02	258.34 ± 0.04	5.97 ± 0.06	16.32 ± 0.02	56.32 ± 0.02
IML	Pink	25.26 ± 0.05	0.04 ± 0.06	949.25 ± 0.20	0.00 ± 0.00	145.28 ± 0.02	33.50 ± 0.06
RML	Purple	28.11 ± 0.04	0.10 ± 0.02	367.58 ± 0.04	0.00 ± 0.00	14.72 ± 0.10	6.13 ± 0.05
RML	Red	27.68 ± 0.03	0.06 ± 0.04	347.42 ± 0.04	0.00 ± 0.00	25.92 ± 0.03	97.63 ± 0.04
RML	Pink	29.70 ± 0.03	0.02 ± 0.01	161.86 ± 0.04	0.00 ± 0.00	363.2 ± 0.16	31.23 ± 0.08

Table 2: Statistical summary of Concentrations of heavy metals from flame absorption spectrophotometry

Parameter	Pb	Cd	Mg	Cr	Zn	Fe
Number of Samples	15	15	15	15	15	15
Number of detectable metal from the total samples	15	7	15	3	15	15
% of Samples with detectable metal	100%	46.67%	100%	20%	100%	100%
Minimum Concentration detected (ppm)	17.04 ± 0.02	0.02 ± 0.01	161.86 ± 0.04	5.97 ± 0.06	12.8 ± 0.12	6.13 ± 0.05
Maximum Concentration detected (ppm)	29.70 ± 0.03	0.10 ± 0.0	1189.73 ± 0.04	7.54 ± 0.08	363.2 ± 0.16	97.63 ± 0.04
Average Concentration (ppm)	24.175	0.023	612.0	1.36	103.05	43.39

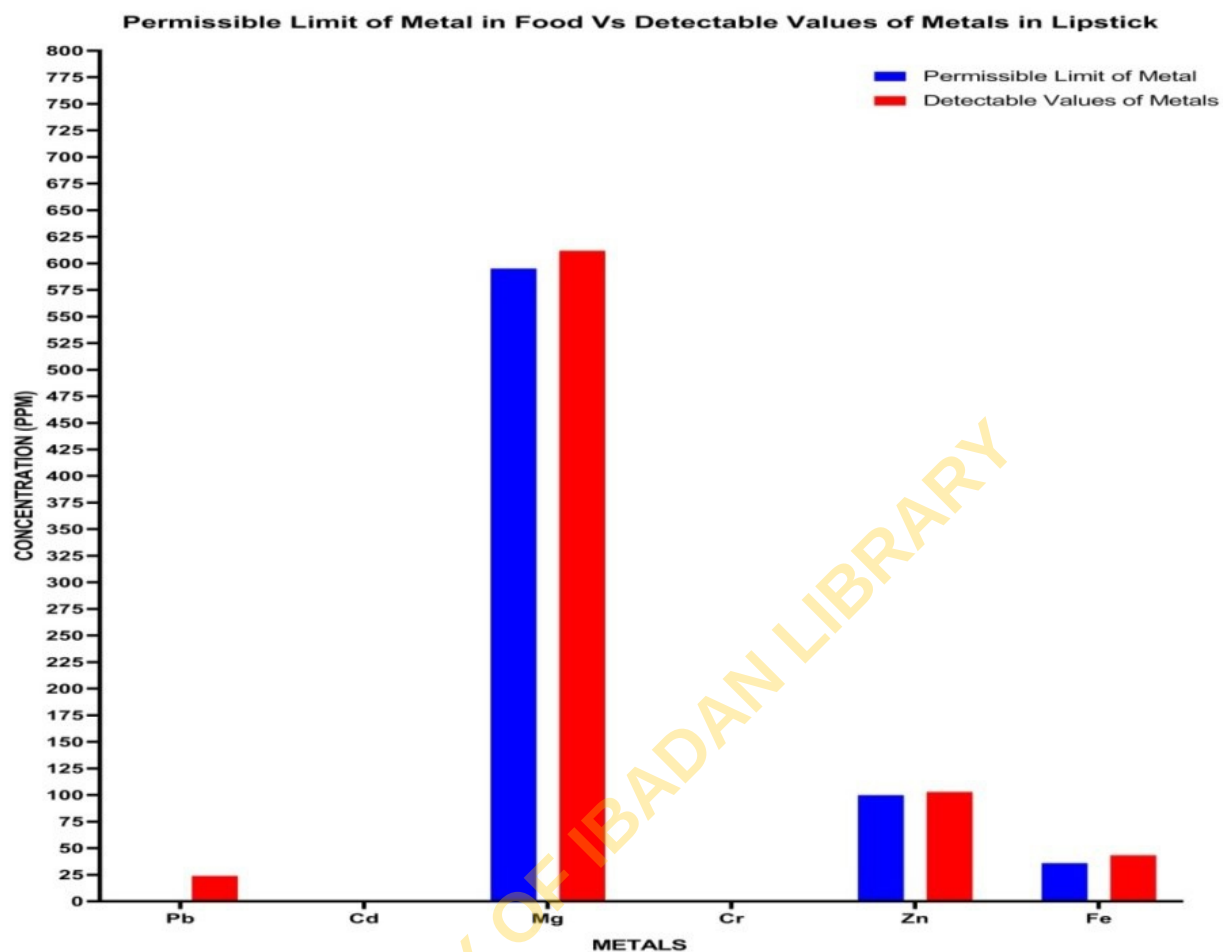


Figure 1: Permissible values of heavy metal versus detectable values.

Table 3: Microbial challenge test on 15 new lipstick samples (concentration in mg/mL)

Clinical isolates	BBL	BBL	NBL	NBL	TBL	TBL	IML	IML	IML	BBL	BBL	BBL	-Ve	+Ve
	100	50	100	50	100	50	100	50	100	50	100	50	100	50
<i>E. coli</i>	20	15	16	0	18	12	18	11	23	16	12	10	20	12
<i>E. coli</i>	14	10	24	0	18	10	17	14	12	10	18	14	18	12
<i>E. coli</i>	11	7	20	0	11	10	20	12	18	12	14	10	29	17
<i>E. coli</i>	20	13	12	0	21	12	23	15	26	18	20	12	20	12
<i>E. coli</i>	14	10	18	0	23	12	24	16	20	14	23	11	20	12
<i>S aureus</i>	28	14	22	18	25	15	30	18	26	15	25	13	28	13
<i>S aureus</i>	23	12	25	12	23	14	27	15	20	12	26	12	20	16
<i>S aureus</i>	20	13	28	18	20	12	28	16	24	11	20	16	20	14
<i>S aureus</i>	28	14	29	17	23	13	18	12	20	13	18	11	28	16
<i>S aureus</i>	20	12	24	15	20	12	32	16	24	16	20	12	20	12
<i>Kleb spp.</i>	20	0	16	10	18	12	22	14	20	13	25	13	30	16
<i>Kleb spp.</i>	14	0	24	13	18	10	28	14	23	13	21	14	27	10
<i>Kleb spp.</i>	11	0	20	15	11	10	19	13	20	12	16	10	28	12
<i>Kleb spp.</i>	20	0	12	10	21	12	28	17	30	17	23	14	18	10
<i>Kleb spp.</i>	14	0	18	14	23	12	29	12	20	12	18	11	32	16
<i>Strept spp</i>	18	11	23	16	12	10	20	12	24	15	23	16	32	18
<i>Strept spp</i>	17	14	12	10	18	14	18	13	21	14	20	15	26	15
<i>Strept spp</i>	20	12	18	12	14	10	29	16	20	16	28	22	22	17
<i>Strept spp</i>	23	15	26	18	20	12	20	14	24	16	20	13	20	12
<i>Strept spp</i>	24	16	20	14	23	11	16	12	20	14	23	14	31	16
<i>Pseudo spp</i>	28	14	22	0	25	15	18	0	23	16	12	10	22	14
<i>Pseudo spp</i>	23	12	25	0	23	14	17	0	12	10	18	14	28	14
<i>Pseudo spp</i>	20	13	28	0	20	12	20	0	18	12	14	10	19	13
<i>Pseudo spp</i>	28	14	29	0	23	13	23	0	26	18	20	12	28	17
<i>Pseudo spp</i>	20	12	24	15	20	12	24	0	20	14	23	11	29	12

Negative control : 50% acetone Positive control: Gentamicin

4. Discussion

A total of fifteen lipsticks namely; BBL, JRL, TBL, IML and RML, 3 samples per type comprises of 5 brands were assessed for concentration of heavy metals and challenged for antimicrobial potential. Lead was detected in varied concentration in all the 15 lipsticks samples examined. The concentration of lead in each lipstick examined were higher than the WHO permissible recommended concentration⁷. The minimum concentration of Pb in this study was recorded to be 17.04 ± 0.02 in a purple color BBL lipstick while the highest concentration was found to be 29.70 ± 0.03 in a pink color RML lipstick sample. The minimum, maximum and average concentrations of Pb obtained from this study exceeded WHO acceptable safety limit of 0.01ppm in candy sweet frequently licked by children and this portends danger due to its toxicity that could penetrate gradually in to the blood of the users by a process of absorption and bioaccumulation.⁸ Lead a zootoxic metal, has no beneficial effect in humans, it is carcinogenic and nerves damaging with no known homeostasis mechanism⁸. Of the 15 samples of lipsticks that are made up of three sample in each of the 5 brands, cadmium were detected in 7 samples with minimum concentration of 0.02 ± 0.10 in BBL purple color lipsticks and maximum concentration of 0.10 ± 0.02 in RML purple color lipsticks. The minimum (0.02 ± 0.01), maximum (0.10 ± 0.0) and average (0.023) concentration obtained in this study exceeded WHO⁹ recommended safe limit of (0.023), as showed in Table 2, which was similar to the study of [10] on the determination of some heavy metals in selected cosmetic products sold in Kano metropolis in Nigeria. Chronic oral exposure to cadmium may cause kidney and bone damage, this heavy metal are known carcinogens that has been associated respiratory system damages.¹⁰ From the safe limit stand point of this products, cadmium concentration composition in these products could be toxic on the user. Magnesium, a component in the synthesis of Deoxyribonucleic acid [DNA], Ribonucleic acid and glutathione was detected in all the 15 samples examined in varied concentrations, with the minimum of 161.86 ± 0.04 in RML and maximum of 1189.73 ± 0.04 in JRL. The concentration of magnesium obtained in this study were below permissible limit in 7 of the 15 samples examined while the remaining 8 samples were above the safe limit of 595.3ppm as recorded by [11] in a study of biscuit as a source of calcium, magnesium, sodium and potassium in nutrition¹¹. Though, Magnesium are required for structural development of bones in human body, its toxicity increases the risk of kidney mal-functioning and renal failure,

diarrhea and abdominal cramping. Chromium were detected from 3; BBL (6.82 ± 0.14), JRL (7.54 ± 0.08) and IML (5.97 ± 0.06) of the 15 sample examined. The concentration detected were above the safe limit of 1ppm recorded by [12] in the international standard for heavy metals in food. Exposure to chromium can results in formation of ulcers, which could persist for months. When broken skin come in contact with chromium compound, a deeply penetrating hole will be formed¹². Exposure to higher amount of chromium in humans can lead to the inhibition of erythrocyte glutathione reductase, which in turn lower the capacity to reduce methaemoglobin to hemoglobin¹³. Zinc is an essential heavy metal in the human body, and its homeostasis reflects a balance between absorption of dietary zinc and loss of zinc from the body. Zinc were detected in varied concentrations in all the 15 samples tested, the minimum and maximum concentrations of the zinc recorded in this study in BBL and RML lipsticks sample range between 12.8 ± 0.12 and 363.2 ± 0.16 , which exceeded the acceptable safe limit 100ppm^[14] which corroborates the study^[15] on exposure assessment and risk characterization from trace elements following soil ingestion by children exposed to playgrounds, parks and picnic areas¹⁶. Zinc is required for the functional integrity of many organ systems, as well as for growth, development, and tissue repair but on exposure to excessive zinc can be harmful and can have pathological consequences¹⁷. Iron, one of the components in lipsticks contents as a colorants and a necessary ingredients for mitochondria and metabolic function were detected in all the 15 lipsticks examined in this study within the minimum and maximum concentrations range of 6.13 ± 0.05 , 97.63 ± 0.04 as elicited in RML and average of 43.39ppm as shown in Table 2 which exceeded the recommended safe limit of 32.6ppm^[18] in a study of heavy metal contents of potato chips and biscuit from Nagpur city An extremely higher level of iron enters into the body crossing the rate-limiting absorption step and becomes saturated. These free irons penetrate into cells of the heart, liver and brain. Some of the heavy metals composition in lipsticks can gradually accumulated and poison the blood since most of these heavy metals are divalent in nature which could replace useful elements in human body¹⁹. A total of 15 lipsticks samples made of 3 samples each in 5 brands were challenged with bacterial of clinical status to determine their antimicrobial activity at 2 different concentrations 50mg/mL and 100mg/mL respectively. Thirteen of the 15 samples of lipsticks examined exhibited antimicrobial property against *Escherichia coli* at both 100 and 50mg/mL

with the exception of BBL and IML that elicited no antimicrobial activities at 50mg/mL concentrations. *Staphylococcus aureus* were found to be inhibited by 13 of the 15 lipsticks samples with the exception of TBL and 1mL beauty at a concentration of 50 mg/mL that had no antimicrobial activity. Eighty seven percent (87%) of the lipsticks samples examined exhibited antimicrobial property against *Klebsiella spp* with exception of BBL and TBL that elicited no antimicrobial property at a concentration of 50 mg/mL. All the 15 lipsticks samples examined in this study exhibited remarkable antistreptococcal activity while *Pseudomonas aeruginosa*, a nutritionally non-exacting bacterial grew at a concentration of 50 mg/mL in 4 of the lipsticks samples; BBL, JRL, TBL and RML of the 15 lipsticks samples examined. The growth of microbes observed from the samples examined in this study could be due to lapses in production protocol, environmental factors, additives or colorants that are metabolizable by bacteria or non-adherence to the norms of quality control ethics which corroborates the study of Afandi^[19] on the antimicrobial properties of crude aqueous *Hylocereus polyrhizus* peel extracts in lipstick formulation against Gram positive and Gram negative bacteria.¹⁹

5. Conclusion

The results of this study indicated the risks associated with possibility of ingesting heavy metals and microbes in minute amount gradually for daily users, which could be health threatening when accumulated beyond the threshold of their standard safety limit. There is therefore, the need for regular and extensive quality control tests on these products to assure safety compliance before delivery to the end-users.

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