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# MICROBIOLOGICAL QUALITY AND PRESERVATIVE CAPACITY OF COMMONLY AVAILABLE COSMETICS IN ZARIA METROPOLIS, NIGERIA

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***Abstract:*** **Ten brands of hair cosmetics were randomly purchased from shops Zaria metropolis, Kaduna state in Nigeria, and were subjected to microbiological assessment for microbial quality and preservative capacity. The culture media used were Mueller-Hinton agar (MHA), Mueller-Hinton Broth (MHB) and Nutrient Agar (NA). These media were used for Sensitivity Test, determination of MIC and MBC respectively. The MIC of the creams was determined using the tube dilution method. Serial dilution of the creams was carried out in a well labeled test tubes using Mueller-Hinton broth as diluents. The isolates used in this study are *Staphylococcus aureus* and *pseudomonad aeruginosa,* as they are of major concern to the Standard Organization of Nigeria in terms of the microbial assessment of hair creams. Results for the sensitivity test showed that all hair creams under study possessed preservative capacity with DAM having the highest anti-microbial efficacy. All the cosmetics displayed adequate preservative capacity evidenced by ability to lower the inherent bio-burden to acceptable levels and to inhibit growth of the tested microorganisms, with DAM having the best anti-microbial activity. The tested cosmetics cannot have detrimental effect on health status of consumers as consequence of their unaltered stability profiles. Therefore, microbiological quality of cosmetics available in other parts of Nigerian market should be assessed and evaluated for consumer's safety.**

***Keywords:*** **Preservative capacity, cosmetics, microbial quality, anti-microbial quality, microorganisms.**

## 1. INTRODUCTION

Millions of consumers use Cosmetic/Personal Care Products (PCP) and their ingredients on a daily basis. PCP produces local (skin, scalp, and eye) exposure and is used in the oral cavity, on the face, lips, eyes, hair and mucosa result in synthetic exposure. In addition, natural and synthetic substances in PCP may produce local effects in human skin, such as irritation, sensitization or photoreactions. These products must be thoroughly evaluated for their safety prior to their marketing due to the significant and relatively uncontrolled human exposure [1]. Cosmetics prior to the 1960s had a good safety record. However, there are instances when this was not the case. In the classical times through the middle ages up to the early 20th century, make-up dyes contained highly toxic heavy metals, such as Lead, Mercury and Cadmium oxides. In the 1930s, thallium-containing depilatory products caused cases of severe and occasionally lethal intoxications[1]. Halogenated salicylanilide-containing cosmetics produced an epidemic of photo-allergic reactions in the UK and elsewhere during 1958 to 1959 [2], and, in the 1950/60s, Zirconium-containing deodorants and hair creams resulted in an outbreak of long-lasting allergic inflammatory skin and hair scalp reactions in consumers in Europe and the US [3]. Cosmetologists reports that besides the toxicities and other adverse effects associated with usage of cosmetics containing harmful chemicals, these ingredients also reduce the microbial flora on the skin, particularly the gram-positive bacterial [4,5], thus increasing vulnerability to pathogenic microorganisms and to secondary microbial infections acquired from the contaminated cosmetics [6,7]. This is the first study to be conducted with regard to microbiological assessment of cosmetics that are available in Zaria metropolis, Nigeria.

## 2. MATERIALS AND METHODS

Ten (10) majorly marketed hair creams given the code (PX, AT, COC, DAM, FM, APP, SW, SM, S-8 and TS) were used for this study, and for each of these ten (10) hair creams selected, three (3) batches comprising of three (3) samples making a total of thirty (30) samples were collected for this study. The samples were bought from major markets (Sabon-Gari central market, Samaru general market, Tudun Wada and Zaria City markets) in Zaria metropolis, Kaduna state. The test organisms used were clinical isolates of bacteria obtained from Department of Microbiology, Ahmadu Bello University, and Zaria. The isolates used in this study are Staphylococcus aureus and pseudomonad aeruginosa, they are of major concern to the Standard Organization of Nigeria in terms of the microbial assessment of hair creams. The culture media used were Mueller-Hinton agar (MHA), Mueller-Hinton Broth (MHB) and Nutrient Agar (NA). These media were used for Sensitivity Test, determination of MIC and MBC respectively. All media were prepared according to manufacturer's instruction. Various concentrations of the creams 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml were prepared by first weighing 0.5g of each of the hair cream dissolved in 10ml of 50% tween 80 (sterile) to obtain 50mg/ml Concentration. A serial dilution of the solution was carried out in different bottles to obtain 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml. The inocula of the test organisms were prepared by first streaking the organisms on sterile nutrient agar plates to obtain discrete colonies of the bacteria. A colony was picked and sub cultured unto sterile nutrient broth and incubated at 37$^{o}$C for 24hours. From the broth culture a loopful of each bacteria suspension was transferred into bottles containing sterile distilled water to obtain a bacteria density of 300 x 106 Colony Forming Unit per millimeter (cfu/ml) as determined by the McFarland Turbidity Standard Scale no.1.

The standardized inoculants of the bacteria isolate 0.1ml were uniformly streaked unto freshly prepared Mueller-Hinton Agar (MHA) plates with the aid of a sterile cork borer (8mm) diameter. The wells were properly labeled according to the number of different concentrations prepared. The wells were then filled with 0.2ml of the cream solution. The plates were left on the bench for about 1 hour for the creams to diffuse into the agar and they were incubated at 37$^{o}$C for 24hours. After the incubation period, the plates were observed for any evidence of inhibition (Zone of Inhibition), which will appear as a clear zone that were completely devoid of growth around the wells. The diameter of the zones was measured using a transparent ruler calibrated in millimeter. The MIC of the creams was determined using the tube dilution method. Serial dilution of the creams was carried out in a well labeled test tubes using Mueller-Hinton broth as diluents. Each standardized organism was inoculated into each tube containing the diluted cream and the broth. A tube containing sterile Mueller-Hinton broth without an organism was used as control. All the inoculated tubes were then incubated at 37$^{o}$C for 24 hours. After incubation period, the tubes were observed for the presence or absence of the growth using turbidity as a criterion. The lowest concentration in the series without visible signs of growth (turbidity) was considered to be the MIC. The results from the MIC were used to determine the MBC. A sterile wire loop was dipped into the test tubes that did not show turbidity in the MIC test. It was then streaked on fresh nutrient agar plates. The plates were incubated at 37$^{o}$C for 24hours. After the incubation period the plates were observed for the presence or absence of growth. This was done to determine whether the antibacterial effect of the creams is bactericidal or bacteriostatic.

## 3. RESULTS AND DISCUSSIONS

### *3.1 Sensitivity Test against S.aureus and P.aeroginosa*

The efficacy of the cosmetics preservative expressed as zones of inhibition (mm) at different concentration varied in the sensitivity carried out on the two micro-organisms. The entire cosmetic were capable of inhibiting growth of all test micro-organisms but at different concentrations. Careful perusal of Table 1 shows that at a concentration of 50mg/ml, DAM has the highest mean zone of inhibition of 24mm, followed by S-8, 19mm, then APP, PX, COC, and FEM has the same mean zone of inhibition of 18mm, followed by TS, SW and AT having the same value of 17mm, lastly is SM with mean zone of inhibition of 16mm. The trend in decreasing mean zone of inhibition is given as DAM > S8 > APP = PX = COC = FEM > TS = SW = AT > SM with DAM having the highest mean zone of inhibition and the more susceptible the micro-organism will be to it than other hair creams as the bigger the diameter of the inhibition zone, the more susceptible is the micro-organism to the anti-microbial agent. At a concentration of 25mg/ml, DAM has the highest mean zone of inhibition of 22mm, then PX, S-8, and FEM having the same value of 16mm, followed by APP, COC, and AT with same value of 15mm, TS and SW having 13mm each, and lastly SM having a value of 12mm. The trend in decreasing mean zone of inhibition at 25mg/l is given as DAM > PX, S-8, FEM > APP = COC = AT > TS = SW > SM. At a concentration of 25mg/ml, DAM maintained the highest value of mean zone of inhibition. At a concentration of 12.5mg/ml, DAM have

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the highest mean zone of inhibition of 20mm, followed by PX with a value of 13mm, then APP, COC, S-8, FEM and AT all having a value of 12mm, while lastly TS, SM and SW having a value of 10mm. The trend in decreasing mean zone of inhibition is given as DAM > PX >APP = COC = S-8 = FEM = AT > TS = SM = SW with DAM exhibiting the highest mean zone of inhibition at this concentration. At concentrations of 6.25mg/ml and 3.125mg/ml all hair creams showed no mean zones of inhibition with the exception of DAM having mean zone of inhibition of 18mm at 6.25mg/ml and 15mm at 3.125mg/ml., this implies that DAM has the highest preservative efficacy in all the concentrations when compared to other hair cream samples as shown in Figure 1. The results also showed that as the concentration increases, the mean zone of inhibition also increases. Similar results were obtained for the sensitivity test for p.aeroginosa as shown in Table 1, the mean zones of inhibition were also concentration dependent, with the trends in decreasing zone of inhibition at a concentration of 50mg/ml as DAM > APP = PX = COC = S-8 = FEM > TS > SW > FEM > SM, concentration of 25mg/ml as DAM > APP = PX = COC = FEM > S-8 > AT > TS = SM = SW, concentration of 12.5mg/ml as DAM > APP = PX = FEM > TS = COC > SM = S-8 = SW > AT, concentration of 6.25mg/ml and 3.125mg/ml no zone of inhibition was observed for all the hair cream samples. AT showed no zone of inhibition at 12.5mg/ml against *p.aeroginosa* but showed a mean zone of inhibition of 12mm at same concentration this showed that *p.aeroginosa* was resistant at that concentration, compared to *S.aureus* which is susceptible at same concentration.

**Table 1: Sensitivity of Test Organisms to Various Concentrations of the Creams**

| | Mean Zone of Inhibition (mm) at the Varying Concentrations of the creams | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Test Organisms | **Varying concentration (mg/ml)** | **APP** | **TS** | **PX** | **COC** | **SM** | **DAM** | **S-8** | **SW** | **FEM** | **AT** |
| *S.aureus* | 50 | 18.0 | 17.0 | 18.0 | 18.0 | 16.0 | 24.0 | 19.0 | 17.0 | 18.0 | 17.0 |
| | 25 | 15.0 | 13.0 | 16.0 | 15.0 | 12.0 | 22.0 | 16.0 | 13.0 | 16.0 | 15.0 |
| | 12.5 | 12.0 | 10.0 | 13.0 | 12.0 | 10.0 | 20.0 | 12.0 | 10.0 | 12.0 | 12.0 |
| | 6.25 | - | - | - | - | - | 18.0 | - | - | - | - |
| | 3.125 | - | - | - | - | - | 15.0 | - | - | - | - |
| | | | | | | | | | | | |
| *p.aeroginosa* | 50 | 16.0 | 15.0 | 16.0 | 16.0 | 14.0 | 22.0 | 16.0 | 15.0 | 16.0 | 15.0 |
| | 25 | 14.0 | 12.0 | 14.0 | 14.0 | 12.0 | 19.0 | 13.0 | 12.0 | 14.0 | 13.0 |
| | 12.5 | 12.0 | 11.0 | 12.0 | 11.0 | 10.0 | 16.0 | 10.0 | 10.0 | 12.0 | - |
| | 6.25 | - | - | - | - | - | 15.0 | - | - | - | - |
| | 3.125 | - | - | - | - | - | 12.0 | - | - | - | - |

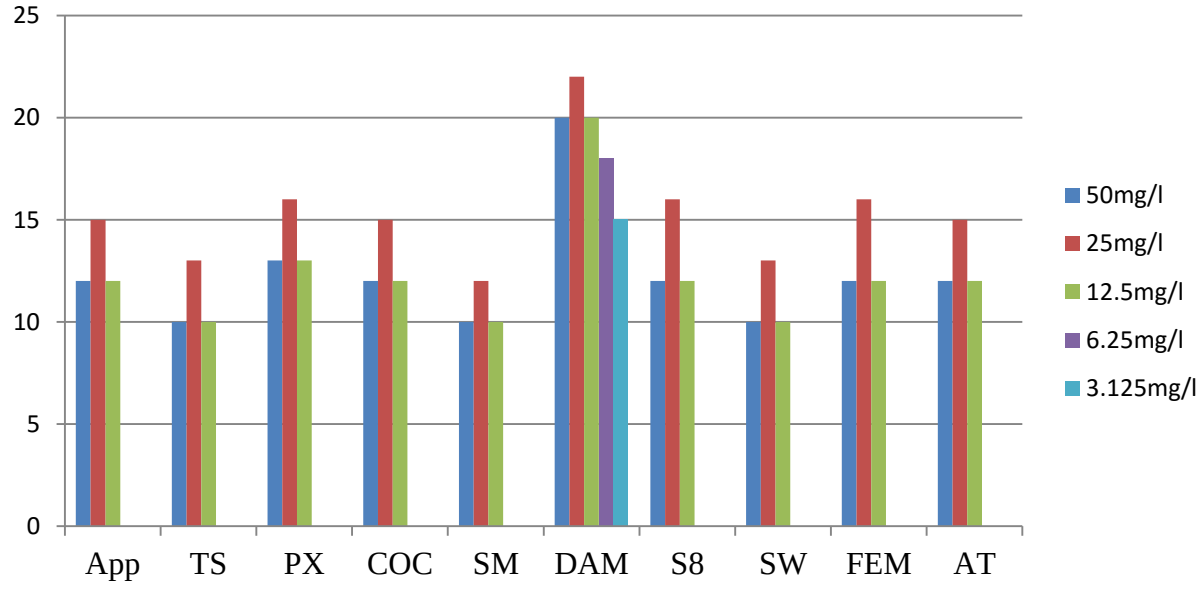


**Figure 1: Mean zones of inhibition for all the hair cream samples**

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**Table 2: Determination of MIC and MBC of the hair cream samples**

| | MIC and MBC at various concentrations of the hair creams | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Test Organisms | **Varying concentration (mg/ml)** | **APP** | **TS** | **PX** | **COC** | **SM** | **DAM** | **S-8** | **SW** | **FEM** | **AT** |
| *S.aureus* | 50 | - | - | - | - | - | - | - | - | - | - |
| | 25 | MBC | MBC | MBC | MBC | MBC | - | MBC | MBC | MBC | MBC |
| | 12.5 | MIC | MIC | MIC | MIC | MIC | - | MIC | MIC | MIC | MIC |
| | 6.25 | + | + | + | + | + | MBC | + | + | + | + |
| | 3.125 | # | # | # | # | # | MIC | # | # | # | # |
| | | | | | | | | | | | |
| *p.aeroginosa* | 50 | MBC | MBC | MBC | MBC | MBC | - | MBC | MBC | MBC | MBC |
| | 25 | MIC | MIC | MIC | MIC | MIC | MBC | MIC | MIC | MIC | MIC |
| | 12.5 | + | + | + | + | + | MIC | + | + | + | + |
| | 6.25 | # | # | # | # | # | + | # | # | # | # |
| | 3.125 | ## | ## | ## | ## | # | # | ## | ## | ## | ## |

**Table 3: Values of Correlation coefficient between physicochemical parameters, Heavy metals and antimicrobial activities against *s.aureus* and *p.aeroginosa* at 95% confidence level**

| | *s.aureus* | | *p.aeroginosa* | |
|---|---|---|---|---|
| Parameter | **MIC** | **MBC** | **MIC** | **MBC** |
| pH | 0.893(0.001) | 0.893(0.001) | 0.893(0.001) | 0.893(0.001) |
| I.V | -0.514(0.129) | -0.514(0.129) | -0.514(0.129) | -0.514(0.129) |
| S.V | 0.212(0.556) | 0.212(0.556) | 0.212(0.556) | 0.212(0.556) |
| P.V | -0.199(0.581) | -0.199(0.581) | -0.199(0.581) | -0.199(0.581) |
| A.V | -0.441(0.202) | -0.441(0.202) | -0.441(0.202) | -0.441(0.202) |
| F.F.A | -0.441(0.202) | -0.441(0.202) | -0.441(0.202) | -0.441(0.202) |
| Pb | 0.412(0.237) | 0.412(0.237) | 0.412(0.237) | 0.412(0.237) |
| Cd | -0.915(0.000) | -0.915(0.000) | -0.915(0.000) | -0.915(0.000) |
| Ni | -0.027(0.940) | -0.027(0.940) | -0.027(0.940) | -0.027(0.940) |
| Zn | 0.322(0.364) | 0.322(0.364) | 0.322(0.364) | 0.322(0.364) |

The p-values are given in parenthesis

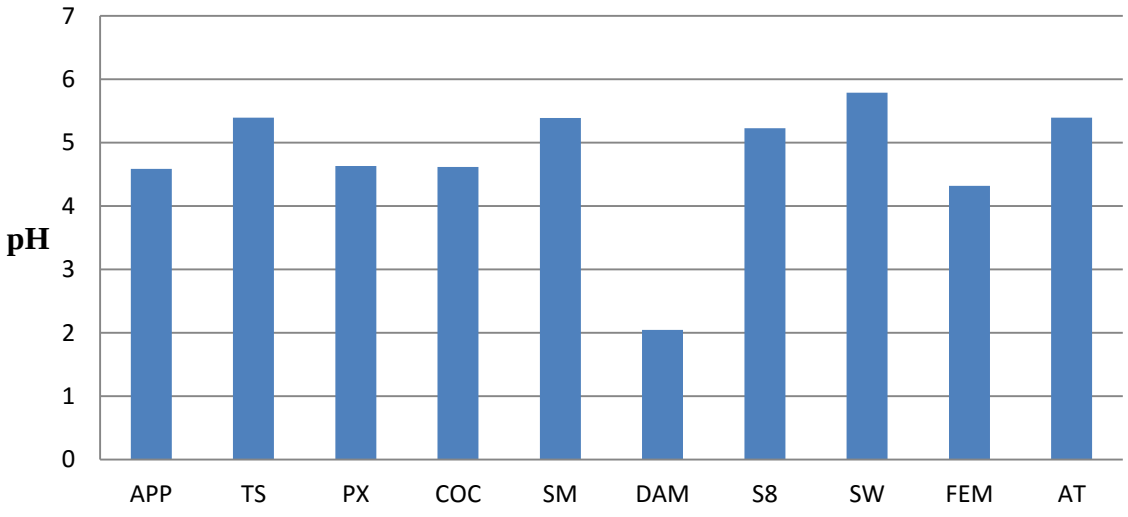


**Figure-2: Distribution of pH among the hair cream samples**

Both micro-organisms were resistant to the anti-microbial activity of the hair cream samples at concentrations of 6.25mg/ml and 3.125mg/ml except for DAM that exhibited a high efficacy at those concentrations against both organisms. All organisms were susceptible to DAM at all concentrations used in this study. Results from this study are also in agreement with reports by [8] on the Microbiological quality and preservative capacity of commonly available cosmetics in Dares Salaam, Tanzania. The sensitivity test carried out against the above two micro-organisms clearly showed that all the hair cream samples under study exhibit anti-microbial activities and to what extent was explained further by the MIC and MBC as shown in Table 1 above.

***3.2 MIC and MBC Test against S.aureus and P.aeroginosa***

In microbiology, minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents [9]. A lower MIC is an indication of a better antimicrobial agent. A MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism [10]. A current definition of the Minimum Inhibitory Concentration, MIC, is "the lowest concentration which resulted in maintenance or reduction of inoculum viability". The determination of the MIC involves a semi-quantitative test procedure which gives an approximation to the least concentration of an antimicrobial needed to prevent microbial growth. In the recent past, the method used tubes of growth broth containing a test level of preservative, into which an inoculum of microbes was added. The end result of the test was the minimum concentration of antimicrobial which gave a clear solution, i.e., no visual growth. The MBC test allows determination of the minimum concentration of an agent necessary to achieve a bactericidal effect. It is worth noting, however, that the duration of time the antimicrobial is in contact with the test organism is quite long for this method, on the order of 18 hours. Thus, the test truly does determine the minimum concentration needed to kill the test organism, when all other parameters are very conducive to biocidal effect.

The MIC and MBC was carried out using each of the hair creams samples at various concentrations say: 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml respectively. Careful perusal of Table-4.5 shows that, at a concentration of 25mg/ml, all other hair creams with exception of DAM showed bactericidal effect and bacteriostatic effect at 12.5mg/ml. Only DAM showed a bactericidal effect at a concentration of 6.25mg/ml and bacteriostatic effect at 3.125mg/ml. In all the hair cream samples, DAM showed the highest bacteriostatic and bactericidal effect against *S.aureus* and since a lower MIC is an indication of a better antimicrobial agent, of all the hair cream samples, it has a better antimicrobial agent. The same trend was observed for the MIC and MBC of the hair cream samples against *p.aeroginosa*, except that for DAM, the bactericidal and bacteriostatic effect was at 25mg/ml and 12.5mg/ml respectively, while other hair creams, showed bactericidal and bacteriostatic effect at 50mg/ml and 25mg/ml respectively, still making DAM with a lower MIC & MBC compared to others having a better antimicrobial agent than them.

At a concentration of 50mg/ml all hair creams tested against S.aureus, had no turbidity growth, while at a concentration of 6.25mg/ml and 3.125mg/ml they all showed slight turbidity/growth and moderate turbidity/growth respectively with the exception of DAM. Against P.aeroginosa, at a concentration of 12.5mg/ml, they all had slight turbidity/growth with the exception of DAM, at 6.25mg/ml, they had moderate turbidity/growth with the exception of DAM with a slight turbidity/growth at this concentration. At a concentration of 3.125, they all had abundant turbidity/growth including DAM. At 95% confidence level, p-value is greater than 0.05, implying that there is no significant relationship between the physicochemical parameters and the antimicrobial activities (MIC and MBC) of the hair cream samples, except for pH which at 95% confidence level, $p < 0.05$, has a significant relationship with the antimicrobial activities of the hair creams. The significant relationship between pH and the antimicrobial activities can be seen in their very strong positive correlation coefficient (r) value of 0.893 and p-value less than 0.05 level of significant as shown in Table 3 above. Similar observation was made with the heavy metals, at 95% confidence level, $p > 0.05$, implying that there is no significant relationship between the heavy metal concentrations and the antimicrobial activities (MIC and MBC) of the hair cream samples, except for Cadmium (Cd) which at 95% confidence level, $p < 0.05$, has a very strong inverse significant relationship with the antimicrobial activities of the hair creams meaning the Cadmium level decreased with an stead increase in the microbial activity. The very strong inverse significant relationship between Cadmium and the antimicrobial

activities can be seen in their very strong negative correlation coefficient (r) value of -0.915 and p-value less than 0.05 level of significant as shown in Table 3 above.

***3.3 Acid Tolerance of Staphylococcus aereus and Pseudomonas aeruginosa***

Bacteria need a physiological pH inside their cells, just like all other living organisms. Their ability to survive in extreme pH (either high or low) depends on their ability to correct for the difference between inside and out. One example of a bacterium that can live in acidic environments is Helicobacter pylori which live in the stomach. It produces high amounts of urease which is an enzyme that degrades urea, and by doing so decreases the acidity (raises the pH). Imagine the bacteria produce a 'cloud' of neutral pH around them to protect them from the acidic environment. There are other bacteria that live in basic pH. *S. aureus* is able to grow in a pH range of 4.0-9.8, with an optimum of 6-7 pH [11]. The minimal values of pH for growth are influenced by other environmental factors. The growth of S. aureus is inhibited by 0.1% of acetic acid and also by the presence of lower (C1-C4) fatty acids [12]. Moreover, *S. aureus* is more sensitive to acidification when salt concentration is increased, although it is a halo tolerant microorganism. Fast acidification down to values unacceptable for growth is the most efficient way of *S.aureus* inhibition. Acids do not have the same inhibition capacity and for a given pH value, the impact on *S. aureus* physiology will vary with the nature of the acid used. Organic acids at pH values equivalent to those obtained by using inorganic acids are more effective against S. aureus. The effectiveness of organic acids generally depends on the concentration of their undissociated form, which is determined by the dissociation constant of organic acids. Thus, acetic acid and propionic acid with pKa of 4.8 and 4.9 (pKa is pH at which the ratio of dissociated to undissociated forms is 50:50) are more inhibitive than lactic acid whose pKa is 3.9 [11]. Complete inhibition of S. aureus is achieved at pH lower than 5.0. An acidic stress and the drop of intracellular pH alter the membrane structure and lead to a decrease in the activity of several enzymes which are pH-sensitive. Non-dissociated form of acid acts as un-couplers of the respiratory chain of *S.aureus*. The protonated form diffuses into the cell at low pH and is followed by a dissociation of the proton. Bacterial growth is then strongly altered because most of the energy available in the cell is used for the de-acidification of the cytoplasm by generating a proton gradient across the cytoplasm membrane [11]. The pH of DAM in Figure-2 indicates that it has an extremely high acidic pH which is a function of its high anti-microbial activity at a concentration of 6.25mg/ml and 3.125mg/ml for MBC and MIC respectively compared with the pH of other hair cream samples. A complete inhibition of *S. aureus* is achieved at pH extremely lower than 5.0 and this was achieved by other hair creams at a higher concentration of 12.5mg/ml and 25mg/ml for MIC and MBC respectively. It can therefore be deduced from our results in Table-1 that DAM has the highest preservative efficacy against *Staphylococcus aureus* partly due to its high acidic pH compared to other hair cream samples. For *p.aeroginosa*, the MIC and MBC were also pH dependent, as DAM having a pH of 2.043±0.006 still prevailed over all other hair creams against *p.aeroginosa* but at a concentration of the bactericidal and bacteriostatic effect at 25mg/ml and 12.5mg/ml respectively, while other hair creams, showed bactericidal and bacteriostatic effect at 50mg/ml and 25mg/ml respectively. Table-1 also showed that all the hair cream samples were more effective against *Staphylococcus aureus*, than against *p.aeroginosa*, with DAM exhibiting more effectiveness than all other hair cream samples against the two mentioned microorganisms. The pH affects the ionization and therefore the binding and interaction of a myriad of molecular processes; this includes very basic things such as nutrient availability. pH also affects the solubility of many substances that bacteria need. There is also no certain pH level for maximum growth for bacteria in general since they all differ slightly in their evolution. Some grow in a very acid environment eg. *H. pylori*. while others do not for example many *Pseudomonas* and *Staphylococcus* [13]. According to Faletra [13], most preservatives like paraben esters, phenoxy ethanol, benzyl alcohol, formaldehyde preservatives, halogenated compounds such as methyl chloro isothiazoline and methyl Isothiazolinone are effective at pH 4-8. It can be concluded that pH really played a vital role in the anti-microbial activity of all the creams.

## 4. CONCLUSION

Quality of cosmetic products especially hair creams in Nigeria largely depends on the quality of the starting materials. The guidelines of good manufacturing practice for cosmetic product (GMPC) have clearly depicted the necessity of products to comply with specifications. This requirement applies to the organoleptic, chemical and physical parameters of the products as well as their anti-microbial activity. The above study calls for incorporation of more efficacious antimicrobial agents in the formulations of hair cream samples so as to guarantee the microbial quality of cosmetics and adherence to the general guidelines as per GMPC.

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