Antimicrobial Activity of Clay against Some Skin Infection Isolates

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Abstract: The rising global threat of resistance of microorganisms to antibiotics is alarming. This has necessitated the use of alternative antimicrobial agents in the treatment of infectious diseases caused by pathogens. Clay has since been under investigation for its antimicrobial and therapeutic properties. This study investigated the physicochemical quality and antimicrobial activity of some clay against some skin infection isolates. The physicochemical quality of the clay samples were determined by standard methods. The test organisms were multidrug resistant *Pseudomonas aeruginosa*, antibiotic-susceptible *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus*, antibiotic-susceptible *Staphylococcus aureus* and *Aspergillus* spp. Clay concentrations of 12.5%, 25% and 50% were prepared for use for determining minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC). Of the 10 screened clay samples for antimicrobial activity, three (3) showed activity with zones of inhibition ranging from 11 mm to 16 mm. The MIC of the clay samples ranged from 25 mg/ml to 50 mg/ml against the tested microorganisms. There was no antimicrobial activity against *Aspergillus* spp. for all clay samples. The MBC for other clay was >50 mg/ml. The physicochemical composition revealed that the clay were high in kaolinite, illite, sulphur, iron and aluminum. This study revealed that clay had antimicrobial activities against the test organisms, perhaps due to their physicochemical composition, suggesting that clay could be an alternative treatment option for skin infections caused by these organisms.
Key word: Clay, antimicrobial activity, skin infection isolates, physicochemical composition.

INTRODUCTION

Clay is stiff, sticky, fine-grained earth that can be molded when wet, and is dried and baked to make bricks, pottery and ceramics. Clay is a soft, freely bound, earthy material containing particles with a grain size of less than 4 micrometres (µm) (Kumari and Mohan, 2021). It is formed as a result of the weathering and erosion of rocks containing the mineral group feldspar (known as the 'mother of clay') over vast spans of time (Kumari and Mohan, 2021). The small size of the particles and their unique crystal structures give clay materials special properties. These properties include: cation exchange capabilities, plastic behaviour when wet, catalytic abilities, swelling behaviour, and low permeability. The authors documented that clay and clay-based minerals have higher application in many industries and processes (Mana *et al*., 2017). Clay has since been under investigation for its antimicrobial and therapeutic properties in pharmaceutical and cosmetic industries (Moraes *et al*., 2017). Clay has been employed as a natural remedy since prehistory. Aside from its healing and soothing properties, clay has been investigated for its antibacterial activities, exemplified by the successful application of French green clay in the treatment of Buruli ulcer, a necrotizing cutaneous infection caused by *Mycobacterium ulcerans* and the antibacterial efficacy of clay leachates against *Escherichia coli* and methicillin-resistant *Staphylococcus aureus* (MRSA) (Otto *et al*., 2016). These studies have stimulated interest in the identification of clays from other localities that possess antibacterial activity and could be utilized as new antibacterial agents (Williams *et al.*, 2011). Only a small proportion of clay minerals have been proven to be antibacterial. In Nigeria, clay-rich soils are derived from erosion of metamorphic and sedimentary rocks, and marine alluvium deposits (Ohashi, 1993). The antibacterial activity of clays is somewhat variable, since no natural clay minerals are precisely the same due to differences in mineralogical and geochemical composition (Ferrell, 2008). However, the antibacterial activity of clay leachates is widely reported to be due to metal ion toxicity released from the clay mineral interlayer. Although, clays have fundamental structural and chemical characteristics in common, each clay mineral has its own unique properties that determine how it will associate with other species. Modern day research has proposed the high healing properties of clay and has been indicated to be effectively used to treat skin infections, stomach ulcers, arthritis. These clays can help in the treatment of various conditions such as hemorrhoids, viral infection, mucus colitis, open wounds, anemia, and acne among other ills (Lafi and Al-Dulaimy, 2011). However, in view of emerging issues of describing clay as an efficient antimicrobial agent against several bacterial pathogens, this study investigates the antibacterial activity of clay from Nigeria against some skin infection isolates.

MATERIALS AND METHODS

Sources of Clay Samples and Preparation: Clay samples of Mamu Formation and Imo Formation origins were obtained from the Department of Geology, University of Benin, Benin City, Nigeria. They were then transported to the laboratory for analysis. Clay samples were pulverized separately using a ceramic mortar and pestle (General Laboratory Standard). Each sample was then sieved using a set of stacked U.S. mesh sieves and a receiving pan after shaking manually for 60 seconds. The pulverized samples collected in the sterile pan were then weighed using an electric weighing balance (WENSAR MAB220T). Ten grams (10 g) of each sample from the various depths and widths were weighed. Weighed samples were then transferred into separate beakers and tagged appropriately. Samples in the tagged beakers were hydrated with 40 ml of deionized water which was measured with a measuring cylinder before stirring thoroughly using a glass rod, then left to settle for about 2 hr. Samples were then filtered using a filter paper and the filtered samples were hydrated with deionized water. A centrifuge was used to spin the samples and the finer particles were separated. These finer particles were collected and placed in the beaker and then in an electric oven at 60°C to dry. The dried samples were pulverized, weighed and hydrated with 5 ml of distilled water for 24 h (Umeaku *et al*., 2019).

Test Organisms: Pure cultures of multi-drug resistant (MDR) *Pseudomonas aeruginosa*, antibiotic-susceptible *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus* and antibiotic-susceptible *Staphylococcus aureus* isolated from infected wounds from patients of the University of Benin Teaching Hospital, Benin City, Nigeria. The cultures were transported to the laboratory in agar slants and then sub-cultured onto nutrient agar plates. The plates were incubated for 24 h. Pure cultures of *Aspergillus* spp. was sub-cultured and maintained on sabouraud dextrose agar, after incubation for 72 h (Islam *et al*., 2008). These inoculi were adjusted to match a specific level of turbidity, equivalent to a 0.5 MacFarland standard, which corresponds to approximately 1×10^8 cfu/ml (colony forming units per milliliter) for bacteria and conidia for fungi. To achieve this, a suspension was made by inoculating 1 ml of sterile water with a sterile inoculating loop of each of the isolates (Islam *et al*., 2008).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Clay Samples: This was achieved using the Kirby-Bauer agar well diffusion. Holes were bored in prepared Mueller Hinton agar plates aseptically with a 10 mm cork borer, after which the base of the bored holes were sealed off with molten Mueller-Hinton agar to prevent leakage. For fungal plates, the medium used was Sabouraud dextrose agar. After solidification of the agar, the entire surface was inoculated generously with inoculum by spreading gently using a sterile swab stick. Different concentrations of clay were prepared viz; 12.50%, 25% and 50%. Then, 0.05 ml of each concentration was aseptically transferred into the designated bored hole. The plates were thereafter incubated for 24 h. For control purposes, ciprofloxacin and ketoconazole were used (10 mg/ml ciprofloxacin for bacteria and ketoconazole for fungi dissolved in 0.1 g of 20% DMSO). After incubation, the diameter of the zones of inhibition was measured and compared to established standards to determine the level of susceptibility of the clay to the tested antimicrobial agents (Jafari-Sales *et al*., 2020). The MBC was determined by plating cell broth dilutions of the MIC and the next two dilutions above the MIC. The streaked plates were incubated for 48 hr. Failure of the organism to grow on the plate after incubation implies that only non-viable cells are present, which translates to bactericidal activity of the clay dilution (Jafari-Sales *et al*., 2020).

Physicochemical Analyses of the Clay Samples: The hydrogen concentration (pH) and electrical conductivity were measured using a microprocessor pH meter (pH 211) and Jenway 4510-conductivity meter respectively. The total carbon was determined by placing 10 g of dried and homogenized clay sample into a clean, carbon-free crucible of known weight. The clay samples were then subjected to high temperature, around 450-550°C, in a muffle furnace. The high heat ensured complete combustion. After the combustion was completed, the concentration of CO_2 released was measured volumetrically (AOAC, 2013).

To determine the total organic carbon content of clay, 10 g of sample was mixed well. The samples were then air-dried to remove any moisture that could affect the accuracy of the organic carbon measurement. Five grams (5 g) of the samples were carefully weighed using an analytical balance (WENSAR MAB220T) and placed into a digestion tube or vessel. Concentrated sulfuric acid (H_2SO_4) was added to the soil sample in the tube to digest the soil and release carbon in the form of carbon dioxide (CO_2). The digestion tube was heated to high temperatures, between 450-550°C. The next step involved measuring the volume of carbon dioxide gas collected during the combustion process. This measurement was done using volumetric analysis by capturing the gas in a known volume of deionized water and measuring the pressure change (AOAC, 2013).

Sulphur, iron and aluminum concentrations were determined using 0.25 g samples, fused with sodium peroxide in a zirconium crucible. The fused samples were acidified with concentrated nitric and hydrochloric acids. The resulting solutions were diluted and then measured by inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma optical emission spectroscopy (ICP-OES) and x-ray fluorescence (XRF). All metals were solubilized. For the XRF samples, 5 g of powdered clay sample was oven-dried at 105℃ and fused into a platinum mold with a commercial lithium tetraborate flux before subsequent analysis (AOAC, 2013).

Statistical analysis of data: The data obtained from this research were analyzed using statistical package for social scientist (version 21), and Microsoft excel (version 2019). Values were expressed as mean ± standard deviation at 95% confidence limit (Ogbeibu, 2015).

RESULTS

Clay has been shown to have some antimicrobial activity against the test organisms. Table 1 shows the antimicrobial activity of the sampled 10 clay varieties. Only three (3) varieties showed activity against the tested isolates. The sample with the highest zone of inhibition (16 mm) was sample 10 from Imegba zone B against multi-drug resistant *Pseudomonas aeruginosa*, antibiotic-susceptible *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus*, and antibiotic-susceptible *Staphylococcus aureus.* Sample 9 from Imegba zone A recorded the lowest zone of inhibition (11 mm) against all tested bacteria, but showed no activity against *Aspergillus* spp. and antibiotic-susceptible *Pseudomonas* *aeruginosa*. Sample 6 from Uzebba zone B and sample 10 recorded 12 mm inhibition zone against *Aspergillus* spp.

Table 1: Antimicrobial activity of clay from different origins against some skin infection isolates

Clay Sample	Zone of Inhibition (mm)				
	A	B	C	D	E
1	-	-	-	-	-
2	-	-	-	-	-
3	-	-	-	-	-
4	-	-	-	-	-
5	-	-	-	-	-
6	12	12	11	12	12
7	-	-	-	-	-
8	-	-	-	-	-
9	-	11	-	11	11
10	12	16	16	16	16
Control	25	14	45	-	35

Key: Control = Ketoconazole and Ciprofloxacin. 1 = Imo shale at 860 – 870 m depth; 2 = Imo shale at 740 -750 m depth; 3 = Imo shale at 1120 – 1160 m depth; 4 = Imo shale at 270 – 280 m depth; 5 = Imo shale at 230 – 240 m depth; 6 = Mamu formation at Uzebba 5B, 7 = Mamu formation at Okpekpe 9, 8 = Mamu formation at Okpekpe 7C, 9 = Mamu formation at Imegba zone A, 10 = Mamu formation at Imegba zone B. A = *Aspergillus* spp., B = Multi drug resistant *Pseudomonas aeruginosa*, C = Antibiotic-susceptible *Pseudomonas aeruginosa*, D = Methicillin resistant *Staphylococcus aureus*, E = Antibiotic-susceptible *Staphylococcus aureus;* - = No zone of inhibition.

The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the tested clay samples against skin wound bacteria and fungus are shown in Tables 2, 3 and 4. No MIC was detected against *Aspergillus* spp. for all three (3) clay samples. For sample 6 clay, 50 mg/ml was the MIC for all test bacteria; while for sample 9, MIC was detected against only antibiotic-susceptible *Staphylococcus aureus* (50 mg/ml). Also, for sample 10, MIC was detected against antibiotic-susceptible *Pseudomonas aeruginosa* (50 mg/ml) and antibiotic-susceptible *Staphylococcus aureus* (25 mg/ml).

Table 2: Minimum inhibitory and bactericidal concentrations of sample 6 (Mamu formation at Uzeba 5B) clay against some skin infection isolates

	Clay Concentrations (mg/ml)				
Test Organisms	12.50	25.00	50.00	MIC (mg/ml)	MBC (mg/ml)
A	-	-	-	Nil	Nil
B	-	-	+	50.00	Nil
C	-	-	+	50.00	Nil
D	-	-	+	50.00	50.00
E	-	-	+	50.00	50.00

Key: A = *Aspergillus* spp., B = Multi drug resistant *Pseudomonas aeruginosa*, C = Susceptible *Pseudomonas aeruginosa*, D = Methicillin resistant *Staphylococcus aureus*, E = Susceptible *Staphylococcus aureus*; + = Zone of inhibition detected; - = No zone of inhibition detected; Nil = No result or MBC > 50 mg/ml.

Table 3: Minimum inhibitory and bactericidal concentrations of sample 9 (Mamu formation at Imegba zone A) clay against some skin infection isolates

	Clay Concentrations (mg/ml)				
Test Organisms	12.50	25.00	50.00	MIC (mg/ml)	MBC (mg/ml)
A	-	-	-	Nil	Nil
B	-	-	-	Nil	Nil
C	-	-	-	Nil	Nil
D	-	-	-	Nil	Nil
E	-	-	+	50.00	Nil

Key: A = *Aspergillus* spp., B = Multidrug resistant *Pseudomonas aeruginosa*, C = Susceptible *Pseudomonas aeruginosa*, D = Methicillin resistant *Staphylococcus aureus*, E = Susceptible *Staphylococcus aureus*; + = Zone of inhibition detected; - = No zone of inhibition detected; Nil = No result or MBC > 50 mg/ml.

Table 4: Minimum inhibitory and bactericidal concentrations of sample 10 (Mamu formation at Imegba zone B) clay against some skin infection isolates

	Clay Concentrations (mg/ml)				
Test Organisms	12.50	25.00	50.00	MIC (mg/ml)	MBC (mg/ml)
A	-	-	-	Nil	Nil
B	-	-	-	Nil	Nil
C	-	-	+	50.00	Nil
D	-	-	-	Nil	Nil
E	-	+	+	25.00	Nil

Key: A = *Aspergillus* spp., B = Multi drug resistant *Pseudomonas aeruginosa*, C = Susceptible *Pseudomonas aeruginosa*, D = Methicillin- resistant *Staphylococcus aureus*, E = Susceptible *Staphylococcus aureus*; + = Zone of inhibition detected; - = No zone of inhibition detected; Nil = No result or MBC > 50 mg/ml.

The physicochemical constituents of the three (3) clay samples selected are shown in Table 5. Sample IM 11B recorded the highest EC, sulphur, iron and aluminum concentrations. Also, UZ 5B recorded the lowest kaolinite content (57.00%), while the highest was recorded by IM 19A (71.30%).

Table 5: Physicochemical composition of clay samples with antimicrobial activity

Samples	**6**	**9**	**10**
Electrical Conductivity (µs)	577	3320	103.20
pH	5.31	5.10	5.23
Total Organic Carbon (%)	1.20	1.40	0.83
Sulphur (%)	0.08	2.97	0.13
Iron (%)	0.97	3.35	1.85
Aluminum (%)	11.16	15.81	15.19
Total Inorganic Carbon (%)	0.01	0.00	0.01
Illite (%)	13.00	11.80	21.80
Kaolinite (%)	57.00	63.40	71.30

Key: Sample 6 = Mamu formation from Uzebba 5B; Sample 9 = Mamu formation from Imegba zone A; Sample 10 = Mamu formation from Imegba zone B.

DISCUSSION

The antimicrobial screening of the ten (10) clay samples revealed that only three (3) possessed antimicrobial activity against the test isolates. This showed that that some clay had antimicrobial activity. The highest zone of inhibition (16 mm) was recorded in sample 10 (Mamu formation at Imegba zone

B) against all the tested organisms. However, 12 mm was recorded against the tested fungus (*Aspergillus* spp.). This points to the fact that clay may possess higher antimicrobial activity against bacteria, than fungi. Similar observations have been recorded in literature (Esteban-Tejeda *et al.,* 2009; Wei *et al.,* 2014) and the reason may be due to the dehydration-resistant nature of fungal spores.

The findings from this research correlated with the works of Haydel *et al*. (2008), who demonstrated bactericidal activity of mineral clay against pathogenic *Escherichia coli*, extended spectrum β-lactamase (ESBL) *Escherichia coli*, *Salmonella* Typhimurium, *Pseudomonas aeruginosa* and *Mycobacterium marinum*, and a combined bacteriostatic/bactericidal effect against *Staphylococcus aureus*, penicillin-resistant *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA) and *Mycobacterium smegmatis*, whereas another mineral with similar structure and bulk crystal chemistry, but CsAr02, had no effect on or enhanced bacterial growth. The works of Lafi and Al-Dulaimy (2011) and Hamilton *et al*. (2019), detected the antimicrobial activity of clay minerals against pathogenic methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolated from wound infections. Also, the study of Behroozian *et al*. (2023) discovered the antibacterial effect of Kisameet clay against multidrug resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*. However, it was opined that the role of clay minerals in the bactericidal process is to buffer the aqueous pH and oxidation state to conditions that promote Fe^{2+} solubility (Williams *et al*., 2011).

The minimum inhibitory concentrations (MIC) of the three selected clay samples with antimicrobial activity showed an MIC range of 25 mg/ml to 50 mg/ml. Interestingly, clay sample 6 which recorded the lowest zones of inhibition measurements during screening showed an MIC of 50 mg/ml against all the tested bacteria. The minimum bactericidal concentration (MBC), which is the lowest concentration at which cells viability were eliminated by at least 99.80%, was assessed for the three selected clay samples. Clay sample 9 was the only clay sample that recorded bactericidal activity at 50 mg/ml concentration. Consequently, the present study has revealed that clay sample 6 exhibited bactericidal effect against *Pseudomonas* and *Staphylococcus*. Clay from the Imegba segments could only cause the inhibition of cell growth, but not their death.

Kaolinite and illite are important constituents of clay, and contribute to the absorption and/or adsorption capacity of clay (Jiang *et al.,* 2010). Since the antimicrobial property of clay is based on its capacity for sorption, this translates to kaolinite and illite directly influencing the sorptive capacity of clay. The higher the concentration of kaolinite and illite, the higher the absorptive capacity (Missana *et al.,* 2008). A high sorption capacity leads to higher antimicrobial activity. This is shown in the findings of the present study where clay sample 10, which has the highest kaolinite and illite concentrations, showed the higher antimicrobial activity, and indeed, recorded the lowest MBC of 25 mg/ml.

Hydrogen ion concentration (pH) is also a very important factor in considering antimicrobial activity. A more acidic pH may be detrimental to bacteria, whereas a more alkaline pH may be detrimental to fungi (Rousk *et al.,* 2010). From the findings of the present study, IM 11B recorded the lowest pH and showed the lowest zones of inhibition against the tested bacteria. Electrical conductivity measures the charge and conductivity of the ions present in clay. The more conductive the clay sample is, the more reactive the metal ions that are present. The antimicrobial activity of clay is decreased by higher electrical conductivity than lower electrical conductivity (Gonzalez-Campos *et al.,* 2013). The type of microorganism also likely determines the degree of the antimicrobial activity. Clay sample 9 recorded the highest electrical conductivity and consequently, elicited the lowest antibacterial activity, and no antifungal activity. The concentrations of sulphur, iron and aluminum were markedly higher in clay sample 9, than in samples 6 and 10. The implication of this may be that the lower the concentrations of these elements in clay, the higher their antimicrobial activity. However, this concurs with previous researches by Arias *et al.* (2018) and Saedi *et al.* (2020), where the presence of sulphur, iron boosted antimicrobial activity. This conflict may be due to the specific medium, and the presence or absence of various other ions in the medium.

CONCLUSION

The menace of microbial infections requires constant innovation around the search for cures and treatment options. The added burden of resistant microbes to the available treatment and management options has given rise to the continual search for new and more effective antimicrobial agent. Clay samples in this study has shown to possess antimicrobial activity against tested bacteria, and the most effective clay for this purpose possessed high concentrations of kaolinite, illite, a higher pH, low electrical conductivity and low concentrations of sulphur, iron and aluminium. These could probably have accounted for the antimicrobial activity of the clay samples, thus suggestive of their use in the treatment of infections caused by the test organisms.

REFERENCES

AOAC (Association of Official Analytical Chemists). (2013). Official methods of analysis 19th Edition. AOAC International, Gaithersburg, MD, USA. 162pp.

Arias, L.S., Pessan, J.P., Vieira, A.P.M., Lima, T.M., Delbem, A.C.B. and Monteiro, D.R. (2018). Iron oxide nanoparticles for biomedical applications: A perspective on synthesis, drugs, antimicrobial activity, and toxicity. *Antibiotics,* 7(2): 46.

Behroozian, S., Zlosnik, J.E.A, Xu, W., Li, L.Y. and Davies, J.E. (2023). Antibacterial activity of a natural clay mineral against *Burkholderia cepacia* complex and other bacterial pathogens isolated from people with cystic fibrosis. *Microorganisms,* 11:1-4.

Esteban-Tejeda, L., Malpartida, F., Esteban-Cubillo, A., Pecharromán, C. and Moya, J.S. (2009). The antibacterial and antifungal activity of a soda-lime glass containing silver nanoparticles. *Nanotechnology,* 20(8): 85-103.

Ferrell, R.E. (2008). Medicinal clay and spiritual healing. *Clays and Clay Minerals*, 56(6): 751-760.

González-Campos, J.B., Mota-Morales, J.D., Kumar, S., Zárate-Triviño, D., Hernández-Iturriaga, M., Prokhorov, Y., Lepe, M.V., García-Carvajal, Z.Y., Sanchez, I.C. and Luna-Bárcenas, G. (2013). New insights into the bactericidal activity of chitosan-Ag bionanocomposite: the role of the electrical conductivity. *Biointerfaces*, 111: 741-746.

Hamilton, A.R., Roberts, M., Hutcheon, G.A. and Gaskell, E.E. (2019). Formulation and antibacterial properties of clay mineral-tetracycline and -doxycycline composites. *Applied Clay Science,* 179:1-6.

Haydel, S.E., Remenih, C.M. and Williams, L.B. (2008). Broad-spectrum *in vitro* antibacterial activities of clay minerals against antibiotic-susceptible and antibiotic resistant bacterial pathogen. *Journal of Antimicrobial Chemotherapy,* 61(2):353-361.

Islam, M.A., Alam, M.M., Choudhury, M.E., Kobayashi, N. and Ahmed, M.U. (2008). Determination of minimum inhibitory concentration (mic) of cloxacillin for selected isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) with their antibiogram. *Bangladeshi Journal of Veterinary Medicine,* 6(1): 121-126.

Jafari-Sales, A., Jafari, B., Khaneshpour, H. and Pashazadeh, M. (2020). Antibacterial effect of methanolic extract of *Rosa damascene* on standard bacteria *Staphylococcus aureus, Bacillus cereus, Escherichia coli* and *Pseudomonas aeruginosa* in vitro. *International Journal of Nature and Life Sci.*, 4 (1): 40-46.

Jiang, M.Q., Jin, X.Y., Lu, X.Q. and Chen, Z.L. (2010). Adsorption of Pb (II), Cd (II), Ni (II) and Cu (II) onto natural kaolinite clay. *Desalination,* 252(1): 33-39.

Kumari, N., and Mohan, C. (2021). Basics of clay minerals and their characteristic properties. *Clay Miner*, 24: 1-29.

Lafi, S.A. and Al-Dulaimy, M.R. (2011). Antibacterial effect of some mineral clays in vitro. *Egyptian Academic Journal of Biological Sciences and General Microbiology,* 3(1): 75-81.

Mana, S.C.A., Hanafiah, M.N. and Chowdhury, A.J.K. (2017). Environmental characteristics of clay and clay-based minerals. *Geology, Ecology and Landscapes,* 1(3):155-161.

Missana, T., Garcia-Gutierrez, M. and Alonso, U. (2008). Sorption of strontium onto illite/smectite mixed clays. *Physics and Chemistry of the Earth,* 33: 156-162.

Moraes, J.D., Bertolino, S.R., Cuffini, S.L., Ducart, D.F., Bretzke, P.E. and Leonardi, G.R. (2017). Clay minerals: properties and applications to dermocosmetic products and perspectives of natural raw materials for therapeutic purposes- a review. *International Journal of Pharmaceutics,* 534 (1-2): 213-219.

Ogbeibu, A. E. (2015). *Biostatistics: A Practical Approach to Research and Data Handling.* 2nd Edition. Mindex Publishing Co. Ltd, Benin. 285 p.

Ohashi, F. (1993). Antimicrobial and antifungal agents derived from clay minerals. *Journal of Antibacterial and Antifungal Agents, Japan,* 21(11): 591–595.

Otto, C.C., Kilbourne, J. and Haydel, S.E. (2016). Natural and ion-exchanged illitite clays reduce bacterial burden and inflammation in cutaneous methicillin-resistant *Staphylococcus aureus* infections in mice. *Journal of Medical Microbiology,* 65 (1): 19-27.

Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Knight, R. and Fierer, N. (2010). Soil bacterial and fungal communities across a pH gradient in an arable soil. *The ISME Journal,* 4(10): 1340-1351.

Saedi, S., Shokri, M. and Rhim, J.W. (2020). Antimicrobial activity of sulfur nanoparticles: effect of preparation methods. *Arabian Journal of Chemistry,* 13(8): 6580-6588.

Umeaku, C.N., Otuadinma, R.N., Ochiabuto, o.m., Onwuasoanya, U.F. and Obeagu, E.I. (2019). Antimicrobial and health potentials of clay. *European Journal of Pharmaceutical and Medical Research,* 6 (8): 25-31.

Wei, H., Guenet, B., Vicca, S., Nunan, N., Asard, H., AbdElgawad, H., Shen, W. and Janssens, I.A. (2014). High clay content accelerates the decomposition of fresh organic matter in artificial soils. *Soil Biology and Biochemistry,* 77: 100-108.

Williams, L.B., Metge, D.W., Eberl, D.D., Harvey, R.W., Turner, A.G., Prapaipong, P. and Poret-Peterson, A.T. (2011). What makes a natural clay antibacterial? *Environmental Science and Technology,* 45(8): 3768-3773.