American Journal of Microbiology and Immunology
Vol. 1, No. 1, 2016, pp. 1-5
http://www.aiscience.org/journal/ajmi

Inhibitory Effect of Selected Ghanaian Clay Leachates on Some Pathogenic Microbes

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Abstract

Clay and other mineral products have been documented as remedies for numerous human diseases throughout history with a number of success stories. In this study, the antimicrobial activity of the aqueous leachates of five clay samples collected from Keta, Anloga, Half-Assini, Sogakope and Savietula, all in Ghana, were examined. The *in vitro* antimicrobial activity of the aqueous leachates were tested against nine microbes using agar well diffusion assay. Of the five clay samples, Sogakope clay leachate possessed the highest antimicrobial activity, showing varying zones of inhibitions against all nine test microorganisms. All other leachates exhibited antimicrobial activity against at least one of the test microbes, except for clay leachate from Anloga which was inactive towards all microbes used in this study. pH and conductivity measurements indicated that Sogakope aqueous leachate had the lowest pH of 2.81 and a conductivity of 54.2 μS/cm while aqueous leachates of Anloga, Keta, Half-Assini and Savietula clays had pH values of 10.27, 7.66, 4.88, 8.01 and conductivities of 94.8, 5.86, 2.02, 91.40 μS/cm respectively. Metal analysis revealed that all clay samples have comparable compositions. Clay deposits may potentially provide cost-effective topical antibacterial treatments.

Keywords

Agar Diffusion, Leachates, Metal Ions, Conductivity, pH

Received: May 17, 2016 / Accepted: May 26, 2016 / Published online: June 17, 2016

1. Introduction

Antibiotics are one of the most important therapeutic discoveries in medical history. Ever since their introduction into medicine, they have changed the way patients with bacterial infections are treated and have contributed to reducing the mortality and morbidity rates from bacterial diseases [1], [2]. Nature provides a vast array of compounds for treatment of infectious diseases. Medicinal plants and organisms from both marine and terrestrial habitats, including fungi and bacteria are a rich source of antimicrobial compounds [3]–[6]. A lot of antibiotics or antimicrobials operate by selectively interfering with the manufacture of one of the huge molecular constituents of the cell; the cell wall, proteins or nucleic acids. Others act by destroying the cell membrane. Some important and clinically useful drugs prevent the production of peptidoglycan, the most significant constituent of the cell wall. [7], [8]. These antibiotics do not affect human cells because human cells lack cell walls.

All around the world, mineral products have a variety of applications, from being used as cosmetics to being used for decorative purposes [9]. In Ghana, clay is commonly used in molding earthen vessels to hold both water and food. They are also used in the preparation of traditional ovens, stoves and in building houses. Baked clay, in small quantities, is consumed by some people (usually pregnant women) to combat nausea.

Mineral products either alone or in conjunction with other products have also been used in treatment of diseases

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throughout history. Galena – lead (II) sulphide (PbS) - is used as a cosmetic, to clean dirt from eyes, improve vision, treat glaucoma and cataracts by Sahrawi refugees of Western Sahara. Charcoal is employed in wound healing preparations. Red hematite is applied around the eyes to reduce the sun's radiation getting into eyes, to treat cataracts, bone fractures and wounds with potassium alum being used to remove sand and dirt from eyes and as a wound cicatrizer and antiseptic in snakebites and scorpion stings [10]

A French humanitarian worker in the Ivory Coast in 2001, treated children suffering from Buruli ulcer with two kinds of French clay. Prolonged treatment of patients with the clay resulted in continued removal of the ulcer, tissue restoration and healing of the wounds [11]. Clay minerals are also included in formulation of several healthcare products mainly being used to stabilize suspensions and emulsions, to support adhesion to the skin, adsorption of greases and as protection against environmental agents [12].

Primarily due to misuse, microorganisms are rapidly developing resistance to most antibiotics currently approved for use in the treatment of human and animal infections [13]. The rise in the recorded cases of newly emerging infectious diseases coupled with the re-emerging of hitherto eradicated infectious diseases have led to an increase in the search of antimicrobial agents with novel mechanisms of action.

In this study, the antimicrobial activity of the aqueous leachates of five clay samples collected from Keta, Anloga, Half-Assini, Sogakope and Savietula in Ghana were examined. The antimicrobial activity of the aqueous leachates were tested against *Escherichia coli (E. coli), Staphylococcus aureus (S. aureus), Bacillus subtilis (B. subtilis), Salmonella typhi (S. typhi), Enterococcus feacalis (E. feacalis), Klebseilla pneumoniae (K. pneumoniae), Streptococcus pneumoniae (S. pneumoniae), Pseudomonas aeruginosa (P. aeruginosa)* and *Candida albicans (C. albicans)* using the agar diffusion method. The study also examined the effects of pH and conductivity of aqueous clay leachates on the antimicrobial activity of the various clay samples. Lastly, the metals in the various clay samples were determined to investigate if they contribute to antimicrobial activity.

2. Methods

2.1. Sample Collection

Clay samples were collected from five (5) different locations in Ghana (Table 1) in November 2014. Samples were dug with a clean plastic shovel and kept in sterile bags. Samples were then transported to the laboratory in an ice chest and stored at 4°C until use.

Table 1. Coordinates of locations where clay samples were obtained.

Location	Latitude (°)	Longitude (°)	Altitude (m)
Keta	5.880486	0.982693	5
Anloga	5.871267	0.826939	8
Half-Assini	5.050148	-2.875372	34
Sogakope	6.004925	0.609377	18
Savietula	5.860938	0.826973	6

2.2. Clay Leachate Preparation

Clay samples used in this work were autoclaved for 1 h at 121°C. 1 g of autoclaved clay was mixed with 20 mL sterile dH_2O. Leachates were obtained by continuously stirring clay mixtures in sterile dH_2O for 6 hours. Subsequently, the hydrated clay mixture suspensions were centrifuged (4000 rpm) for 30 minutes at 4°C to separate insoluble and soluble fractions. The leachate was collected by decanting the centrifuged solution.

2.3 Antimicrobial Assay

2.3.1. Microorganisms

Microbial strains used in this study were obtained from the Department of Pharmaceutics and Microbiology, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi, Ghana

Eight strains of bacteria and one fungal strain were used to assay the antimicrobial activities of the various clay leachates. The microbial strains included four (4) Gram negative bacteria *(E. coli, S. typhi, K. pneumoniae, P. aeruginosa)*, four (4) Gram positive bacteria (*S. aureus, B. subtilis, E. feacalis, S. pneumoniae)* and one fungus *(C. Albicans)*.

2.3.2. Preparation of Agar Plates

14 g of nutrient agar was weighed into a 500mL volumetric flask and topped up to the mark with sterile water followed by sterilization in an autoclave at 121°C for 15 minutes. Molten agar was then stabilized in a water bath at 45°C. 20mL of molten nutrient agar was seeded with 100 µL of appropriate test microorganism and poured into a sterile Petri dish and allowed to solidify. The procedure was repeated for the other test microorganisms. After solidifying, size 6 cork bore was used to create wells in the 9 agar plates, each seeded with different test microorganisms.

2.3.3. Diffusion Well Assay

The nine microorganisms were cultured on nine different agar plates. 100µL portions of each clay leachate were pipetted into each well. Ciprofloxacin was used as the standard antibiotic (positive control). The plates were incubated at 37°C for 24 hours. The zone of inhibition was measured in millimeters (mm) as the distance from the center of the disc to the edge of the clear zone. The assay was repeated three times and the averages of the three experiments taken.

2.4. pH and Conductivity of Clay Leachates

Conductivity and pH measurements were done with a Jenway 3540 pH and Conductivity meter (Jenway, Essex, England). The pH electrode was calibrated with standard buffer solutions of pH 4.20 and 7.62 before use. 50 mL of distilled water was added to 1 g of clay sample to create a clay suspension. The pH electrode was then dipped into the clay suspension and measurements taken after values stabilized on the pH meter.

For conductivity measurements, the conductivity cell was calibrated with 0.01 M KCl solution and the electrode dipped into the prepared clay suspensions to measure their conductivity. All experiments were performed in triplicate.

2.5. Metal Analysis

Clay samples were digested by using the nitric acid-hydrogen peroxide method. Clay samples were oven dried at 105 - 110°C. The dried clay (0.5 g) was slurried with 0.5 mL of water to minimize sample splash and facilitate rapid reaction with the acid. 10 mL of conc. HNO_3 was added to the slurry and heated at 100°C for 2 hours. The mixture was cooled for 15 minutes and 3 mL of 30% H_2O_2 was added drop wise to the mixture. Heating was continued for another hour with intermittent gentle swirling, followed by cooling to room temperature. The cooled digestate was filtered into a 25 mL volumetric flask and topped up to the 25 mL mark with deionised water. A blank sample was prepared in similar manner without the clay. Metals were determined by atomic absorption spectrophotometry. The external calibration method was used for the quantitative analysis of the samples. Working standard solutions of analyte metals were prepared from their respective standard stock solutions. Working standards were prepared in 0.1% HNO_3. To monitor consistency in the performance of the instrument, working standards and blanks were run as samples at regular intervals. All sample analysis was performed in triplicates. Standards and samples were analysed with an Analytik Jena NovAA 400P Atomic Absorption Spectrometer (AAS) (Analytik Jena, Germany). The levels of metals present in clay samples were expressed as mean of metal concentration (mg/kg).

3. Results and Discussions

3.1. Results

Leachates of clay samples obtained from five different locations in Ghana were assayed against a number of microbes to evaluate their antimicrobial activities. Table 2 summarizes the results of the antimicrobial tests. Clay leachate from Sogakope was observed to possess broad spectrum antimicrobial capabilities, displaying activities against all test organisms. The highest activity was recorded against *B. subtilis*, with an inhibition zone of 13 mm. the least inhibition zone by the Sogakope clay leachate was 2 mm against *S. aureus*. Savietula clay leachate inhibited the growth of *B. subtilis, S. typhi, S. aureus* and *S. pneumoniae.* All other test microbes were unaffected. The growth of only *C. albicans* (6 mm) and *S. typhi* (14 mm) were inhibited by Half-Assini leachate. Keta clay leachate was active against only *K. pneumoniae* whereas clay from Anloga had no activity against any of the organisms tested in this study. Interestingly, the highest antimicrobial activity (inhibition zone of 14 mm) was recorded against *S. typhi* by 2 different clay samples, Half-Assini and Savietula.

Table 2. Zones of inhibition of clay leachates against test microorganisms.

Zone of inhibition (mm)						
Microorganism	**Half Assini**	**Anloga**	**Sogakope**	**Keta**	**Savietula**	**Ciprofloxacin**
P. aeruginosa	-	-	8	-	-	25
E. faecalis	-	-	4	-	-	37
C. albicans	6	-	8	-	-	11
E. coli	-	-	7	-	-	30
B. subtilis	-	-	13	-	6	18
S. typhi	14	-	8	-	14	22
S. aureus	-	-	2	-	4	24
K. pneumoniae	-	-	8	11	-	15
S. pneumoniae	-	-	9	-	7	29

Both pH and conductivities of the clay leachates were determined (Table 3) to examine if they played any role in the observed antimicrobial activities. The most active leachate, Sogakope recorded a pH of 2.81 and a conductivity of 54.2 µS/cm. Half-Assini leachate was also acidic (pH of 4.88) while Keta clay was largely neutral (pH 7.66). Basic clay leachates were Savietula (8.01) and Anloga (10.27). Interestingly, the most basic leachate (Anloga) recorded no activity against any test microbe. Conductivities of the various leachates ranged from 2.02 µS/cm to 94.8 µS/cm.

Table 3. pH and conductivity of clay leachates.

Location	pH	Conductivity µS/cm
Sogakope	2.81	54.2
Anloga	10.27	94.8
Keta	7.66	5.86
Half-Assini	4.88	2.02
Savietula	8.01	91.40

Metal analysis was carried out to determine some of the chemical constituents of the various clay samples used in this study. Of the metals analyzed (Zn, Fe, Cr, Cu, Ni, Ag, Mn and Mg), Ag was not detected in any of the samples (below detection limit), as can be seen in Table 4. Mg and Fe were the most abundant while Zn was present in the least amount.

Table 4. AAS metal analysis of clay samples.

Location	Mean Concentration (mg/kg)							
	Zn	Fe	Cr	Cu	Ni	Ag	Mn	Mg
Sogakope	8.4	4414.6	27.8	57.8	49.0	-	6.5	731.4
Anloga	7.2	4193.9	30.1	3.3	46.4	-	14.3	5175.2
Keta	8.9	4188.1	26.7	4.1	35.7	-	22.1	3793.4
Half-Assini	15.1	4431.5	25.8	31.4	51.6	-	6.2	1623.6
Savietula	7.3	3714.1	13.2	23.4	21.3	-	27.4	5557.8

(-) implies not detected.

3.2. Discussions

Antimicrobial activities of clay samples from various locations around the world have been previously reported [11], [14], [15]. In a similar vein, leachates of clay samples obtained from various locations in Ghana have showed significant antimicrobial activity against the test microorganisms used in this study. All leachates were active against at least one microorganism, except for clay samples from Anloga which displayed no antimicrobial activity. pH and metal ion concentrations have being widely speculated to be responsible for the observed antimicrobial activities of clay leachates . A study on the *in vitro* antibacterial properties of natural clay mixtures identified that the pH of clay, presence or absence of certain metal ions, ion solubility, osmotic strength and temperature were all contributing factors towards the toxicity of the clay sample [11], [14], [16]. To investigate pH effects, the pH of clay samples used in this study were determined. The high pH of clay from Sogakope (pH 2.81) could potentially be responsible for its broad antimicrobial activity. The highly basic clay sample (Anloga) recorded no antimicrobial activity against any of the test microorganisms.

In general, the surface of many clay minerals is negatively charged [17]. Free exchange of positively charged moieties including metal cations therefore occurs on regular basis. In aqueous media, these metal ions can be released from the clay surface to the nearby media [14]. It is therefore believed that the antimicrobial activity of some clay leachates is possibly derived from the action of these metal cations. It has also being demonstrated that pH plays significant role in the availability of metal ions in solutions [18]–[20]. In particular, Fe, Cu, Ni and Zn are known to possess significant bactericidal activity in *in vitro* studies [14]. In this study, the concentrations of the metals determined among the various clay samples did not differ significantly. Ag was not detected in any of the samples, similar to work done by Otto and Haydel [14]. It is however worthy to note that even though total ion concentration accurately predicts metal ion toxicity, it is not an accurate barometer for the prediction of bactericidal and antimicrobial activity. This possibly explains why differences in antimicrobial activity of clay samples existed even though metal concentrations were largely similar.

4. Conclusions

Five clay samples were tested against nine pathogenic microbes to examine their antimicrobial activities. Of clay samples tested, leachates from Sogakope demonstrated the highest antimicrobial activity with clay from Anloga possessing no activity at all. The other clay samples had varying activities on the various test organisms. The low pH of the Sogakope clay could be responsible for the observed antimicrobial activity. Metal analysis of the various samples revealed the presence of similar metal ions in all clay samples. Ag, was absent in all samples. Together, these results provide preliminary evidence that natural clay deposits in Ghana could potentially provide cost-effective topical antimicrobial treatments and/or possibly lead to the discovery of new antibacterial mechanisms that may ultimately benefit health care delivery systems worldwide.

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