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Full Length Research Paper

Metal content determination and antimicrobial properties of ochre from North-central Nigeria

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Metal content of the ochre was determined by Flame photometry and atomic adsorption spectroscopic technique while the antimicrobial activity of ochre from North-Central Nigeria against selected organisms were investigated using standard methods. The results revealed the presence of Fe, Mg, Na, K, Ca, Mn, Cu, and Zn in ochre. Fe had the highest concentration (1122.7 mg/kg) followed by Mg (193.0 mg/kg). The lowest concentration was that of Zn followed by Cu at 0.65 and 3.8 mg/kg respectively. Sensitivity tests of ochre against *Staphylococcus aureus*, *Streptococcus pyrogenes, Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli* and *Dermatophilus congolensis* revealed that ochre had the highest zone of inhibition against *Dermatophilus congolensis* compared to other pathogens. The MIC and MBC at 50% of the compounding with culture of 1000 µgcm^{-3} were found to be favourable. The study therefore showed that ochre could be a good agent in the fight against infections associated with *D. congolensis.*

Key words: Ochre, metals, North-Central Nigeria, antimicrobial properties.

INTRODUCTION

Ochre is a mineral of clay and iron oxide found in hematite and limonite. It exhibits various colors ranging from red through purple, brown and orange to yellow, depending on its chemical composition. Its color is yellow when hydrated in its natural state, but changes to red when oxidized or burned (Erlandson et al., 1999; Williams et al., 2011). Naturally occurring ochre is reddish in colour and is like clay in appearance. The various hues of colours in ochres are among the earliest pigments used by mankind. The colours are derived from mineral oxides composed mainly of hydrated iron oxide (Fe_2O_3). The amount of iron oxide (Fe_2O_3) in ochre is quite variable ranging from 20 to 70%. However, the elemental and phase analysis of red and yellow earthy pigments (ochres) indicated the presence of iron, potassium, and titanium, calcium, magnesium and sodium (Grygar et al., 2003). Ortega-Aviles et al. (2001) reported that elemental analysis of ochre paint revealed a substantial concentration of O_2, Ca and C, followed by Si, Mg, Al, Fe, and K in lower concentration. The presence of these elements through the dissolution of ochre in soil or water can negatively affect the environment at high concentrations. Apart from this, ochre was also found to be useful in medicine and for decorations among other uses. These provide the basis for chemical analysis of ochre found in North-Central Nigeria.

Ochre is considered as an earth color, ranging from golden yellow to dull red or burnt sienna. Analysis of ochre by Gryger et al. (2003); Heal et al. (2004) and Green and Watling (2007) showed that ochre consist primarily of the oxides and hydrated oxides of iron (hematite and goethite), silicon (quartz), aluminum (clay) and manganese.

Ochre is one of the materials used medicinally and otherwise since of pre-historic times (Erlandson et al, 1999). In addition to its uses, there is some evidence that ochre had practical functions. For example, ochre has been shown to have medicinal purposes as an antifungal

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agent and inhibits collagenase, making it ideal for tanning, softening and colouring leather (Audouin and Plisson, 1982; Wadley et al., 2004).

The prevalence of systemic skin infections has increased significantly during the past decade. Only a limited number of antimicrobial agents such as polyenes and azoles, and the recently introduced caspofungin acetate are currently available for the treatment of life-threatening skin infections (Hoang, 2001). For this reason, the development of new antimicrobial agents, preferably with novel mechanisms of action, is an urgent medical need.

The objective of this research therefore, was to assess the elemental composition as well as its antimicrobial activity of ochre against selected bacteria, namely *Staphylococcus aureus*, *Streptococcus pyrogenes, Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli* and *Dermatophilus congolensis.*

MATERIALS AND METHODS

Samples collection, identification and pretreatment

Ochre samples used in this study were obtained from different location in Jos (9°55' latitude and 8°54 longitude), Plateau State, while Mariga (30 20'' latitude and 110 3'' longitude) and Bida (9°06'N and 6°01'E) ochre were obtained from markets in Niger State, North Central, Nigeria. Sampling was done at random within these areas. A composite sample was made from the random ones where the representative samples were collected for this study. The samples identification was done by the Geology Department, Federal University of Technology, Minna. The samples were then air-dried and sieved through 2 mm sieve, ground, labelled and stored in polyethylene bags.

Digestion of ochre samples for metal determination

1 g of grounded sample of ochre was weighed into 125 cm^3 of Erlenmeyer flask, which was washed with nitric acid and distilled water. 4 cm^3 of perchloric acid, 25 cm^3 of concentrated nitric acid and 2 cm^3 of sulphuric acid were added to the sample. This was heated on a hot-plate under an acid fume hood until white or colourless fumes appeared. The content obtained was cooled and 45 cm^3 of distilled water was added and further heated and cooled and filtered into 100 cm^3 volumetric flask. The digested sample (filtrate) was then stored in plastic container for analysis.

To determine K and Na by flame photometer, the absorbance was set at 767 nm. The standard solutions were used to standardize the analyzer after which the samples were aspirated into the flame and transmittance was recorded for each sample (Ogugbuaja, 2000).

Extraction of ochre samples

150 g of the powdered ochre samples was weighed and macerated with 200 cm^3 of chloroform for a period of 24 h. The extract was collected and filtered into a beaker, which was evaporated to dryness in a water bath, and then allowed to cool. The residue was air dried and the procedure repeated for methanol and petroleum ether extracts.

Antimicrobial activity

Source of microorganisms

Pure isolates of *S. aureus, S. pyrogenes, P. aeruginosa, S. typhi, E. coli* and *Dermatophilus* were obtained from the stock culture of the Microbiology Laboratory of Federal University of Technology, Minna.

Susceptibility test for the organisms

The organisms were inoculated on nutrient agar plates surfaces with 3 h old culture swabbing using sterile swab stick. A sterile 4 mm cork borer was used to bore holes on the prepared plate. The plates were inoculated at 37°C for 24 h. Zone of inhibition were then recorded.

Determination of minimum inhibitory concentration and minimum bactericidal/fungicidal concentration

For MIC, two-fold serial dilutions of the extracts were performed. Each inoculum was prepared in its respective medium and density was adjusted to 0.5 McFarland standards (10^8 CFU/cm^3) and diluted to 1:100 for the broth micro dilution procedure. The test organisms were inoculated into each test tube which was then incubated at 37°C and the tube with the lowest dilution which had no detectable growth was considered as the MIC after 24 h. MBC was determined by sub-culturing the test dilutions on to a fresh solid medium and incubated further for 24 h. The highest dilution that yielded no bacterial or fungal growth on solid medium was taken as MBC/ MFC (Suffredini et al., 2004; Doughari et al., 2007).

RESULTS, DISCUSSION AND CONCLUSION

The results showed that the ochre contained Fe, Ca, Mg, Mn, Zn, K, Cu and Na in various proportions (Table 1). Fe had the highest concentration (1122.7 mg/kg) followed by Mg (193.0 mg/kg). Zn and Cu were lower in concentrations 0.65 and 3.8 mg/kg respectively. The concentration of Fe obtained indicates that these ochre samples which are mainly oxides of Fe are largely responsible for the activity of the ochre against the tested pathogens. This result is in agreement with what was obtained by Gryger et al. (2003) who earlier reported that ochre contains Fe in higher proportions to other metals. The concentrations of the metals obtained from this research can effectively inhibit the growth of *D. congolensis* as reported by Shelley et al. (2007) and Adekunle and Ikumapanyi (2006). The analysis revealed that there are variations in the concentrations of the metals detected. The variations can be attributed to difference in weather of the sampling sites. Leaching, mineral deposits and activities of man are other factors that can also influence the elemental composition of the samples (Gryger et al., 2003). Jos, for instance was a mining region which enhances deposit of pyrite (FeS_2) resulting into deposit of ochre.

Table 2 shows the susceptibility test conducted on the ochre samples. Results showed that there was no activity against *S. aureus*, *S. pyrogenes, P. aeruginosa, S. typhi,*

Table 1. Metal content in ochre samples from Jos, Mariga and Bida, North Central Nigeria (mg/kg).

Element	Jos	Mariga	Bida
Na	49.33±0.02	65.3±0.05	82.6±0.11
Mg	193±0.06	430±0.03	414.5±0.01
Mn	71.6±0.08	157.7±0.06	131.3±0.16
K	7.6±0.02	79.3±0.04	3.6±0.05
Ca	56.1±0.09	4.7±0.0.11	18.7±0.07
Fe	1122.7±0.03	1145±0.14	1005.6±0.12
Cu	3.8±0.01	8.4±0.01	7.3±0.01
Zn	0.65±0.02	0.93±0.12	3.0±0.04

Table 2. Antibacterial activity of Ochre from Jos, Mariga and Bida North Central, Nigeria.

Zone	Organisms	Zones of inhibition (mm)			
		25%	50%	75%	100%
Jos	*D. congolensis*	20	15	21	13
Mariga	*D. congolensis*	17	15	12	8
Bida	*D. congolensis*	13	10	8	7

Table 3. Minimum inhibitory concentrations (MIC) of Ochre from Jos, Plateau State, Nigeria.

Organism	25%	50%	75%	100%	Control
D. congolensis	1250	1000	1250	1500	NI

NI= No inhibition.

and *E. coli.* It was observed that all the ochre samples were very efficacious against *D. congolensis.* This could be due to the fact that there was a high level of Fe in ochre resulting in high inhibitory effect. The ochre sample from Jos exhibited higher inhibitory property of 20.0 mm at 25% concentration than that from Mariga, 17.0 mm while that from Bida was 13.0 mm (Table 2). These results can be attributed to the comparatively higher concentrations of the composites of the ochre sample from Jos. This inhibitory property reduces as the extracts became less concentrated by dissolution with ethanol as solvent. The most surprising thing was that at 50% concentration ochre (Jos) and (Mariga) were found to be active against *D. congolensis* at zone of inhibition of 8.0 mm while, Bida ochre inhibited it at 10.0 mm. As the concentration increases the degree of the activity against the organisms reduces. This therefore means that the ochre was active at low concentration. Recently, it has also been reported that iron in clay kills bacteria by generating radicals that attack cell components (Chem. World, 2011; Williams, 2011) and as such it can be inferred that the high level of Fe in ochre is mainly responsible for its observed antibacterial activity against *D. congolensis.*

Apart from Fe, other metals like Zn, Mg and Cu, etc can exhibit effective antimicrobial activity. This confirmed the statement made earlier by Rahman et al. (2005) that metal ions play vital roles in various biochemical reactions by acting as cofactors for many enzymes. The inhibitory activity of ochre against the *D. congolensis* could also result from alkaloids and glycosides detected in the sample as reported by Prasad et al. (2004).

Results of MIC and MBC determination revealed that the highest MIC value (50%) was obtained from samples from the Jos ochre (Tables 3 and 4). These results further suggest that the MIC values for the sample were lower than their MBC values, justifying that ochre inhibited growth of the test microorganisms while being bactericidal at higher concentrations.

In conclusion, locally available material like ochre, which is eco-friendly, can be used for the management of skin infections associated with *D. congolensis*. Further investigations should be conducted with a view to developing antibacterial formulations based on the

Table 4. Minimum bactericidal concentrations (MBC) of Ochre from Jos, Plateau State, Nigeria.

D. congolensis	0.5 cm^3	0.75 cm^3	1.0 cm^3	1.25 cm^3	1.5 cm^3	1.75 cm^3
25%	+	+	+	-	-	-
50%	+	+	-	-	-	-
75%	+	+	+	-	-	-
100%	+	+	+	+	-	-
Control	+	+	+	+	+	-

+ = Growth, - = Inhibition (no growth).

toxicological experiments.

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