Inhibitory Effect of Termite Mound Soil Leachates on Some Human-Wildlife Pathogens

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Abstract: Although, there are reports of soil ingestion by wildlife (geophagy), but reasons for action remained elusive. A predominant hypothesis posits geophagy as source of medication for wildlife. This hypothesis albeit tested on different soils, but has sparsely been documented for termite mound soil (TMS). This study compared antibacterial susceptibility of aqueous leachates of four geophagic TMS1-4 from different sources with controls; a composite sample of forest soil (C1) and an aqueous solution of streptomycin sulphate (C2), against predominant human-wildlife pathogens; *Staphylococcus aureus (S. aureus)*, *Escherichia coli(E. coli)* and *Salmonella typhi (S. typhi)*. All TMS1-4 and C1-2 exhibited range of 1.33±0.58 to 8.25±2.87 inhibition zones (IZ) against all tested pathogens. While TMS2 (5.00±1.00) and C2 (5.00±1.00) gave similar IZ against *S. typhi*, C2showed significant highest IZ (8.25±2.87) against*E. coli*. The exhibition of IZ in all TMS supports medication hypothesis. Hence, wildlife of the study area may be consuming TMSs for self-medication. Further studies may be needed to investigate properties of TMS responsible for exhibition of IZ.

Keywords: Antibiotics,Geophagy, Hypothesis, Pathogens and Termite mound soil.

INTRODUCTION

Termite mound (TM) isa landscape ecosystem comprising lots of plants, animals and microorganisms interacting with one another.Termite guts have been reported to habour a number of microorganisms of economic importance, which have great impact on the formation and composition of TMs (Ayitso and Onyango, 2016; Chandra and Kumar, 2017; Enagbonma and Babalola, 2019;Mahdi *et al.*, 2020). Besides, the high nutrients composition and clay content of termite mounds (TMs) have contributed to their wide range of use in various fields of endeavour such as agriculture, building construction, biotechnology and pharmaceutical industries (Rodríguez-Rojas. *et al*, 2015; Borquay *et al.*, 2016).

Although, the stimuli behind the deliberate act of consuming soil by both man and animals (geophay or geophagia) are yet to be completely understood, however, many previous studies/reviews on mineral licks have reported the consumption of termite mound soil (TMS)for various purposes; as nutrient supplements and/or as medications (Krishnamani and Mahaney, 2000; Young *et al.*, 2011;Pebsworth *et al.*, 2019). Most recent unpublished work of Egbetade (2021) in Old Oyo National Park (OONP) has demonstrated how kobs and patas monkey utilized TMS using camera trap. Over the years, the purposes for which mammals and other important taxas ingest soil have generated lots of controversy, thus creating a huge debate among the scientists. Except for the report of Ketch (2001) and Bisi-Johnson *et al.* (2013), studies investigating etiology of geophagy through microbiological evaluation of mineral licks are scanty, most especially for TMS.

Even the few available reports on wildlife geophagy had concentrated mainly on the analysis of physico-chemicals and mineralogical properties of the consumed soil (Eksteen and Bornman, 1990; Ayotte *et al.*, 2006; Slabach *et al.*, 2015), excluding the antibacterial susceptibility testing of the soil.

Studies have shown that microbes of certain soils (most especially soil-containing clay) have inhibitory effect on certain pathogens (Lafi1 and Al-Dulaimy, 2011;Borquay*et al.*, 2016).However, this inhibitory effect has rarely been evaluated for TMs, even though they considerably appear to contain clay (Mahaney *et al.*, 1996; Aufreiter *et al.*, 2001). The commonly utilized antibiotics for treatment of various ailments in man and animals have suffered great resistance, thus becoming a global problem (Morrison *et al.*, 2017). Since TMs are home to various beneficial microorganisms, most especially microbes-producing antibiotics (Ayitso and Onyango, 2016), they may therefore have capabilities to be utilized as potent sources of new strain of antibiotics for exploitation in pharmaceutical industries.

Unlike various studies, where inhibitory properties of isolates from certain soils were tested against specific pathogens, this study explore the possibilities of inhibitory activities of selected TMs directly against three common human-animal pathogenic organisms in comparison with two controls; forest soil (FS/Control 1= C1) and pharmaceutical streptomycin sulphate (SS/Control 2= C2). If TMs were to act as natural medication for wildlife, it is therefore expected that TMs will generate some inhibitory zones that maybe equal to that of the selected pharmaceutical antibiotics (C2), but more than that of the forest soil (C1). It is hoped that the result will serve as a baseline data for understanding the possibilities of TMs not only for being used for developing new drugs, but alsofor giving a reason for its being utilized by wildlife as self-medication.

MATERIALS AND METHODS

Study Area

Among the seven National Parks in Nigeria is Old Oyo National Park (OONP), located in the Southwest (in Oyo State) at latitude 8° 15' and 9° 00'N and longitude 3° 35' and 4° 42'E and established by decree 46 of 1999 (Fig. 1). Though, the number of national parks in Nigeria has presently been increased to seventeen (Alarape, 2021). Of the former seven parks, because of the area occupied (2,512 km^2), OONP was known to be the fourth largest park, next to Gashaka Gumti National Park in Taraba State (6,731 km^2). At present, it however retains its fourth position amongst the 17 national parks. For easy management, OONP is divided into five ranges; Marguba range (in Sepeteri town), Tede (in Tede town), Yemoso (Ikoyi town), Sepeteri (in Igboho town) and Oyo lle (in lgbeti town). It has several natural mineral licks utilized by wildlife, tourists, hunters and some members of the community (Adewale and Alarape, 2020).

Sample Collection

Five soil samples were collected, four from randomly selected four different termite mounds (TMS1-4) that showed signs of geophagy from among the already identified TMS from the previous unpublished work of Egbetade (2021). A sterilized hand trowel, zip lock bag and hand glove were used for the soil collection. Soils were dug and collected from the top, middle and bottom layers at the four purposively selected spots surrounding each of the TMs and thoroughly mixed together to form a composite sample. Sub-samples of the soils were then taken for laboratory analysis. The fifth soil sample was also collected randomly from adjacent forest soils (FS) with soil auger at 10-15 cm depth. Ten core soil samples were collected from FS area (20m by 30m) and then bulked together to form a composite sample, serving as control (C1). In all and for each, not less than 5g of composite samples were collected in a well labeled zip lock bag, stored in cooler-containing ice-flakes for preservation during transportation to the laboratory for analysis.

pH of Soil Samples (TMS and FS)

The pH of each sample of soil was determined using glass electrode pH meter in soil/water suspension 1:2 according to McLean *et al.* (1982).

Leachates Preparation of Termite Mound Soil (TMS) and Forest Soil (FS)

Leachates of TMS1-4and FS (C1) were prepared by adopting the procedure described by Russell and Furr (1977) and Otto and Haydel (2013). To generate suspension, leachates were prepared by dissolving 1g of TMs or FS in 10ml of sterile distilled water in a test tube [i.e10% TM/FS in distilled water or 1% (wt/vol.)]. This is equivalent to 0.1g of TMS or FS per 1ml of sterile distilled water). To separate the suspension into soluble and insoluble fractions, the test tube was vortexed three minutes each time. Then, the aqueous supernatant (leachate) was collected and sterilized by passing them through a 0.22 mm Whatman filter and thereafter serially diluted into ten-fold. Streptomycin sulphate (SS) was also prepared at a concentration of 1mg/ml (wt/vol.) of sterile distilled water.

Antimicrobial Susceptibility Testing

An 18 hours overnight bacteria cultures (on nutrient broth at 37°C) including Gram positive *Staphylococcus aureus*, Gram-negative *Salmonella typhi* and *Echerichia coli* were standardized using McFarland standard (10^8 CFU/ml of 0.5 McFarland standards). Hundred microliters (100 µl) of each bacterial suspension were spread on Mueller-Hinton agar using sterile glass spreader. The antimicrobial activities of TM1-4 and the controls (C1-2) were determined against *S. aureus, S. typhi* and *E. coli* using agar well diffusion methods as described by Russell and Furr (1977), with some modifications (by using soil leachates in addition to antibiotics). One hundred micro liters (100µL) of each of the supernatant from each dilution were added into Mueller-Hinton agar plates, allowed to equilibrate and incubated at 37°C for 24h. The plates were then observed for inhibition zones (IZ). The sensitivity of each tested media (TMS1-4) was then compared with that of the controls (C1-2).

Statistical Analysis

Statistical Package for Social Sciences (SPSS) software, version 20 was used to evaluate the result of antimicrobial experiments, carried out in triplicate, as mean ±standard deviation ($\overline{x}$±S.D) and a p-value $<$ 0.05 was considered significantly different.

RESULTS

Results obtained showed that pH of the samples varied across different locations (Table 1), though not significantly different. The pH of the samples range between 2.11±0.78 and 4.00±1.22 indicating acidic conditions, with TMS3 having the highest acidity and TMS2 with the lowest acidity. Figure 2 showed the inhibitory activities of soil extracts on the test strains. All TMS (TMS1-4) and the controls (C1-2) exhibited varying inhibition zones (1.33±0.58 - 8.25±2.87) mm against all the tested pathogenic microbes (*S. aureus, E. coli and S. typhi*). Of all the TMSs, TMS2 had its highest inhibition zone (5.00±00) mm on*S. typhi*, which is the same with that of C2 and higher inhibition zone (4.00±00) mm on*E. coli*. The highest inhibition zones (IZ) for C1 and C2 on *E. coli* were 2.75±1.00mm and 8.25±2.87mm respectively. Although, that the lowest IZ (1.33±0.58) mm exhibited by TMS3 on *E. coli* is lower than the highest IZ exhibited by C1 (2.75±1.00) mm, and that C2 significantly have the highest IZ on *E. coli*(8.25±2.87)mm and *S. aureus* (4.00±0.00)mm than other media does not limit the potential of TMSs in producing highly effective antibiotic-producing factors. Apart from the aforementioned, majority of the media have IZ ranges from 2mm to 3mm on all the tested pathogens.

DISCUSSION

Geophagy is one of the practices of highly developed wildlife and humans alike. Scientists have speculated various factors responsible for the phenomenon (Krishnamani and Mahaney, 2000). There have been extensive investigations to elucidate factors driving wildlife towards geophagy. Inhibitory effects of TMS as one of the reasons behind geophagy have not been widely reported. This study thus, predicts etiology of geophagy using inhibitory characteristics of the ingested soil. Though, the results obtained showed very low inhibitory zones (IZ) for all the investigated TMSs on the tested gram negative and gram positive bacteria, when compare with that of the control-2 (Streptomycin sulphate). However, these low IZs of the TMSs doubled the IZs of C1 in some cases (e.g TMS2 against *S. typhi* and TMS3 against *E. coli*), though in most cases lessthan that of the expected ranges of the C2 (4.00± 0.00 - 8.25 ± 2.87) mm. Like the C2 (having IZ of 8.25 ± 2.87) mm, it is likely that the constituent antibiotic producing organisms of the TMSs can also perform better than the expectation, if isolated before being used for susceptibility testing. Still, the low IZs of the TMSs may likely be enough to supports the hypothesis that TMS could be efficacious against certain disease (e.g diarrhea) among wildlife (Pebsworth *et al.*, 2019). More so, the IZs produced is proportional to the concentration of the leachates (0.1g/1ml).An increase in the concentration of the TMSs leachates (beyond 0.1g/1ml) may produce IZs values that could be equal/greater than that of the C2. Until the minimum quantity of the antimicrobial geophagic soil is established, the low IZs produced by the 0.1g/1ml leachates of TMSs in this study may not be disregarded as insufficient to support the existing hypothesis that wildlife may be consuming the TMSs for medication purpose.

The samples (TMS1-4) showed a significant difference ($p = 0.00$, $\alpha = 0.05$) in inhibitory properties with C2 (SS), but not with C1 (FS) against the test organisms. Sample C1, albeit, not a geophagic soil, but also exhibited inhibitory effects (though, not more than 2±1.00mm on the pathogenic microbes). This is not surprising as several authors have reported IZ in a non-geophagic soil from soils other than TMS (Lafi1 and Al-Dulaimy, 2011; Kaur *et al.*, 2014). While this suggests no reason as to why wildlife will choose TMS in preference to C1 for consumption, however, extracts of all tested soils, particularly TMSs having IZ greater than 2±1.00mm could be harnessed for the production of innovative antibacterial drugsfor the treatment of captive wildlife and human diseases. Among these reports include the report of Lafi1 and Al-Dulaimy (2011) showing the inhibitory activities of four different mineral clay soils on *S. aureus* and *Pseudomonas aeruginosa* with three samples exhibiting antibacterial effect only against *S. aureus*. Borquaye *et al.* (2016) *in vitro* tested for the inhibitory activities of clay against nine microbes in China and found almost all of the sample clay exhibiting antimicrobial activities against the tested microbes.

Several studies assayed anti-microbial effects of soil against pathogenic microbes have shown that low pH, mineral content, clay content and antimicrobials composition (Lafil and Al-Dulaimy, 2011; Otto and Haydel, 2013; Sethi *et al.*, 2013;Borquaye *et al.*, 2016; Begum *et al.*, 2017a, Begum *et al.*, 2017b; Williams, 2019) were predictive factors. The inhibitory activity by TMS in this study could be due to its low pH. This was consistent with previous investigation (Borquaye *et al.* 2016).

CONCLUSION

It can be concluded that wildlife in the study area may be consuming the geophagic soils (TMSs) for self-medication as the investigated TMSs possess inhibitory zones which is lower and/or equal to that of the pharmaceutical control (Streptomycin sulphate).

Further study may be required to isolate and identified the antibiotic-producing constituents of the TMSs. TMS may have the potential for supplying its yet unidentified antibiotic-producing microbes for the treatment of various human-wildlife diseases.

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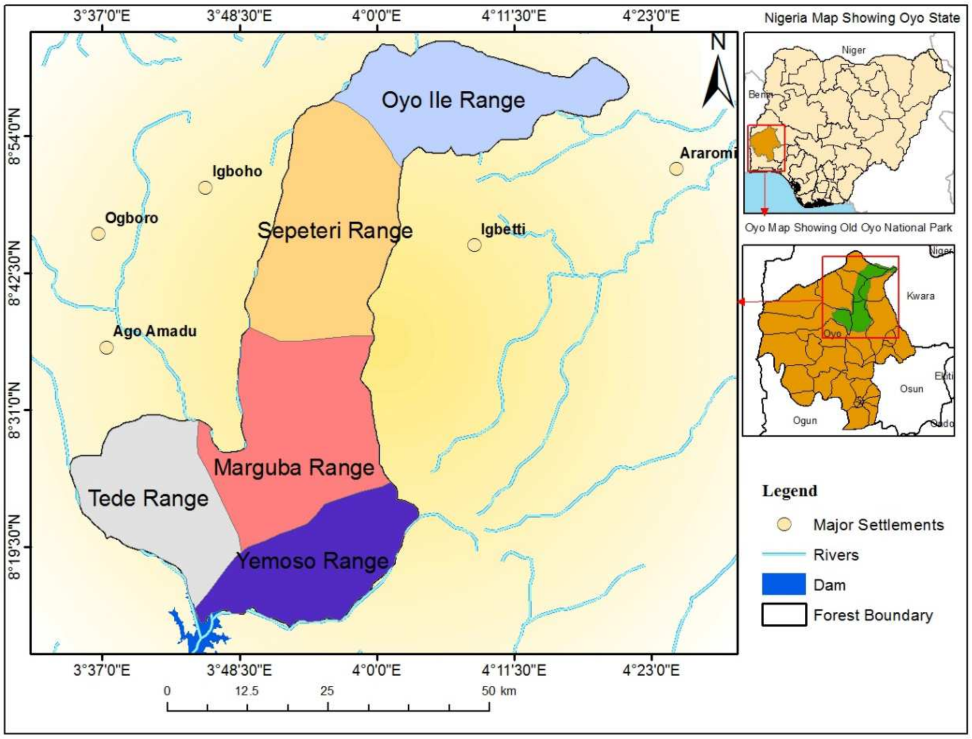

Figure 1: Map of Old Oyo National Park, Adapted from Akinsorotan (2017)

Table 1: Location and pH of Termite Mound Soil Samples Collected from Old Oyo National Park

Sample	**Longitude**	**Latitude**	**pH($\bar{x}$±S.D)**
TMS1	N08.27116	E003.46450	2.33±0.71^{a}
TMS2	N08.27159	E003.46479	4.00±1.22^{a}
TMS3	N08.27171	E003.46456	2.11±0.78^{a}
TMS4	N08.27115	E003.46448	2.78±0.97^{a}
FS(C1)	N08.28272	E003.45801	2.11±0.60^{a}

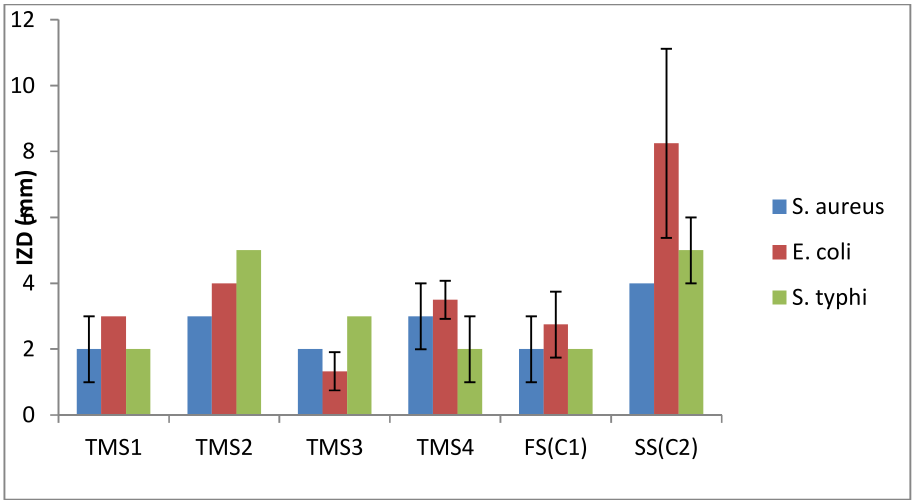

Figure 2: Susceptibility Test of Selected Organisms against Termite Mound Soils,Forest Soil and Streptomycin Sulphate

IZD: Inhibitotry Zone Diameter, TMS: Termite Mound Soil, FS; Forest Soil, SS: Streptomycin Sulphate, C1: Control 1, C2 : Control 2

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