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Processing and Evaluation of Locally Sourced Kaolin for Pharmaceutical Production

Adebowale O. Adeluola, Emmanuel C. Ezeobiora, Udoma E. Mendie

Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Lagos, Lagos, Nigeria.

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ABSTRACT

Cheaper and readily available natural clay deposits in Nigeria, may serve as good sources of pharmaceutical raw material. This work sought to process, characterize and evaluate locally sourced kaolin for drug production. Kaolin samples were locally sourced from Abia state and Ogun state of Nigeria. Purification was done through wet processing and chemical treatment of the samples. Chemical leaching was done using 2mol/l, 4mol/l and 8mol/l concentrations of hydrochloric acid with subsequent boiling for 6 hours. They were subjected to identification test for kaolin , carbonate test and iron limit test. The physicochemical properties were assessed. Samples were characterized using X-Ray Flourescence (XRF), Fourier Transform Infrared (FT-IR), X-Ray Diffraction (XRD) and micrograph. The raw, treated and standard kaolin samples were assessed for microbiological quality. The physicochemical properties and microbial limit tests for their formulated suspensions were also assessed.

All the treated powdered samples, complied with pharmacopoeial standards for kaolin. The micrograph showed that the particles are small, clustered and granular. *Bacillus subtilis*, *E. coli*, *Aspergillus spp*. and *Histoplasma spp*. were identified only in the raw kaolin samples, while the Acid treated samples had acceptable pharmacopoeial standards for microbial limit . XRF, XRD and FT-IR results revealed that 2 mol/l HCl treated samples gave better pharmaceutical grade kaolin. The suspensions formulated from the treated kaolin samples showed good physicochemical properties and microbial limit. This research reveals that Wet Processing Technique and 2 mol/l HCl leaching is suitable for processing locally sourced kaolin for drug production.

Keywords: Kaolin, wet processing, acid leaching, microbial limit.

Introduction

The purity of excipients for Pharmaceutical production has much to do with their origin and method of processing. They are produced from a great variety of different source materials, for example: Minerals (talc, kaolin, calcium and sodium phosphates, sodium chloride) according to Dankward Jakel and Martin Keck, 2000.[1] Irrespective of origin and form, excipients for use in Pharmaceutical production must go through a refining process that must confer the level of purity required for pharmaceutical applications. Microbial contamination of excipients is most frequently caused by contaminated source materials (e.g., grain), processing aids, or insufficient cleaning of equipment. Among the processing aids, water is by far the most important source for contamination. Microbial contaminants in excipients range from pathogenic microorganisms such as *Salmonella sp*., *Escherichia coli* and *Staphylococcus aureus to* fungi, and yeasts. The presence of these contaminants may lead to infection of patients, degradation of active ingredients and or drug formulations made from them, presence of pyrogens and mycotoxins in the formulations, the presence of residues from antimicrobial treatment and contamination of pharmaceutical-processing equipment. For immune-compromised patients, high microbial counts can be critical, even in the absence of pathogenic species. Requirements for the microbiological purity of excipients vary according to the type of drug product to be made. All excipients must therefore comply with general specifications so that their drug products or pharmaceutical preparations meet the requirements for microbiological purity.[2]

Kaolin, a fine, soft white or off-white mineral, resulting from decomposition of clays and other rocks is named after the hill in China (Kao-ling) from which it was mined for centuries.[3] Kaolin is a major ingredient in medicines to alleviate stomach upsets by its adsorbent properties to bind Gastro intestinal (GI) toxins and control diarrhea. It has been added to dusting powders, used as a tablet excipient and as a coating on sores caused by radiation treatments and as a drying agent on skin. It serves as an emollient and drying agent when applied topically. Kaolin as a raw material is commonly used in cosmetics and pharmaceuticals.[4] It may also be used in pharmaceutical preparations as a filtering agent to clarify liquids. Evidence abound suggesting that kaolin may be useful in the decoloration of dye waste water using electromagnetic method.[5]

Generally, kaolin is one of a group of fine clay minerals with the chemical composition of $Al_2O_3.2SiO_2.2H_2O$ which means two-layer crystal (silicon-oxygen tetrahedral layer joined to alumina octahedral layer) exist alternatively. Chemical compositions of kaolin mineral are same. Kaolinite is the principal constituent of kaolin. Its chemical structure is $Al_2Si_2O_4(OH)_4$ (theoretically 39.8% alumina + 46.3% Silica + 13.9% water). The pH of kaolinite obtained from Nigeria is acidic.[6]

Materials and Methods

Kaolin ore samples used in this work were obtained from two different sources:

*Corresponding author. E mail: debodelu@gmail.com; aadeluola@unilag.edu.ng
Tel: +2347056004859

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1. Lakiri village, Mile 7-Ajebo Road, ObafemiOwode Local Government (6°57′N 3°30′E), Ogun state (OG).
2. Ohia village in Umuahia **(5°32′N 7°29′E)** North L.G.A, Abia state (AB).
Light kaolin (reference standard) from Bristol Scientific Company UK was used as control (SD).

Processing procedure
A wet processing method employed by Mohamed *et al*., 2015 was adopted.[7] A total of 2kg of each kaolin ore sample was manually reduced in size by milling using mortar and pestle. The milled powder in each case was dispersed in sterile distilled water to form the slurry. The coarse grits (>45 µm) were removed by settling procedures and use of vibrating screens. The resultant slurry in each case were fed into the centrifuge to separate the slurry into fine and coarse particle size fractions. The fine particle size fractions were decanted and filtered using vacuum pump filter. The filtered fine kaolin was allowed to dry in an air drier. About 20 g of each dried kaolin sample was weighed and treated with 2 mol/L, 4 mol/L and 8 mol/L concentrations of hydrochloric acid heating to boiling point for 6 hours for each acid concentration.[7] The treated kaolin is washed three times with sterile distilled water, filtered and dried.

Isolation and characterization of microbial groups in the kaolin samples
Isolation: sterile Tryptone soya broth (TSB) was used for enrichment. Streaking of culture was done on Tryptone soya agar (TSA) with sterile wire loop and incubated at 35 ± 2ºC.[2]
Characterization: The method used by Zhang *et al*[8] was adopted. This was based on colonial morphology, microscopy and biochemical tests. Gram staining, spore staining, motility test, catalase, oxidase, methyl red, indole tests and carbohydrate fermentation tests were carried out and analysed using the Advanced Bacterial Identification Software (ABIS) version 12.8 for identification of the organisms.

Physicochemical properties
The macroscopic and organoleptic properties of each sample of raw kaolin ore and the various samples of processed kaolin were evaluated for texture, colour, odour and taste.
pH readings of 20% w/v slurry of each kaolin sample was determined using a pH meter.[9]
The density and specific gravity of each sample were determined using a graduated cylinder as described by James Rutter.[10]
FT-IR characterization of each kaolin sample was done by placing 10 mg sample of each kaolin sample on the optical eye of the table top Bruker FTIR, platinium-ATR, Alpha model machine and was scanned within 60 seconds.
The XRD characterization was carried out using a Philips PW1700 Series X-Ray Diffractometer. The diffraction angle was scanned from 10^o to 60^o 2θ, at a step size of 0.05^o and a rate of 5.00 ^{o}/min using CuKα filtered radiation at 40 kV and 40 mA.
The XRF characterization for each sample was carried out using Philips PW1606 X-Ray Fluorescence spectrometer at voltage of 40KV and current of 350µA.
Microscopic studies of kaolin powder samples were done under microscope by mounting flips of samples on the glass slides with a drop of glycerol and enclosed with a cover slip.

Formulation of kaolin suspension
All the kaolin powder samples were used to make formulations of kaolin suspension as described in British Pharmacopoeia (BP)[11] and pharmaceutical practice hand book by Judith and Jennies, 2014.[12] Table 1 gives the details of the formulation used.

Evaluation of kaolin suspension
pH evaluation
pH of the suspension was obtained using Seven Easy Mettler Toledo pH meter with code number GmbH 8603.

Table1: Formulation table.

Ingredients	Master Formular	In 100 mL
Light kaolin	200 g	20 g
Light mag. carbonate	50 g	5 g
Sodium bicarbonate	50 g	5 g
Peppermint emulsion conc.	25 mL	2.5 mL
Chloroform water, DS	500 mL	50 mL
Water for preparation	1000 mL	100 mL

Measurement of sedimentation volume (F)
The sedimentation volume (F) of each suspension was determined by measuring the volume of the sediments in the suspension placed in the measuring cylinder for 7 days as described by Azubuike *et al*, 2017[13] F was calculated thus:
$F = V_u / V_0$
Where $V_u = volume\ of the\ sediment$
$V_0 = total$ volume of the suspension

Rheological assessment
The method used by Azubuike *et al.*, 2017[13] was adopted.

Viscosity
The viscosity of the suspension was assessed using a DV-E digital viscometer. Spindle 3 was used and the selected numbers of RPM used were 10, 20, 30, 40 and 60.

Flow rate
A volume of 25ml of the suspension was withdrawn using a 25 mL pipette. The time for the suspension to flow through a 25 mL pipette was recorded using stop watch. The flow rate was calculated using:

Flow rate (ml/s) = volume from pipette (ml)/flow time (sec.).

Redispersibility
A fixed volume of each suspension (50 mL) was kept in calibrated containers and stored at room temperature. At various time intervals (1, 5, 10, 15, 20 days), each container was shaken vigorously to re-disperse the sediment and the presence or absence of undispersed sediment was recorded.[13]

Microbiological evaluation
Microbiological assessment of the kaolin samples was carried out by using the method described in United States Pharmacopeia (USP).[14]
Eosine methylene blue agar (EMBA) was used for the detection of *Escherichia coli*, Cetrimide agar (CET) was used for the detection of *Pseudomonas aeroginosa*, Salmonella Shigella agar (SSA) was used for the detection of *Salmonela* and *Shigella spp*, Tryptone soy agar (TSA) was used for total aerobic microbial counts (TAMC). These plates were incubated at 35ºC ± 2ºC and observed daily for 72 hours. Sabouraud dextrose agar (SDA) was used for detection of total yeast and mould (TYMC) counts. The plates for determination of TYMC were incubated at 27 ± 2°C and these plates were observed daily for one week.[14]

Microbial limit test on kaolin suspensions
A 10 mL volume of each suspension was pipetted using a sterile pipette and transferred into 90ml of 3% tween 80 to make 1:10 dilution. From this, a further 1:10 dilution was made in 90ml of 3% tween 80 to make 1:100 dilutions for each suspension. A 1 ml volume of each dilution was aseptically transferred into appropriately labelled petri dishes. 19ml of Tryptone Soy-Agar (TSA), Sabouraud Dextrose Agar (SDA) and Eosin Methylene Blue Agar (EMBA) was added to each sample in the petri-dishes as labeled for Total Aerobic Microbial Count, Total Yeast and Mould Count and Test for presence of *E. coli* respectively.[15, 16]
The SDA plates for TYMC were incubated at 25°C while the TSA plates and EMBA plates for TAMC and test for absence of *E. coli* were incubated at 37°C. The plates were observed daily for 72hours for bacterial load and 7days for fungal load. The colonies were counted and the dilution factor was used to compute the cell concentrations in colony forming units per g (cfu/g) of the sample.

Results and Discussion

Organoleptic characteristics

The organoleptic properties of kaolin clay samples; the colour, taste, odour, texture of kaolin samples is presented in Table 2.

Abia clay (sample AB) was noted to have grayish white colour while that of Ogun clay (Sample OG) was pinkish white. The treated samples appeared off white in colour. The standard Kaolin powder was off-White in colour. Samples AB and OG were very gritty with earthy odour whereas the treated samples and the standard were non-gritty and retains slight earthy odour which increases when in contact with water. Sample AB has higher density and specific gravity than sample OG (Table 3). This can be attributed to high iron content of sample AB. Upon acid treatment, both density and specific gravity of the samples decreased.[17-19]

pH

The pH of 20% slurry of each kaolin sample was determined. The result showed that in raw forms, sample AB is more acidic than sample OG as presented in Table 3. All the treated kaolin samples are acidic as well as the standard. The pH range of pharmaceutical grade kaolin was given to be between 4 to 8 values.[10]

Some pharmacopoeial properties of the kaolin sample are shown in table 3

Characterisation

X-ray flourescence analysis

This reveals the percentage elemental content of each kaolin sample. The results for the elemental analysis are presented in Table 4. Permitted Daily Exposure (PDE) for some of the elements were compared with quantities found in a maximum daily dose of 262 g of kaolin taken orally as indicated in Table 5.

From the results in Table 4, percentage of the elemental impurities were reduced with acid treatment in nearly all the Samples tested. The level of reduction was also found to increase with increasing concentration of the hydrochloric acid used. This is due to segmental degradation of the clay sheet upon acid treatment.

In the case of silicone, the concentration of the element increased with increase in the concentration of the hydrochloric acid. There was successive leaching of the aluminium ion from the octahedral layer causing concomitant increase in silicon value.[20- 22]

Both the Abia and Ogun samples have virtually similar elemental characteristics except their variations in their iron and silicon content. The Abia sample (AB) gave higher iron content when compared with the Ogun (OG) sample and the standard. However, sample OG has higher silicon percentage value when compared with AB as presented in Table 4.

From a maximum daily dose of 262 g of kaolin, the daily exposure to all the elemental impurities were well within limits of the permitted daily exposure (PDE) limits of elemental impurities for calcium, copper, lead, chromium and nickel as obtained from table 5. In all cases, the treated samples gave lower elemental impurities than the Standard (SD).

FT-IR analysis

This elucidates the functional groups available in the kaolin samples. The results of FT-IR for Abia and Ogun state sourced kaolin are presented in figures 1 and 2 respectively.

The bands (cm^{-1}) observed at 3620, 3650 and 3690 show Al-O-H_{str} (structural HydroxyI groups). Bands at 3395 and 1640 correspond to H-O-$H_{bending}$; 1113, 1023 and 989cm^{-1} bands indicates S_i-$O_{str.}$, 907cm^{-1} indicates Al-Al-$OH_{str.}$, 777cm^{-1} indicates Al-Mg-$OH_{str.}$; 747cm^{-1} indicates Si-O-$Al_{str.,}$ band 669cm^{-1} indicates Si-O_{str} while band 521cm^{-1} shows Si-O-Al (figures 1 and 2). This corresponds to IR bands of kaolinite clay with possible assignment.[23]

When the clays were treated with acid, there was not much variation in the peak pattern for 2M acid; however, there was loss of bands at 3395.40cm^{-1} and 1640.60cm^{-1} of the raw samples AB and OG; with further increase in acid strength, the peak intensity was found to decrease progressively as shown in figures 1 and 2.

For 4M and 8M acid treatment, the structural hydroxyl vibration band is extremely weak indicating penetration of protons into the kaolin layers and attack on the structural hydroxyl group resulted in the dehydroxylation and leaching of Al^{3+} from the octahedral layer.[18]

The bands observed at 3390 cm^{-1} to 3445cm^{-1} and 1633cm^{-1} to 1645cm^{-1} are assigned to the bending vibration mode of high amount of water on the surface of the clay. This peak was found to reduce after acid treatment and was found to be absent in case of 8M treated clay due to structural disintegration.

The band observed at 1113cm^{-1}, 1020cm^{-1} to 1030cm^{-1} and 1005cm^{-1} to 980cm^{-1} are assigned to Si-O stretching modes.[22]

The FTIR result is in clear agreement with the XRF study which indicates segmental degradation of the clay sheet upon acid treatment.

X-ray diffraction analysis

This reveals the mineralogical content of kaolin samples. The results of XRD analysis are presented in figures 3 and 4 for Abia and Ogun state sourced kaolin respectively.

Both standard and untreated clays show well defined reflection at 2θ value 12^0 and 25^0 which corresponds to the values of 7.180A^0 which are typical characteristic peaks of kaolinite clay. Again, peaks corresponding to 2θ value at 34-36^0, 38-42^0, 45-50^0 and 54^0were observed for both Ogun and Abia clays, which may vary for Kaolinites from different origin.[24]

Upon acid treatment, the peak intensity of both clays was found to have decreased progressively as shown in figures 3 and 4. This could be due to structural disorder that occured owing to acid treatment that produced a partial dissolution of Al^{3+} cations from the octahedral sheet. At low acid strength (OG–2M–HCL and AB–2M-HCL), the reflection of the original kaolinite phase becomes narrower. The narrowing of the peak may be related to the increase of crystallite size and/or decrease in the mean lattice strain.[19]

It is also observed that both clays treated with high acid strength (AB–8M-HCI and OG-8M-HCl) showed reduction in the entire well–defined peak in the X- ray diffractograms. This indicates that these clays become more amorphous in nature and there is a large degree of structural disorderliness in these samples since leaching is quite severe in this acid strength, the layered structure of clay mineral disintegrates to give an amorphous phase. This corresponds to similar results that are described in other literatures.[17,18]

Micrograph

This reveals the samples' images from Olympus Digital Camera Microscope with magnification 10 x 100 x 1. The micrographs for Abia and Ogun sourced kaolin samples are shown in Figures 5 and 6 respectively.

Microbiological assay

Evaluation of raw kaolin samples (AB and OG) for TAMC and TYMC showed that they harbor numerous bacteria and fungi that were shown to be "Too Numerous to Count (TNTC). The samples treated with different concentration of acids showed drastic reduction in total counts (Table 6). When assessed using MacConkey agar, raw sample of kaolin (sample AB and OG) showed few numbers of suspected E. coli bacteria. While upon assessment of the treated samples, and standard there were no noticeable colonies found after 72hours incubation at 37ºC.

The results for TAMC, TYMC and test for absence of E. coli are shown in Tables 6 and 7.

Gram-positive and Gram-negative rods were identified. Upon biochemical tests, they were found to be Bacillus subtilis and E. coli. The morphological and microscopic studies of fungi on sabourand dextrose agar showed that sample AB is contaminated with Aspergillus spp., while sample OG was contaminated with *Aspergillus spp*. and *Histoplasma spp*.

Table 2: Organoleptic characteristic of kaolin samples.

Organoleptic properties	Observations
Colour	Sample AB was noted to be grayish white while sample OG was pinkish white. The treated clays appeared off-white in color.
Taste	All the samples tasted muddy.
Odour	All the samples had an earthy odour.
Texture	The raw samples were rough and clumpy while the treated samples were non-gritty fine powders.

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Table 3: Solubility, Density, pH and Specific gravity of kaolin sample.

Sample code	Solubility in water	Solubility in Ethanol	Density (g/cm^3) ± SD	pH ± SD	Specific gravity ± SD
Pharmacopoeial standards (Codex)	Insoluble				
AB	Insoluble	Insoluble	1.2910 ± 0.13	5.71±0.09	1.3990 ± 0.03
OG	Insoluble	Insoluble	0.7920 ± 0.069	6.71 ±0.034	0.8587 ± 0.06
SD	Insoluble	Insoluble	0.4447 ± 0.10	5.91 ± 0.01	0.4822 ± 0.004
AB-2M-HCl	Insoluble	Insoluble	0.9064 ± 0.088	5.27 ± 0.013	0.9828 ± 0.004
AB-4M-HCl	Insoluble	Insoluble	0.6902 ± 0.045	5.10 ± 0.03	0.7484 ± 0.005
AB-8M-HCl	Insoluble	Insoluble	0.5245 ± 0.024	4.71 ± 0.02	0.5687 ± 0.051
OG-2M-HCl	Insoluble	Insoluble	0.5881 ± 0.069	6.31 ± 0.02	0.06376 ± 0.008
OG-4M-HCl	Insoluble	Insoluble	0.5442 ± 0.043	5.79 ± 0.01	0.5810 ± 0.008
OG-8M-HCl	Insoluble	Insoluble	0.4640 ± 0.037	5.23 ± 0.03	0.5031 ± 0.001

Key: AB-Abia sourced sample, OG- Ogun sourced sample, SD- Standard sample,
AB-2M-HCl and OG-2M-HCl are Abia and Ogun samples treated with 2 M HCl,
AB-4M-HCl and OG-4M-HCl are Abia and Ogun state samples treated with 4 M HCl,
AB-8M-HCl and OG-8M-HCl are Abia and Ogun state samples treated with 8 M HCl.

Table 4: Elemental analysis

Samples	**Al%**	**Si%**	**Ca%**	**K%**	**Mn%**	**Tc%**	**Fe%**	**Cu%**	**Pb%**	**Zn%**	**Cr%**	**Co%**	**Ni%**	**S%**	**P%**
Pharmaceutical codex standards	40	47							Absent						
AB	47.2680	39.4260	0.0870	1.4190	0.0410	0.7760	3.1982	0.0630	Absent	0.1210	0.0080	0.0790	0.0760	0.4180	0.3860
OG	47.2680	40.4260	0.0870	1.4190	0.0410	0.7760	2.9820	0.0630	Absent	0.1210	0.0080	0.0790	0.0760	0.4180	0.3860
AB-2M-HCl	45.2680	41.4260	0.0870	1.3390	0.0310	0.6560	3.1820	0.0590	Absent	0.1130	0.0060	0.0660	0.0590	0.3180	0.2986
AB-4M-HCl	34.4316	58.7426	0.0192	0.1439	0.0291	0.2965	3.1679	0.0315	Absent	0.0599	0.0048	0.0471	0.0394	0.1613	0.1366
AB-8M-HCl	28.9268	65.0426	0.0176	0.0919	0.0141	0.1776	3.1492	0.0263	Absent	0.5481	0.0263	0.0379	0.0276	0.1141	0.0986
OG-2M-HCl	43.1896	47.9426	0.0541	0.7419	0.0352	0.4576	2.5982	0.0443	Absent	0.0981	0.0047	0.0521	0.0621	0.3418	0.2386
OG-4M-HCl	33.9168	60.5426	0.0432	0.6419	0.0319	0.3776	2.4382	0.0321	Absent	0.0721	0.0032	0.0443	0.0498	0.2848	0.1306
OG-8M-HCl	29.3926	64.2726	0.0387	0.4194	0.0227	0.2436	2.4102	0.0263	Absent	0.0582	0.0019	0.0362	0.0376	0.1418	0.0986
SD	39.2280	48.1320	0.7870	1.4790	0.0720	0.7760	3.1690	0.0650	Absent	0.0720	0.0310	0.1160	0.0850	0.3980	0.3470

Key: AB-Abia sourced sample, OG-Ogun sourced sample, SD- Standard sample,
AB-2M-HCl and OG-2M-HCl are Abia and Ogun samples treated with 2 M HCl,
AB-4M-HCl and OG-4M-HCl are Abia and Ogun state samples treated with 4 M HCl,
AB-8M-HCl and OG-8M-HCl are Abia and Ogun state samples treated with 8 M HCl.

Table 5: Table of Permitted Daily Exposure (PDE) and their corresponding maximum daily intake of elemental impurities.

Samples	Ca%	Cu%	Pb%	Cr%	Co%	Ni%
PDE – oral (µg/day)	5	3000	5	11,000	50	220
Daily exposure from a maximum daily dose of 262g kaolin	46.1 – 227.9 µg	68.9 – 170 µg	NIL	5.2 – 78.6 µg	94.84 – 303.92 µg	72.31 – 222.7 µg
Permitted oral concentrations (POC) for elemental impurities (µg/g)		300	0.5	11,000		

Key: Ca – Calcium, Cu – Copper, Pb – Lead, Cr – Chromium, Co – Cobalt, Ni - Nickel *Evaluation of kaolin suspension.*

Table 6: TAMC, TYMC and Test for specific micro-organism.

Sample code	TAMC (cfu/g)	TYMC (cfu/g)	Detection of *E. coli*
Allowable Limits	$\leq 10^3$cfu/g	$\leq 10^2$cfu/g	Absent
AB	TNTC	TNTC	Present
OG	TNTC	TNTC	Present
SD	0	0	Absent
AB-2M-HCl	5×10^1	5×10^1	Absent
AB-4M-HCl	5×10^1	5×10^1	Absent
AB-8M-HCl	5×10^1	5×10^1	Absent
OG-2M-HCl	0	0	Absent
OG-4M-HCl	5×10^1	0	Absent
OG-8M-HCl	0	5×10^1	Absent

Key: AB-Abia sourced sample, OG- Ogun sourced sample, SD-Standard sample,
AB-2M-HCl and OG-2M-HCl are Abia and Ogun samples treated with 2 M HCl,
AB-4M-HCl and OG-4M-HCl are Abia and Ogun state samples treated with 4 M HCl,
AB-8M-HCl and OG-8M-HCl are Abia and Ogun state samples treated with 8 M HCl.

pH, sedimentation volume and flow rate
The pH, sedimentation volume and flow rate of kaolin suspension were evaluated, and the results presented in Table 8.
The pH of kaolin suspensions produced are all alkaline. The sedimentation volume (F) of formulated suspensions ranges from 0.70 - 0.75. This shows the stability of the suspension as their sedimentation volume approaches unity (Table 8). The flow rates ranged from 0.7446 - 0.7516 mL/s. when compared to the standard value using students' t-test at 0.05 significant level, there were no statistically significant difference between values obtained from suspensions of the SD, AB and OG samples.

Viscosity
The iscosity of kaolin suspensions determined at different RPM showed decrease in viscosity as the RPM increases (figure 7). This indicates shear thinning. This is comparable with the standard formulation which shows decrease in viscosity with increasing RPM values. Figure 7 reveals the graphical relationship between viscosity and RPM.

Microbial limit test for formulated suspensions
The AMC for oral suspensions samples falls within the acceptable limit of not more than 10^2 cfu/ml. All formulations except OG_2 passed the test for TYMC when compared with the official limit standard of not more than 10^1cfu/ml[17] (Table 9).
The results for TAMC and TYMC in suspensions from the kaolin samples are presented in Table 9.

Table 7: Isolation and characterization of microbial groups in samples AB and OG.

Samples	Gram Staining	Shape	Spore Forming	Motility	Catalase	Oxidase	MR	Indole	Gas	Citrate	Glucose	Lactose	Mannitol	ABIS Software identification Bacillus base closest Taxa.
AB	+	Rod	+	+	+	–	–	–	–	+	+	+	+	*Bacillus subtilis* = 92%
														Paenibacillus pectinilyticus = 92%
OG	+	Rod	+	+	+	–	–	–	–	+	+	+	+	*Bacillus pumilus* = 91%
														Paenibacillusvalidus = 90%

Samples	Gram staining	Shape	Spore Forming	Motility	Catalase	Oxidase	MR	Indole	Gas	Citrate	Glucose	Lactose	Mannitol	ABIS Software identification Enterobacteracea base closest Taxa.
AB	–	Rod	–	+	+	–	+	+	+	–	+	+	+	1. *Eschericha coli* = 99%
														2. *Escherichia decarboxylata* = 98%
OG	–	Rod	–	+	+	–	+	+	+	–	+	+	+	3. *Escherichiahermannni* = 95%
														4. *Citrobacter farmeric* = 91%

Key: AB – Kaolin sample from Abia; OG – Kaolin sample from Ogun; + Positive; - Negative.

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Table 8: pH, Sedimentation volume (F), and flow rate of kaolin suspension.

Sample code	pH ± SD	$F = V_U/V_0$ ± SD	Flow rate (mL/s) ± SD
Pharmacopoeial standards			
AB_1	9.73 ± 0.14	0.75 ± 0.02	0.75 ± 0.02
AB_2	9.47 ± 0.25	0.75 ± 0.01	0.75 ± 0.01
AB_3	9.43 ± 0.14	0.74 ± 0.02	0.75 ± 0.01
OG_1	9.51 ± 0.06	0.71 ± 0.99	0.74 ± 0.45
OG_2	9.50 ± 0.08	0.71 ± 0.07	0.74 ± 0.01
OG_3	9.44 ± 0.09	0.70 ± 0.01	0.74 ± 0.01
SD	9.59 ± 0.06	0.72 ± 0.02	0.75 ± 0.01

Key: AB_1-suspension from 2M-HCl treated Abia kaolin,
AB_2- suspension from 4M-HCl treated Abia kaolin,
AB_3- suspension from 8M-HCl treated Abia kaolin,
OG_1, OG_2, OG_3 are suspensions from 2M-HCl, 4M-HCl and 8M-HCl treated Ogun kaolin respectively,
SD- suspension from standard kaolin.

Table 9: TAMC, TYMC and Test for specific micro-organism in kaolin suspensions.

Sample code	TAMC (cfu/mL)	TYMC (cfu/mL)	Test for *E. coli*
Allowable Limits	$\leq 10^2$cfu/ml	$\leq 10^1$cfu/mL	Absent
AB_1	4×10^1	1×10^1	Absent
AB_2	6×10^1	1×10^1	Absent
AB_3	5×10^0	5×10^0	Absent
OG_1	6.5×10^1	1×10^1	Absent
OG_2	6.0×10^1	5.5×10^1	Absent
OG_3	5.5×10^1	5×10^0	Absent
SD	5×10^0	5×10^0	Absent

Key: AB_1-suspension from 2M-HCl treated Abia kaolin
AB_2- uspension from 4M-HCl treated Abia kaolin
AB_3-suspension from 8M-HCl treated Abia kaolin
OG_1, OG_2, OG_3 are suspensions from 2M-HCl, 4M-HCl and 8M-HCl treated Ogun kaolin respectively
SD – standard kaolin suspension.

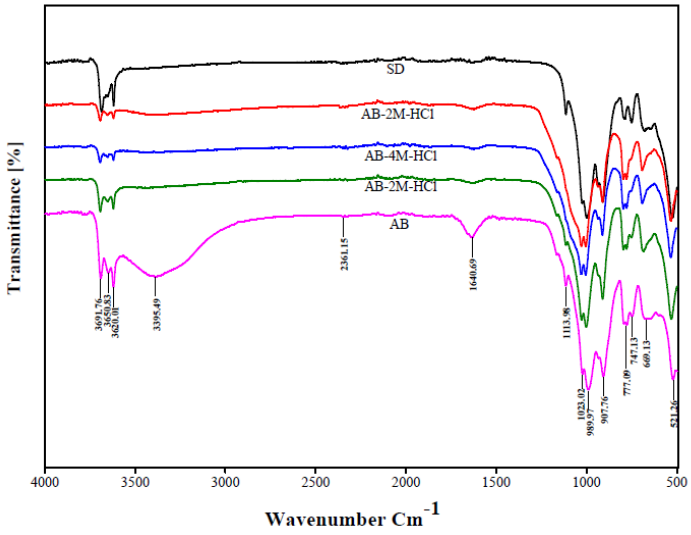

Figure 1: FT-IR results of raw and treated Abia kaolin samples.

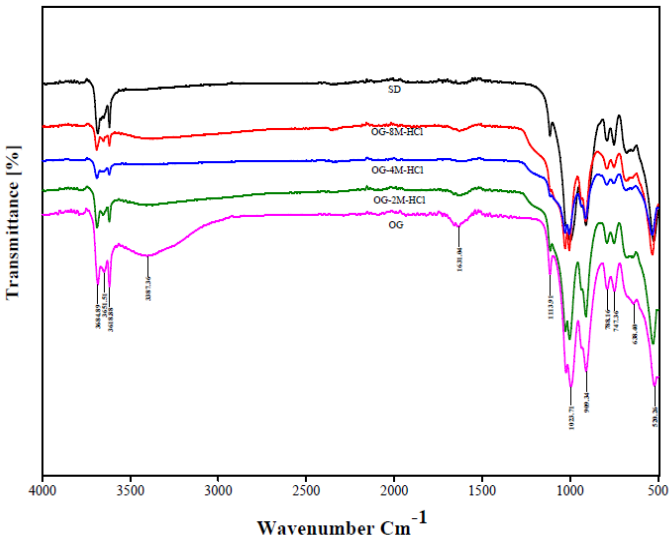

Figure 2: FT-IR results for raw and treated Ogun kaolin samples.

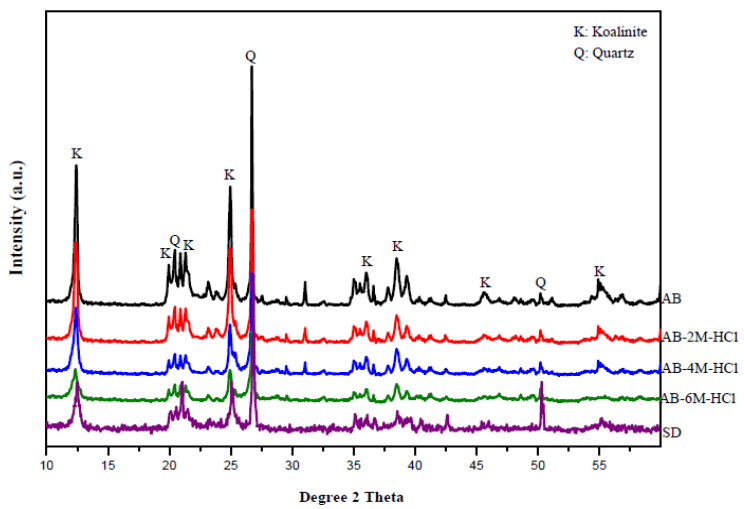

Figure 3: XRD results for raw and treated Abia sourced kaolin samples.

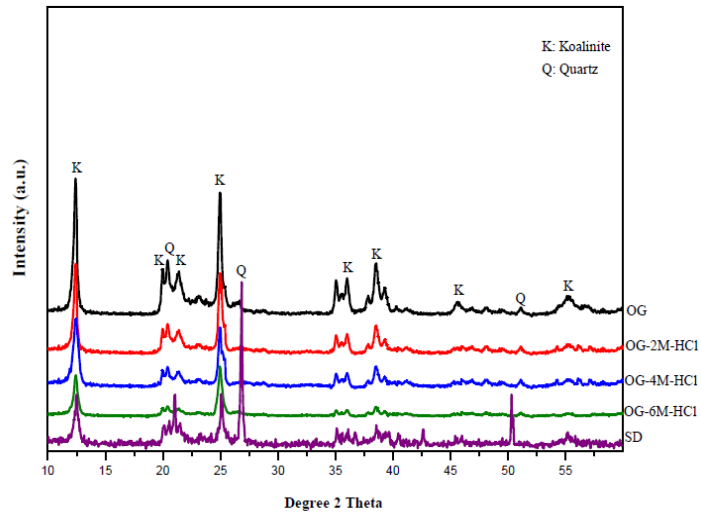

Figure 4: XRD result for raw and treated Ogun kaolin samples.

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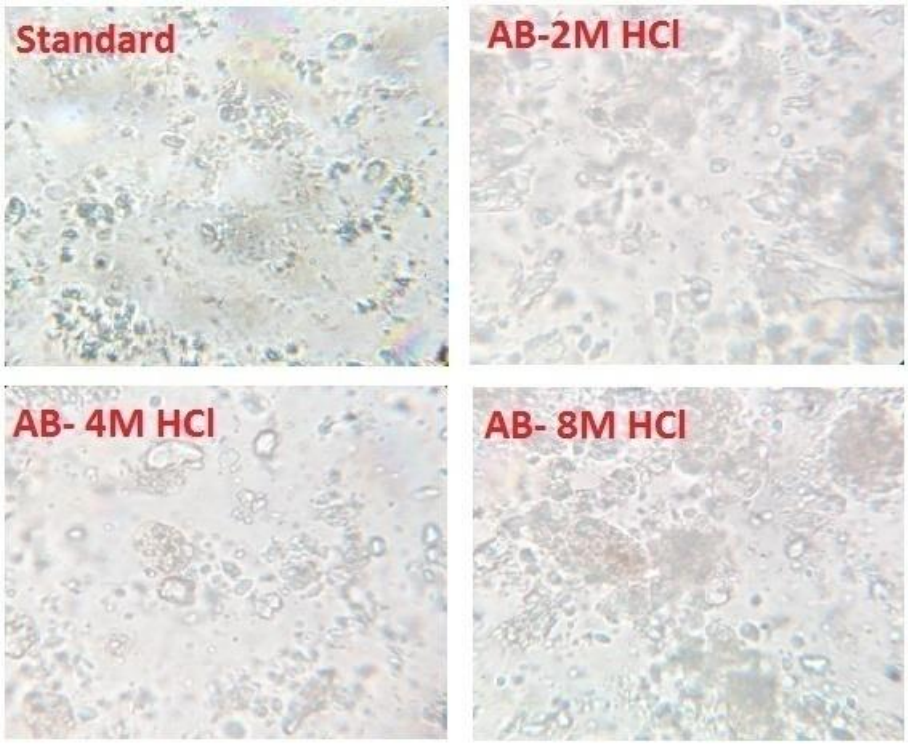

Figure 5: Micrograph for the standard sample and Abia sourced kaolin

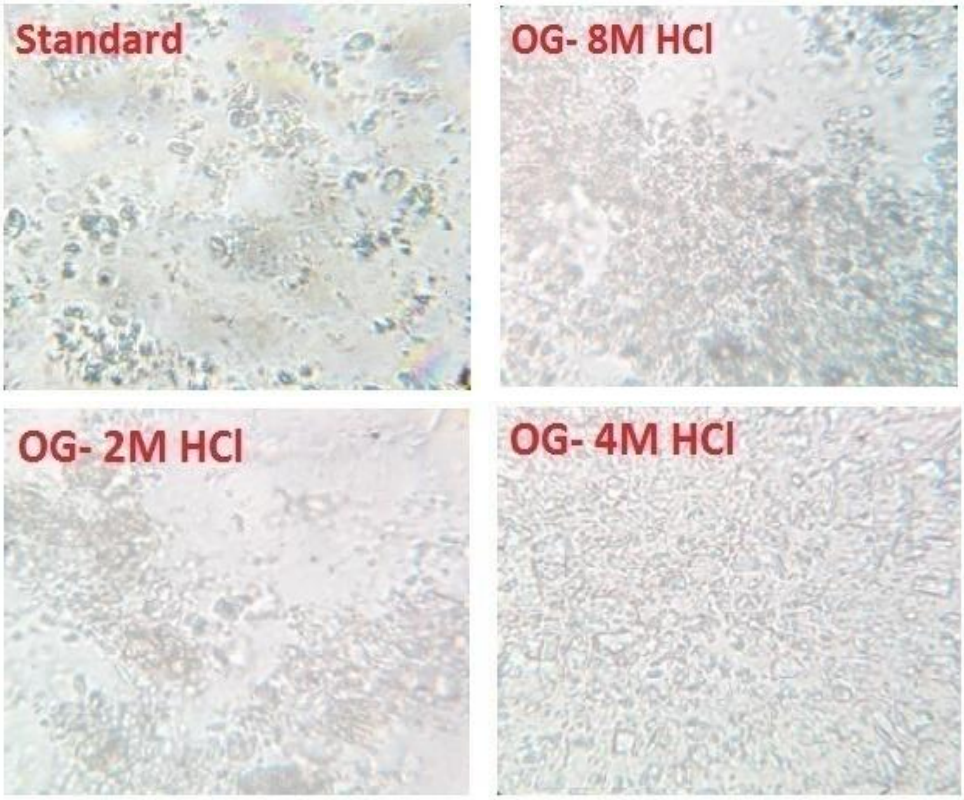

Figure 6: Micrograph for Ogun sourced kaolin.

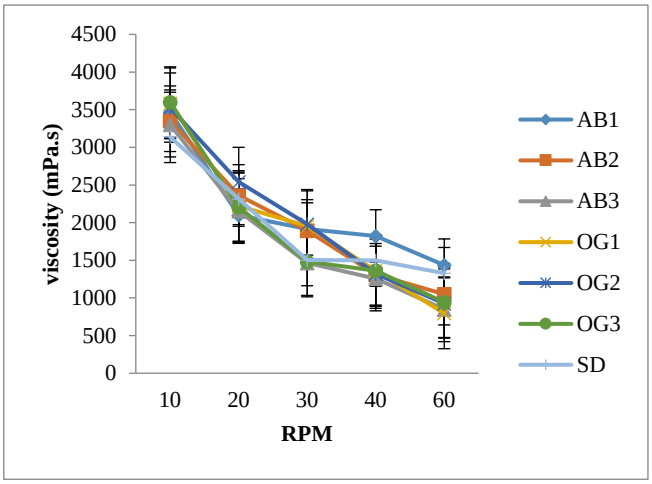

Figure 7: A graph of viscosity (mPa.Sec) against RPM.

Conclusion

From the processing, analysis and characterization of kaolin samples from Abia and Ogun States, it can be concluded that these locally sourced clays can be employed in drug production after wet processing technique and leaching with hydrochloric acid. The major microbial contaminants of raw kaolin samples found to be *Bacillus subtilis*, *E. coli*, *Aspergillus spp*. and *Histoplasma spp*. were absent and the microbial loads of the samples were greatly reduced after hydrochloric acid treatment. Wet processing and leaching of both kaolin samples (AB and OG) with hydrochloric acid at 2 mol/l concentration gave better pharmaceutical grade kaolin when compared with the standard sample hence could be employed in processing locally sourced kaolin for drug production.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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