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Original Article

# Effect of pore size and morphology of activated charcoal prepared from midribs *of Elaeis guineensis* on adsorption of poisons using metronidazole and *Escherichia coli* O157:H7 as a case study

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ABSTRACT

Agricultural waste obtained from *Elaeis guineensis* mid ribs can provide a veritable source of materials which can be used as precursor materials for the production of pharmaceutical grade activated charcoal. The pore size and surface morphology of activated charcoal defines the types of molecules that could be adsorbed unto it, as surface morphology plays a significant role in determining the surface availability and areas of adsorption.

The activated charcoal samples prepared from *Elaeis guineensis* via either physical or chemical activation was characterized via surface area using the BET method and subsequently pore structure and size analyzed by scanning electron microscopy (SEM).

Physically activated *Elaeis guineensis* fronds activated with nitrogen gas had wide spread microporosity with micropore volume of 0.232 cc/g compared to the chemically activated with 1M and 3M phosphoric acid respectively. The commercial activated charcoal/metronidazole combination in the in vitro- pharmacodynamic model reflected no re-growth after 4 hours, however for charcoal formulated from *Elaeis guineensis* via chemical activation with 3M phosphoric acid and metronidazole no regrowth was seen at the second hour and this was maintained throughout the duration of the experiment.

Increased macroporosity enhanced bacterial adsorption and this was further facilitated by the presence of antibacterial metronidazole in the in vitro pharmacodynamic model. Activated charcoal produced from agricultural waste obtained from *Elaeis guineensis* dried mid ribs consisting of increased macroporosity with mixed meso/micro porosity and antibacterial metronidazole form the best model for bacterial adsorption and will be useful in the treatment of diarrhea caused by *E. coli* O157:H7.



## 1. Introduction

Activated charcoal (AC) is an effective, nonspecific adsorbent of a wide variety of drugs and chemicals and thus inhibits gastrointestinal absorption of these agents. Studies have shown the effectiveness of AC in adsorption of microorganisms [1,2]. These adsorptive

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characteristics however vary with the physical characteristics such as internal total available surface area and micro/meso/macro-pore volume of the activated charcoal.

Pore size distribution has been used to describe the internal structures and adsorption capacities of activated charcoals [3]. Chemical functional groups and the internal surface areas account for, and are associated with, highly active surface properties of the activated charcoal, and these areas typically range from 500 $m^2/g$ to 3000 $m^2/g$. Due to varying methods of preparation, the pore sizes of the activated charcoal can be categorized as being micropores (width < 2 nm), mesopores (width = 2–50 nm), or macropores (width > 50 nm); the differences in the size of their width openings being a representation of the pore distance.

Most organic materials rich in carbon that do not fuse upon carbonization can be used as precursor materials for AC preparation [4]. For selection of raw material for preparation of porous carbon, several factors are taken into consideration, including high carbon content, low inorganic content (i.e. low ash), high density and sufficient volatile content, the stability of supply, potential extent of activation, and inexpensive materials [5].

Varying raw materials have been used in the production of AC [6–8]. Depending on the characteristics of the raw material as well as the mode of preparation, AC exhibiting varying porosity can be obtained. *Elaeis guineensis* is a species of palm that is native to West African countries including Nigeria and Ghana and it is cultivated for the fruit that supplies oil for the food and cosmetic industries [9]. It has now become of great economic value in countries like Malaysia, which is the second largest exporter of palm oil in the world [9]. The branches are usually an agricultural waste in countries like Malaysia, however in Nigeria some of the branches recovered are used in making brooms and brushes. Even in this case, the mid ribs of these branches still serve as agricultural waste, and they are used as precursor materials in the production of AC characterized for pharmaceutical purposes of adsorption of toxins and bacteria.

Metronidazole is a nitroimidazole derivative and a commonly used antibiotic in the treatment of anaerobic infections associated with diarrheal disease as well as *Clostridium difficile* infections. Diarrhea is a common cause of death in developing countries and the second most common cause of infant death worldwide, as a result of loss of fluid, which can cause dehydration and electrolyte disturbances such as potassium deficiency or other salt imbalances. Diarrheal disease is estimated to have caused 1.1 million deaths in people aged $\geq$ 5 years in Africa in 2013 [10,11]. The treatment primarily targets three mechanisms. Adsorption of causative organisms where adsorbents cause the invading microorganisms to adhere to their large surface area. These organisms are expelled via increased intestinal motility that characterizes the condition. Antibiotics act by attacking the cellular structure, as well as the inherent biological processes of the microorganisms causing cell lysis and death. Drugs can slow intestinal motility through a direct effect on the nerve endings and/or intramural ganglia of the intestinal wall. Metronidazole in sub- saharan Africa is misused in treatment of diarrhea caused by susceptible and resistant organisms and thus is used as a drug of choice to evaluated the synergistic potential of an adsorbent and an anitmicrobial in treatment of Escherichia coli O157:H7 associated diarrhea.

The present study evaluated the surface morphology of AC formulated from agricultural waste obtained from *Elaeis guineensis*. The method of preparation of the AC samples will influence their surface characteristics, thus the need to precisely evaluate the effects of pore size on adsorption of *Escherichia coli* O157:H7, an enterohemorrhagic starin of diarrhea-causing bacteria via *in-vitro* pharmacodynamic modeling. We also investigated the effect of pore size and morphology of AC prepared from midribs of *Elaeis guineensis* on adsorption of poisons using metronidazole and *Escherichia coli* O157:H7.

## 2. Materials and methods

Activated charcoal (AC) was obtained from Friendemann Schmidt Chemicals (Parkwood, Australia). Metronidazole powder was obtained from Nacalai Tesques (Kyoto, Japan). Phosphoric acid and potassium hydroxide were obtained from Sigma–Aldrich (St. Louis, MO, USA). Luria–Bertani (LB) broth L2897 was obtained from Becton Dickinson (Sparks, MD, USA), and trypticase soy agar (TSA) plus 5% sheep blood from Becton Dickinson (Cockeysville, MD, USA). *Escherichia coli* O157:H7 (Migula) Castellani and Chalmers ATCC 43895 were obtained from Sigma–Aldrich. Methyl tert-butyl ether was purchased from SECO (Aston, PA, USA). Formic acid, hydrochloric acid, ammonium hydroxide, ammonium chloride, and liquid chromatography (LC) grade water (Omnisolv) were purchased from EMD Millipore (Billerica, MA, USA). Methanol [LC-mass spectrometry (MS) grade] was purchased from Fisher Scientific (Fair Lawn, NJ, USA) and acetonitrile (LC-MS grade) was purchased from ACROS Organics (Morris Plains, NJ, USA). Dried mid ribs of *Elaeis guineensis* were obtained from the oil palm plantation in the grounds of the University of Nottingham Malaysia Campus, Semenyih, Malaysia and identified and filed as herbarium specimen unmcddl201, and utilized as raw material to produce and characterize AC.

### 2.1. Preparation of AC from dried midribs of Elaeis guineensis plant

The plant material was crushed using a Retsch SM 100 comfort grinder, 230 V/50 Hz and sieved into varying particles sizes. The particles that passed through a < 2 mm sieve were used for the production of AC (Figure 1). Prior to processing, the crushed plant material was washed thoroughly with distilled water in a Millipore Q Pod machine to remove debris and sand and then dried in an oven at 70 °C for 6 hours [5,7]. The samples were divided into two batches: one for physical activation alone (PB1), and the second batch underwent chemical activation with 1 M phosphoric acid (PB2) or 3 M phosphoric acid (PB3) as shown in Table 1.


**Fig. 1.** Washed, dried, and crushed samples of *Elaeis guineensis* dried midribs.

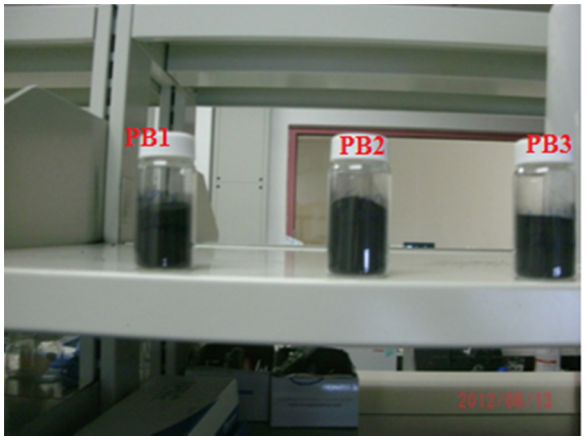


**Fig. 2.** Activated charcoal samples prepared from the midribs of *Elaeis guineensis* palm fronds.

**Table 1**
Process variables in activated charcoal production from midribs of the branches of *Elaeis guineensis* oil palm plant.

| Oil palm plant | Activation process |
|---|---|
| **PB1** | Physical activation at 600 °C in a tubular furnace using nitrogen gas in a tubular furnace |
| **PB2** | Impregnation with 1M $H_3PO_4$ followed by activation at 600 °C in an open top muffle furnace |
| **PB3** | Impregnation with 3M $H_3PO_4$ followed by activation at 600 °C in an open top muffle furnace |

### 2.2. Impregnation and formulation of AC

The raw samples were impregnated with phosphoric acid overnight prior to activation in the open top muffle furnace. To achieve impregnation, dried crushed plant material was poured into beakers containing 1 M and 3 M phosphoric acid of 85% purity, stirred thoroughly before being dried in the Memmert oven at 110 °C for 24 hours [5–8]. The ratio of acid to material was 1:1.

The samples were carbonized for 4 hours. The temperature was raised from room temperature to 400 °C with a heating rate of 10 °C/min and then increased to 600 °C at a rate of 40 °C/min. After the retension time since heating in a furnace was via destructive distillation, the samples were cooled rapidly in an ice bath, thus preventing further biomass burning. The AC was refluxed in distilled water to remove all metals, ions and ash, followed by washing 12 times with distilled water to remove the dehydrating agent. Fifty milliliters of KOH (used for cleansing) was poured into a beaker containing the cooled carbonized sample. Excess chemicals were removed by magnetic stirring followed by solid separation via filtration. Subsequently obtained samples were rinsed sequentially with warm distilled water, followed by cold distilled water until the pH of the wash solution fell to 6–6.5. This was followed by samples drying at 105 °C in the Memmert oven for 24 hours [8,9]. The resultant AC was soaked in distilled water for 24 hours and dried at 105 °C in the oven for 24 hours. The AC underwent particle size reduction using an ultracentrifugal mill, Retch ZM 200, 230 V/50 Hz. Powdered AC (Figure 2) size range was obtained through impact and shearing effects between the rotor and the fixed ring sieve and stored in glass vials in a desiccator [5].

### 2.3. Thermo-gravimetric analysis

Thermo-gravimetric analysis (TGA) was performed using Perkin Elmer Pyris 6 TGA at a heating rate of 25 °C/min. A sample of ~100 mg was heated from 30 °C to 120 °C. The instrument was purged with nitrogen flow at a rate of 20 mL/min.

### 2.4. Scanning electron microscopy

A Quanta 400F scanning electron microscope (FEI, Hillsborough, OR, USA) was used to determine the pore size and morphology of the surface area of AC by using a JEOL JSM-6360LA and Oxford Instruments Software XT microscope control systems (JEOL USA, Inc.11 Dearborn Road Peabody, MA). A minute amount of the activated charcoal sample was placed on a labelled aluminium stub.

### 2.5. Determination of surface area using Brunauer, Emmett, and Teller method

The Brunauer, Emmett, and Teller (BET) method was used to measure the surface area of the AC. Analysis was done using a Micromeritics ASAP 2020 Surface Area and Porosity Analyzer (Norcross, GA 30093-2901, U.S.A.). BET surface areas of the activated charcoal samples were determined using the BET equation, assuming that the surface area occupied by each physisorped argon molecule was 0.146 $nm^2$ [13]. The argon adsorption data were recorded automatically and presented as a plot of volume of argon adsorbed versus relative pressure [10].

Zero point three grams of each corresponding activated charcoal sample was measured and degassed under vacuum (300 mmHg) until the pressure was stable at $6 \times 10^{-6}$ torr, and was put in the sample tube and left at a constant heat of 250 °C for 10 hours. The degassed sample was then cooled and weighed. Surface area analysis was performed

once and the weighed samples were transferred to the analysis port [8].

### 2.6. Effect of pore size on adsorption of Escherichia coli O157:H7 via in vitro pharmacodynamic modeling

This experiment was conducted using a modification of the in vitro model previously described [1,14,15]. Six concentration time kill curve were used as a representation of the adsorptive effect of activated charcoal on *Escherichia coli* O157:H7 exposed to 200 mg metronidazole, metronidazole–AC combination, PB1, PB2, PB3, and PB3 - met or AC for 24 hours. A growth control experiment was conducted in triplicate.

*Escherichia coli* O157:H7 ATCC 43895 strain was utilized. Several colonies of each isolate were incubated aerobically overnight in 50 mL LB broth. The overnight culture was diluted 1 in 10 in fresh warm LB broth ~30 minutes before the experiment, to allow the bacteria to attain exponential growth. The initial bacterial inoculum was $10^6$ CFU/mL for all the experiments.

A 1-mL inoculum of *Escherichia coli* followed by bolus injection of metronidazole alone, metronidazole–AC, PB1, PB2, PB3, or PB3–metronidazole was introduced into each anaerobic chemostat chamber. Utilizing the desired concentration for 24 hours, each experiment and the growth control was run in triplicate. The desired half-life of metronidazole, that is, 8 hours, was achieved via simulation of a monoexponential pharmacokinetic process. This process was obtained via simultaneous introduction of antibiotic-free LB broth being introduced via a peristaltic pump with an equal volume of drug-containing broth being displaced from the chemostats into a waste reservoir at a predetermined rate.

Antimicrobial susceptibility testing using metronidazole was performed in triplicate for each isolate *Escherichia coli* O157:H7 ATCC 43895 prior to the concentration time–kill experiments as previously described previously [1]. The 96-well plates inoculated with *Escherichia coli* were incubated for 16–20 hours at 36 °C in ambient air. Minimum inhibitory concentrations (MICs) were reported as the concentration in the first clear well (no growth). Colonies present at 24 hours were frozen at –20 °C in sterile defibrinated sheep blood until they were needed and were then subcultured onto fresh agar plates for at least 2 consecutive days prior to susceptibility testing.

#### 2.6.1. LC-tandem MS

The actual concentrations of metronidazole in batched samples stored in LB broth (frozen at –20 °C) were determined via LC-tandem MS [16], which were linear over a range of 50–500 ng/L ($R^2 \geq 0.9998$) with the inter- and intraday precision being 3.68% and 8.75%, respectively.

#### 2.6.2. Pharmacodynamics and antibiotic carryover

At 0 hours, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, 12 hours and 24 hours, 1-mL samples were removed from the model for quantification of the bacterial density by serial dilution techniques. The possibility of antibiotic carryover was evaluated for each antimicrobial agent–bacterium combination using saline dilution techniques. TSA plus 5% sheep blood (Becton Dickinson, Cockeysville, MD, USA) was streaked in triplicate with 100 μL of broth spiked with metronidazole to final concentrations of 5 μg/mL, 50 μg/mL, and 100 μg/mL. After 15 minutes, each plate was cross-streaked with a standardized quantity of *Escherichia coli* (100 CFU). After 48 hours incubation, plates were visually inspected for a zone of growth inhibition and quantitatively analyzed for viable cell counts. Colony counts from drug-exposed plates were compared with those from growth control plates. Antibiotic carryover was considered significant if a zone of growth inhibition was evident and/or if drug-exposed plates contained fewer colonies than growth control plates.

After aerobic incubation for 24 hours, at 37 °C, the numbers of *Escherichia coli* CFU on each plate were counted visually. Plates were then quantified to determine viable bacterial counts; the dilution that revealed between 30 colonies and 300 colonies per plate was used for constructing time–kill curves. Inoculation of a plate with 100 μL of an undiluted sample resulted in a lower limit of bacterial quantification of 300 CFU/mL [10,14,15]. Time–kill curve data were plotted as logarithmic declines in CFU/mL versus time. The curves were evaluated by visual inspection for time to a 3 log10 unit decline in bacterial numbers and total logarithmic decline in bacterial numbers. Because of small sample sizes, inferential statistics were not applied to these data. Time–kill curves were constructed by plotting the log CFU/mL versus time in hours.

## 3. Results and discussion

### 3.1. Effect of temperature on prepared AC samples studied using TGA

The AC samples did not show any significant weight loss until the temperature exceeded 750 °C, when a 30% weight loss was experienced for sample PB1. PB3 experienced the least weight loss (Figure 3; $p > 0.01$). This was due to the fact that during the carbonization process, tar and other volatile compounds were removed via the dehydrating effect of 3 M phosphoric acid, and during the reflux stage of the recovery of the activated charcoal after activation. The commercial AC powder was thermally stable with weight loss of < 20% at 750 °C.

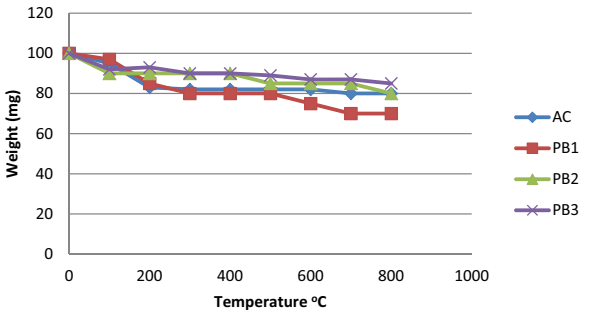


**Fig. 3.** Thermal stability of activated charcoal prepared via physical and chemical activation of *Elaeis guineensis*.

### 3.2. Susceptibility testing, pharmacokinetics, and effect of pore size on adsorption of Escherichia coli O157:H7 via an in vitro pharmacodynamic model

The pre-exposure MIC of metronidazole for *Escherichia coli* isolates was > 64 mg/L [17–19]. This showed that the hemorrhagic *Escherichia coli* strain was not susceptible to metronidazole. The in vitro MIC for most strains of metronidazole-susceptible organisms is ≤ 1 μg/mL. A bacterial isolate may be considered susceptible if the MIC for metronidazole is not > 16 μg/mL. An organism is considered resistant if the MIC is > 16 μg/mL [19].

Drug concentrations attained in the model determined by LC-tandem MS compared to the expected concentrations allowed verification of the values for the simulated pharmacokinetic parameters. The actual metronidazole concentrations in these experiments ranged from 47.99 ± 0.92% to 92.78 ± 0.77% of the expected concentration with half-lives ranging from 6.4 ± 0.28 hours to 9.3 ± 0.19 hours. Antibiotic carryover was not evident, since colony counts from drug-exposed plates did not differ significantly from those of growth control plates ($p < 0.01$) [15].

Varying raw materials have been used in the production of AC [6–8]. Depending on the characteristics of the raw material as well as the mode of preparation, AC exhibiting varying porosity can be obtained. *Elaeis guineensis* is a plant native to West Africa. In Nigeria, it is cultivated for the fruit that supplies oil for the food and cosmetics industries, and it has now become of great economic value in countries like Nigeria, Ghana, and Malaysia, the world's largest exporter of palm oil. The branches are usually an agricultural waste in these countries, however, in Nigeria the branches are used to make brooms. Even in this case, the midribs of these branches still form agricultural waste. This resulted in its use as the precursor material for the production of AC specifically characterized for pharmaceutical purposes of adsorption of toxins and bacteria (Table 2).

The pore characteristics of the AC are determining factors in its rate and ability to adsorb toxins. The AC produced in the present study was the lower activating temperature type, that is, L-type AC, for which a temperature of 600 °C was utilized. Increasing the concentration of phosphoric acid from 1 M to 3 M decreased the overall percentage yield of the activated charcoal due to the facilitation of volatile matter release [4,20]. The acid acts as a dehydrating agent that inhibits tar formation, which characteristically clogs the mesopores and micropores. Once these materials are liberated via chemical activation, the percentage yield reduces but a more defined pore structure can be seen via scanning electron microscopy (SEM). Associated with this was a corresponding increase in the total pore volume (Table 3). The physical activation carried out for PB1 was done in the presence of nitrogen gas, which ensured defined microporosity. However, here, predominant microporosity was obtained. The surface area and micropore analysis were obtained from Micromeritics ASAP 2020 Surface Area and Porosity Analyzer using argon adsorption and BET surface area of the AC samples, prepared with the assumption that surface area occupied by per physisorped argon was 0.146 $nm^2$ [13]. The average pore radius for PB3 was the highest at 12.95 Å, thus reflecting a predominance of macroporous structures in the AC sample. PB2 had more uniform division between macroporosity and mesoporosity than seen in PB1. SEM also showed some microporosity. PB1 that was activated physically using nitrogen gas showed a predominance of micropores with a volume of 0.232 $cm^3/g$ and average pore radius of 4.16 Å (Table 3). The commercial AC used as the standard was microporous in nature and had an average pore radius of 2.42 Å. A large number of activated charcoals from agricultural materials were reviewed, which suggested that the surface area can be as high as that obtained in this study (Table 3) [20,21]. SEM was used to study the surface morphology of the AC samples (Figure 4). Figure 4A shows an AC sample with well-developed and orderly hexagonal pores, which corresponded to microporous sizes. This was evaluated utilizing microscope XT measurement software. The activated charcoal prepared via physical activation with nitrogen gas showed abundant and defined microporosity. The use of phosphoric acid in the chemical activation process led to organized smooth

**Table 2**
Analysis of formulated activated charcoal against United States Pharmacopoeia (USP) specification.

| Parameters | PB1 | PB2 | PB3 |
|---|---|---|---|
| **Microbial limits** | Nil growth | Nil growth | Nil growth |
| **Loss on drying at 120 °C (≤ 15%)** | 12.5 ± 0.43 | 10.45 ± 0.21 | 5.45 ± 0.83 |
| **Residue on ignition (≤ 4%)** | 4.33 ± 1.22 | 1.88 ± 0.32 | 1.01 ± 0.65 |
| **Dechlorination (< 10 ppm)** | 1.1 | 1.99 | 1.98 |
| **Heavy metals (< 0.005%)** | 0.0001 | 0.00032 | 0.00035 |
| **Un-carbonized constituents** | Nil | Nil | Nil |
| **Adsorptive power** | No turbidity | Slight turbidity | No turbidity |

**Table 3**
Influence of chemical and physical activation on surface characteristics and yield of AC prepared from *Elaeis guineensis*.

| AC | Activation temperature (°C) | BET surface area ($m^2/g$) | Micropore volume ($cm^3/g$) | Average pore radius (Å) | Total pore volume ($cm^3/g$) | Yield (%) |
|---|---|---|---|---|---|---|
| **AC (commercial)** | NA | 2058 | 0.482 | 2.42 | 0.795 | — |
| **PB1** | 600 | 1496 | 0.232 | 4.16 | 0.918 | 41.9 |
| **PB2** | 600 | 2100 | 0.596 | 10.99 | 1.127 | 39.8 |
| **PB3** | 600 | 2153 | 0.578 | 12.95 | 1.577 | 37.5 |

AC = activated charcoal; BET = Brunauer, Emmett and Teller; NA = not applicable; PB1 =; PB2 =; PB3 = .

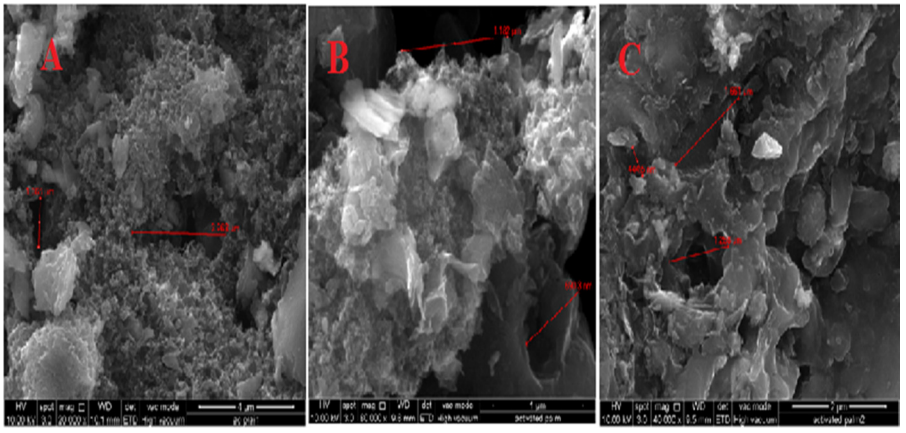


**Fig. 4.** Surface morphology of activated charcoal prepared form *Elaeis guineensis* using (A) physical activation with $N_2$ as the activating agent (PB1), (B) using chemical activation with 1 M phosphoric acid as the activating agent (PB2), and (C) using chemical activation with 3 M phosphoric acid as the activating agent (PB3).

surfaces on the surface of the crystalline carbon characterized by macroporosity. Samples PB2 and PB3 showed enhanced pore sizes that facilitated adsorption of large molecules. Metronidazole is adsorbed by the micro- and mesopores, while *Escherichia coli* O157:H7 is adsorbed by the macropores.

The number of bacteria adsorbed as a function of time was explored using the combined time–kill curve against *Escherichia coli* O157:H7 (Figure 5). The number of bacterial cells exponentially increased with the amount in the initial inoculum for the control. The time–kill curve for metronidazole against *Escherichia coli* was almost a replica of the curve for growth control [1,15]. Confluent bacterial growth was observed using metronidazole alone when compared to metronidazole combined with AC or PB3 (Figure 6). AC alone in the pharmacodynamic model produced a 1 log killing of the bacteria and this was increased with a combination of AC with metronidazole. When AC (AC, PB1, and PB3) was used alone, significant regrowth occurred above the lower limit of accuracy (LLA) of bacterial counting. Significant regrowth (increase in the number of bacteria to quantifiable levels following a 3-log killing) did not occur. In the case of metronidazole–AC combination,

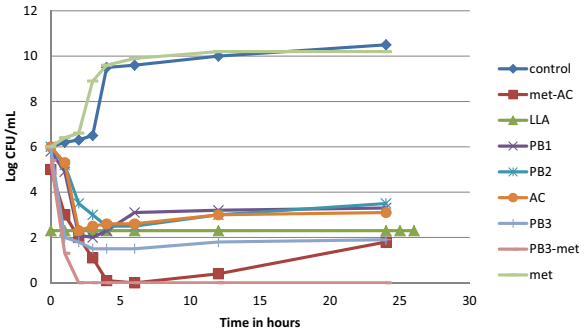


**Fig. 5.** Combined time–kill curve against *Escherichia coli* O157:H7 from exposure to AC (i.e., commercially available AC), AC prepared from *Elaeis guineensis* PB1, PB2, PB3, AC and metronidazole, and PB3 and metronidazole.
AC = activated charcoal; LLA = lower limit of accuracy; met = metronidazole; PB1=; PB2=; PB3 = .

there was no regrowth after 6 hours. However, for PB3 and metronidazole (Figure 4), no regrowth was observed at 2 hours, and this was maintained throughout the experiment. This reflected a decrease in the number of bacteria

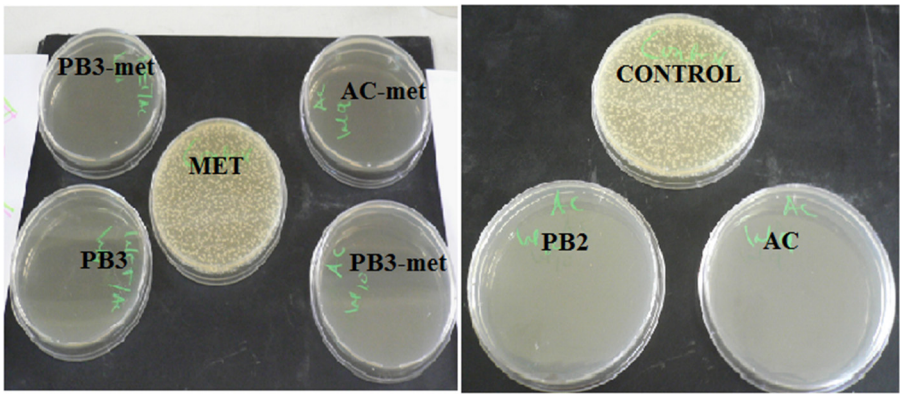


**Fig. 6.** Bacterial cultures of *Escherichia coli* O157:H7 in Petri dishes 6 hours after plating from the in vitro pharmacodynamic model.
AC = activated charcoal; met = metronidazole; PB1=; PB2 =; PB3 = .

to an unquantifiable level following a 3 log killing. To determine the rate of killing or adsorption, points were used for the determination of the slope until regrowth occurred.

PB3 showed good adsorptive capacity in the absence of metronidazole (Figure 4C). The rate of killing was rapid and the values for log CFU/mL were well below the LLA by 1 hour. At 12 hours, there was a slight increase in the log CFU/mL, although throughout the experiment, the values did not exceed the LLA.

The adsorption capacity of *Escherichia coli* to the AC can be directly linked and attributed to pore size. The AC samples with predominantly macroporous structures on the surface had the best adsorptive capacity (i.e., PB2 and PB3) and this was enhanced by the presence of metronidazole. This synergistic activity inhibited regrowth of the organism. PB1 had large micropores with narrow mesopores (Figure 4A). This characteristic pore size distribution of activated charcoal is as a result of utilizing nitrogen as the activating agent. PB3 had the highest adsorptive capacity for bacteria compared with commercial AC, which was predominantly microporous, thus facilitating accessibility of the bacteria to the adsorptive surface (Figure 5).

## 4. Conclusion

The midribs of *Elaeis guineensis* fronds as a form of agricultural waste have been physically and chemically activated to produce AC. AC morphology was directly dependent on the method of preparation. The surface area varying from 0.232 $cm^3/g$ to 0.578 $cm^3/g$ for micro pore volume and 0.795 $cm^3/g$ to 1.577 $cm^3/g$ for total pore volume were obtained, with the pores which were mainly microporous in the samples activated physically while increased macroporosity was associated with chemical activation. Synergistic interaction between metronidazole and PB3 which had increased macroporosity enhanced bacterial adsorption in the *in vitro* pharmacodynamic model causing a significant reduction in the starting inocula of *Escherichia coli* O157:H7. AC consisting of increased macroporosity with mixed meso/microporosity and antibacterial metronidazole form the best model for bacterial adsorption and will be useful in the treatment of diarrhea caused by *Escherichia coli* O157:H7.

## Conflict of interest

The authors declare no conflict of interest.

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