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African Journal of Biotechnology

Full Length Research Paper

Adsorption of lead, cadmium, and mercury ions in aqueous solution using groundnut and sheanut shells biochars

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The adsorption performance of toxic ions and effect of initial pH using groundnut shells and sheanut shells biochars were studied to ascertain the practical and theoretical basis of the use of biochars to remediate water pollution. Biochars were prepared using groundnut and sheanut shells as the feed stocks. The removal efficiencies of lead (Pb) were 100%, > 65.90% for cadmium (Cd) and 96.70% for mercury (Hg) in mono systems of 5, 10, 25 and 50 mg/l. The maximum Langmuir capacity ranged from 400 to 2000 mg/g for Cd and 232.56 to 312.50 mg/g for Hg by biochars. The adsorption of toxic metal ions by groundnut and sheanut shells biochars showed similar removal efficiencies. Adsorption of Pb, Cd and Hg were effective in the varied pH which was largely dependent on the characteristics of groundnut and sheanut shells biochars determined by the pyrolysis conditions and nature of feed stock. Langmuir isotherm was the model that best fit the adsorption of toxic metal ions onto the biochars. Groundnut and sheanut shells biochars have proven to be good candidates for the remediation of water polluted with toxic metals in mono systems.

Key words: Groundnut and sheanut shells biochars, lead, mono systems, removal efficiencies.

INTRODUCTION

Lead, cadmium, mercury are usually considered as priority contaminants due to their toxicity, persistent nature and are released in a large concentration into the environment (Wang and Chen, 2014). The use of biochar to remove toxic metals from water is essential and beneficial for environmental protection and human life. Biochar has received increasing attention due to its cost and associated physico-chemical properties such as structured carbon matrix, wide surface area, and high porosity in recent years (Lebrun et al., 2019). The exceptional surface chemistry of biochar such as high surface area, high aromaticity, different functional groups

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and high alkalinity aid its high immobilisation/removal abilities for toxic metals in soil/water (O'Connor et al., 2018).

With the increasing environmental pollution, it is essential to pursue eco-friendly, economically feasible, sustainable and efficient solutions to solve the persistent global problems of food security, environmental pollution, and energy and resource shortages (Chen et al., 2020). Biochar in recent years, has been broadly applied for water treatment (Xing et al., 2020), waste management (Yang et al., 2020), and other purposes owing to its high structural stability, rich porous structure, and large surface area (O'Connor et al., 2018). Cadmium removal efficiency by biochars from *Eichornia crassipes* were more than 90 to 99.24% (Li et al., 2016). Percentage of Pb (II) removal was up to 99% in 40 min from aqueous solution using bamboo biochar and calcium sulphate (Hassan and Kaewsichan, 2016). Cd, Hg and Pb removal efficiency in mono system in lower concentrations was almost 100% onto biochars derived from groundnut, sheanut shells, and a combination of both of them (Duwiejuah et al., 2018).

Harmful components such as polycyclic aromatic hydrocarbons, toxic metals, perfluorochemicals, dioxins, and environmentally persistent free radicals may be produced due to the improper biomass feedstocks selection, conditions and methods preparation although biochar has been widely regarded as an eco-friendly material (Xiang et al., 2021). Recently, most studies are on the negative effects of biochar in the environment because of its potentially toxic components and several interactions with environment (Cui et al., 2021). Cadmium, mercury and lead occurrence in the environment is a major problem due to their toxicity and ability to cause disease. Cd, Hg and Pb are among the notorious toxic metals that has become a considerable global problem in the environment particularly in developing countries with insufficient resources and technological challenges. The utilisation of groundnut and sheanut shells biomasses will be cheaper in terms of cost, efficient and ecologically sustainable adsorbent for Pb, Cd and Hg removal which will guarantee food safety and drinking water by restoring the water contaminated in environment. Hence, the study investigated the adsorption performance of Pb, Cd and Hg ions and effect of initial pH and adsorbent dosage using groundnut shells and sheanut shells biochars.

MATERIALS AND METHODS

Preparation of biochars

Groundnut and sheanut shells were used to produce biochars (Plates 1 and 2) in the Agricultural Sub-sector Improvement Programme Laboratory. The feed stocks were collected from a processing centre, Nyohini, in the Tamale Metropolis. Groundnut and sheanut shells were kept in separate earthen pots and then transferred into a Gallenkamp muffle furnace with internal dimensions of 18" × 8.5" × 7.5" High. The feed stocks were converted into biochar under a limited oxygen condition. The slow pyrolysis of groundnut shell was done at 350 ± 5°C for 60 min (GB350: groundnut shells biochar produced at 350 ± 5°C) and fast pyrolysis at 700 ± 5°C for 45 min (GB700: groundnut shells biochar produced at 700 ± 5°C) in a muffle furnace. The slow pyrolysis of sheanut shell was done at 350 ± 5°C for 180 min (SB350: sheanut shells biochar produced at 350 ± 5°C) and fast pyrolysis at 700 ± 5°C for 90 min (SB700: sheanut shells biochar produced at 700 ± 5°C) in a muffle furnace. The difference in residence time of pyrolysis of groundnut and sheanut shells are due to their difference in lignocellulosic biomass (Duwiejuah, 2017). After the pyrolysis step, the biochars were left to cool, crushed and sieved through 2 mm and used for the experiment.

Stock solution preparation and mono aqueous solutions

In the experiments, all chemicals used are at analytical purity and were obtained from Lab Aid, Accra and used without any further treatment. Distilled water was used in all the experiments. Lead nitrate (Pb $(NO_3)_2$: grade; GR, assay; 99.50%), cadmium nitrate ($Cd(NO_3)_2$ grade; reagent CAS, assay; 99.99%), and mercury chloride ($HgCl_2$ grade; ACS reagent, assay; ≥ 99.50%) were obtained from Lab Aid in Accra, Ghana. The preparation of stock solutions were done by dissolving accurately weighted 1.60 g of lead nitrate, 1.93 g of cadmium nitrate and 1.35 g of mercury chloride in deionised water to obtain solutions of 1000 mg/L concentration. Molecular weight of $Pb(NO_3)_2$ (331.21 g/mol), $Cd(NO_3)_2$ (236.42 g/mol) and $HgCl_2$ (271.50 g/mol) were calculated and divided by atomic weight of Pb (207.20 g/mol), Cd (122.41 g/mol) and Hg (200.60 g/mol), respectively to obtain amount of compounds containing 1 mg (1000 ppm) of Pb, Cd, and Hg. Toxic metal solutions were prepared in a 1000 mL volumetric flask. Serial dilutions of the stock solution were done to obtain targeted concentrations of 5, 10, 25 and 50 mg/L.

Experiment for mono metal systems

The experiment was conducted from November to December, 2019. Adsorption studies in the mono metal system of Pb (II), Cd (II), and Hg (II) by GB350, GB700, SB350, SB700, GS350 (combination of groundnut and sheanut shells biochars produced at 350 ± 5°C) and GS700 (combination of groundnut and sheanut shells biochars produced at 700 ± 5°C) were carried out. 100 ml of toxic metal solution of concentration levels of 5, 10, 25 and 50 mg/L with varied adsorbent masses of 2, 4, 8 and 10 g, respectively were agitated at room temperature of 25°C in an orbital shaker (Rotabit orbital shaker with 20 - 230 rpm rotational) with speed rate of 14.6 ± 1 U/min for 60 min. The mono systems experiments were carried out at varied pH solutions that ranged from 5.48 to 7.70. Sufficient time of 60 min was provided for the system to ascertain equilibrium at 25°C constant room temperature. After settling, elute was filtered through a Whatman's qualitative filter paper with 125 mm Ø particle retention size. Elutes were preserved in an ice chest and transported to the University of Ghana, Ecological Laboratory, for analysis. The toxic metals' analysis was done using the Perkin Elmer PIN Accle 900T Graphite Atomic Absorption Spectrophotometer (Waltham, United States of America).

Calculation of mono metals removal efficiency

The adsorption capacity of toxic metal $\mathbf{Q_e}$ (mg of toxic metal per g of biochar) for each adsorption system was carried out using Equation 1:

$$Q_e = \frac{C_o - C_e}{M} \text{ x V} \quad (1)$$

Plate 1. Groundnut shells biochar.
Source: Authors

Plate 2. Sheanut shells biochar.
Source: Authors

and the adsorption efficiency of cadmium, mercury and lead, $\mathbf{Q_e}$ (mg/g) in percentage was determined using Equation 2:

$$Q_e = \frac{(C_o - C_e)V}{M} \times 100\% \quad (2)$$

where C_o is initial concentrations (mg/L), and C_e represents final concentrations (mg/L), V is volume of metal solutions (L) and M is the mass of biochar (g).

Adsorption isotherm using Langmuir and Freundlich models

This study employed Langmuir model (Langmuir, 1918) and Freundlich model (Freundlich, 1906) for the data fitting. Langmuir model explains the quantitative monolayer formation of toxic metal on the biochar, outer surface and subsequently no further adsorption occurs. Langmuir model assumes uniform adsorption energies on the surface and no toxic metal transmigration in the plane of the biochar surface. Based on these assumptions, Langmuir then exemplified the following Equation 3:

$$Q_e = \frac{Q_{max} K_l C_e}{1 + K_l C_e} \quad (3)$$

Adsorption parameters of Langmuir model were assessed by changing the Langmuir Equation 3 to a linear form:

$$\frac{1}{q_e} = \frac{1}{Q_o} + \frac{1}{Q_o K_l C_e} \quad (4)$$

Table 1. Removal efficiency of toxic metals using biochars produced during slow and fast pyrolysis (n = 72).

Metal	Conc (mg/L)	Slow pyrolysis			Fast pyrolysis		
		GB350	SB350	GS350	GB700	SB700	GS700
Pb	5	100	100	100	100	100	100
Pb	10	100	100	100	100	100	100
Pb	25	100	100	100	100	100	100
Pb	50	100	100	100	100	100	100
Cd	5	98.24	92.06	100	98.96	97.98	96.24
Cd	10	98.78	98.00	100	98.70	97.00	98.60
Cd	25	98.47	91.95	97.76	99.49	98.82	99.11
Cd	50	96.61	76.90	87.25	99.30	99.36	99.26
Hg	5	99.76	99.94	99.98	99.90	99.36	100
Hg	10	99.78	99.70	99.88	99.49	96.79	100
Hg	25	99.98	99.99	99.94	100	100	99.87
Hg	50	99.99	99.98	100	100	100	100

Source: Authors

where Q_{max} is the adsorption maximum capacity determined by Langmuir model; Q_e = the quantity of toxic metal adsorbed per gram at equilibrium by the biochar (mg/g); Q_o = maximum capacity of monolayer coverage (mg/g); K_l = constant of Langmuir isotherm (l/mg); and C_e = the toxic metal (adsorbate) equilibrium concentration (mg/L^{-1}). The values of K_l and Q_{max} were determined using the slope and intercept of the plot of Ce vrs C_e/Q_e (Langmuir, 1918). The important feature of Langmuir isotherm may be the expression of equilibrium parameter (R_L), which is a dimensionless constant denoted as separation factor (Webber and Chakravarti, 1974).

$$R_L = \frac{1}{1+K_l C_0} \quad (5)$$

where K_L = the constant that is associated to the energy of adsorption (Langmuir constant) and C_0 = initial concentration of absorbate. The R_L is mostly used to ascertain whether the process of an adsorption was thermodynamically favourable or not: when R_L = 0, an irreversible adsorption; when $0 < R_L < 1$, favourable adsorption; when $R_L = 1$, a linear adsorption; when $R_L > 1$, an unfavourable adsorption (Gorgievski et al., 2013).

Adsorption isotherm of Freundlich is often used to describe the heterogeneous surface characteristics of adsorption (Hutson and Yang, 2000). Freundlich constants signify the nonlinearity degree between solution concentration and adsorption, and extent of the adsorption, respectively. The data usually fit the proposed empirical Equation 6 by Freundlich:

$$Q_e = K_F C_e^{1/n} \quad (6)$$

where Q_e = the quantity of toxic metal adsorbed per gram at equilibrium by the biochar (mg/g); K_F = Freundlich isotherm constant (mg/g); C_e = the adsorbate equilibrium concentration (mg/L); and n = adsorption intensity. Linearising equation is given as:

$$\log Q_e = \log K_F + {}^1/_n \log C_e \quad (7)$$

The constant K_F is an estimated capacity of adsorption indicator, whilst $^1/_n$ is a function of the adsorption strength in the process of adsorption (Voudrias et al., 2002). If value of $^1/_n$ is below (>)1 indicates occurrence of a normal adsorption. If $^1/_n$ is above (<) 1, it shows co-operative adsorption and if n = 1 then the partition amongst the two phases are independent of the concentration (Mohan and Karthikeyan, 1997).

RESULTS AND DISCUSSION

The lead removal efficiency at concentrations of 5, 10, 25 and 50 mg/L were 100% for groundnut shell biochar (GB350), sheanut shell biochar (SB350) and combination of groundnut and sheanut shells biochars (GS350), groundnut shell biochar (GB700), and sheanut shell biochar (SB700) and combination of groundnut and sheanut shells biochars (GS700) (Table 1). The removal rate of Pb was highly effective by the groundnut and sheanut shells biochars. This may be due to O-containing functional groups that play a vital role in modifying Pb mobility. This is because biochar derived under low temperature pyrolysis gives high amount of O-containing functional groups which generally showed good efficiency for toxic metal stabilisation (Ahmad et al., 2014). Also, biochars produced under high temperature had higher removal rates due to the presence of high pores and surface area. Biochars vary greatly in properties and capacity to adsorb the toxic metals. Other studies

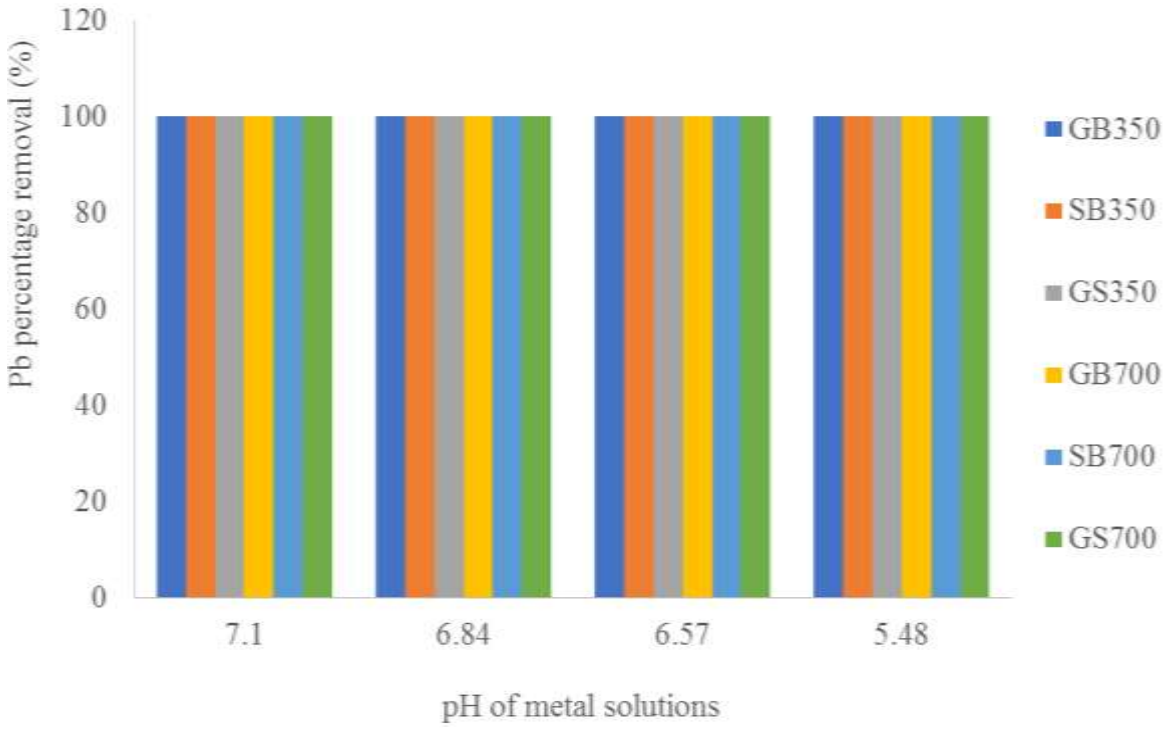

Figure 1. Effect of pH on Pb adsorption in mono system using biochars produced during slow and fast pyrolysis
Source: Authors

conducted with different experimental conditions also show high percentage removal of Pb ion using various natural adsorbents such as chalf (85%), sun flower husk (86%), rice husk (90%), tea waste (98%), sesame husk (100%) (Surchi, 2011), bamboo biochar and calcium sulphate (99%) (Hassan and Kaewsichan, 2016), rice husk biochar (almost 90%) (Sanka et al., 2020) and rice and corn husk-based sorbents (>90%) (Rwiza et al., 2018). Mechanisms of non-electrostatic are deliberated as dominating for Pb (Clemente et al., 2017).

Cadmium removal efficiency at concentrations of 5, 10, 25 and 50 mg/L by groundnut shell, sheanut shell, and the combination of groundnut and sheanut shells biochars produced at temperatures of 350 ± 5 and 700 ± 5°C ranged from 76.90 to 100% and 96.24 to 99.49%, respectively (Table 1). The groundnut and sheanut shells biochars produced during fast pyrolysis recorded the highest Cd removal rates. Similar studies showed Cd^{2+} removal of almost 100% for municipal sewage sludge biochar (Chen et al., 2014), 90 to 99.24% for *Eichornia crassipes* biochars (Li et al., 2016), 80.60 to 96.90% for orange peel biochars (Tran et al., 2015), and 86.60% for soybean straw and 99.20% for peanut husk biochar (Cheng et al., 2016). Also, wheat straw biochar removal efficiency of Cd was up to ~90% (Cui et al., 2019) and almost 100% by palm waste biochar (700 °C) from Cd polluted solutions which was attributed to functional groups (such as $-CO_3^{2-}$ and –OH through surface precipitation and surface complexation, respectively) of the biochar (Usman et al., 2016).

The removal efficiency of mercury ions in the aqueous solution with concentrations of 5, 10, 25 and 50 mg/L by biochars produced from groundnut shell, sheanut shell and the combination of groundnut and sheanut shells at a temperature of 350 ± 5°C ranged from 99.70 to 100% (Table 1). The biochars produced from groundnut shell, sheanut shell and the combination of groundnut and sheanut shell at temperature of 700 ± 5°C had removal efficiency ranging from 96.79 to 100% at the concentrations of 5, 10, 25 and 50 mg/L in the prepared aqueous solution (Table 1). The adsorption of toxic metals ions by groundnut and sheanut shells biochars produced during the slow and fast pyrolysis showed similar removal efficiencies. Comparable, high removal of 67.77 to 95.96% for Pb^{2+} and 92.12 to 99.05% for Cd^{2+} from drinking water by biochar obtained from peanut shell, “chonta” pulp and corn cob calcined at 500, 600 and 700°C, respectively (Puglla et al., 2020).

Effect of pH on the adsorption of biochars towards mono metal ions

The pH of solutions of 5.48 to 7.10, 7.30 to 7.70 and 6.20 to 6.91 in the mono systems (Figures 1 to 3) were more effective for Pb, Cd and Hg removal. This was largely

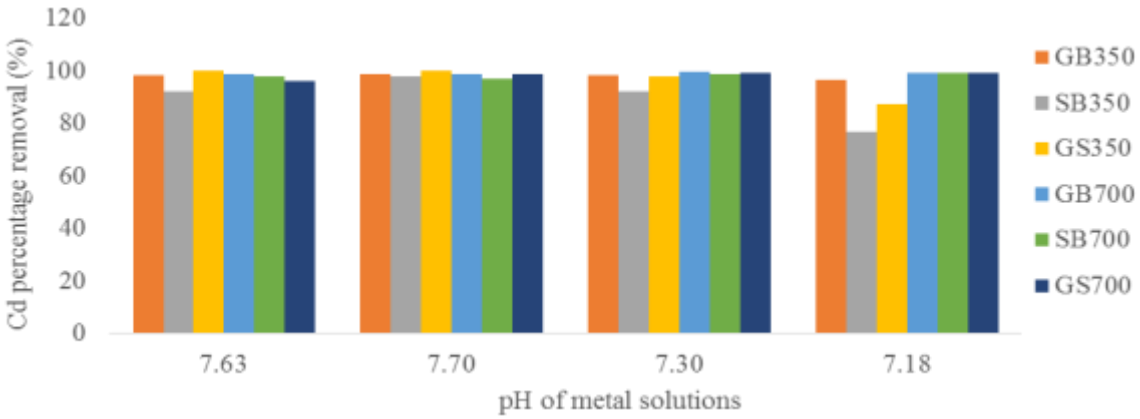

Figure 2. Effect of pH on Cd adsorption in mono system using biochars produced during slow and fast pyrolysis.
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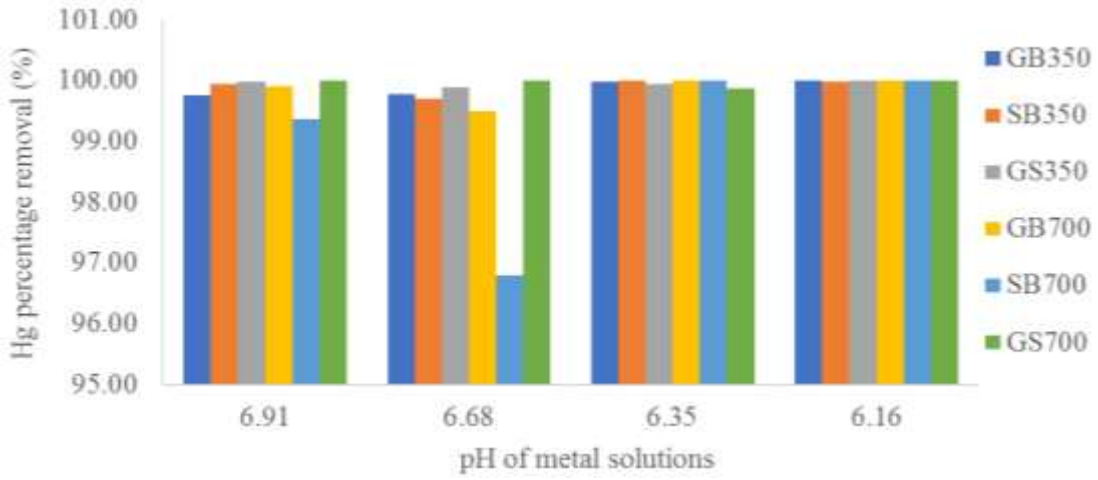

Figure 3. Effect of pH on Hg adsorption in mono system using biochars produced during slow and fast pyrolysis.
Source: Authors

dependent on the characteristics of groundnut and sheanut shells biochars determined by the pyrolysis conditions and nature of feed stock. The nutrient content, structure of porosity, phenolic content and pH of biochar depend on the raw material type, temperature and duration of the pyrolysis process (Xu et al., 2021). The surface properties, the adsorbed toxic metal ions distribution, and functional groups protonation on adsorbent are affected by pH of solution (Sun et al., 2014).

Effect of biochar dosage on adsorption performance

The study revealed at biochar dosage of 2 g/5 mg/L, 4 g/10 mg/L, 8 g/25 mg/L and 10 g/50 mg/L Pb removal efficiency was 100% (Figure 4), 76.90 to 100% for Cd (Figure 5) and 96.79 to 100% for Hg for the biochars (Figure 6). Dosage of biochar affects toxic metal removal in an aqueous solution. The experiment showed the various dosages were effective for metals removal. High amount of biochar in process of adsorption can guarantee availability of more sites and specific surface areas for adsorption, which usually contribute to great adsorption capacity. The increases in number of sites available for adsorption is followed by increase of an adsorbent specific surface area (Thavamani and Rajkumar, 2013). This causes the higher removal efficiencies by the groundnut and shea nut shells biochars under the various dosages and elevated contaminations limits. The toxic metal ions were nearly completely adsorbed at perspective dosages, and

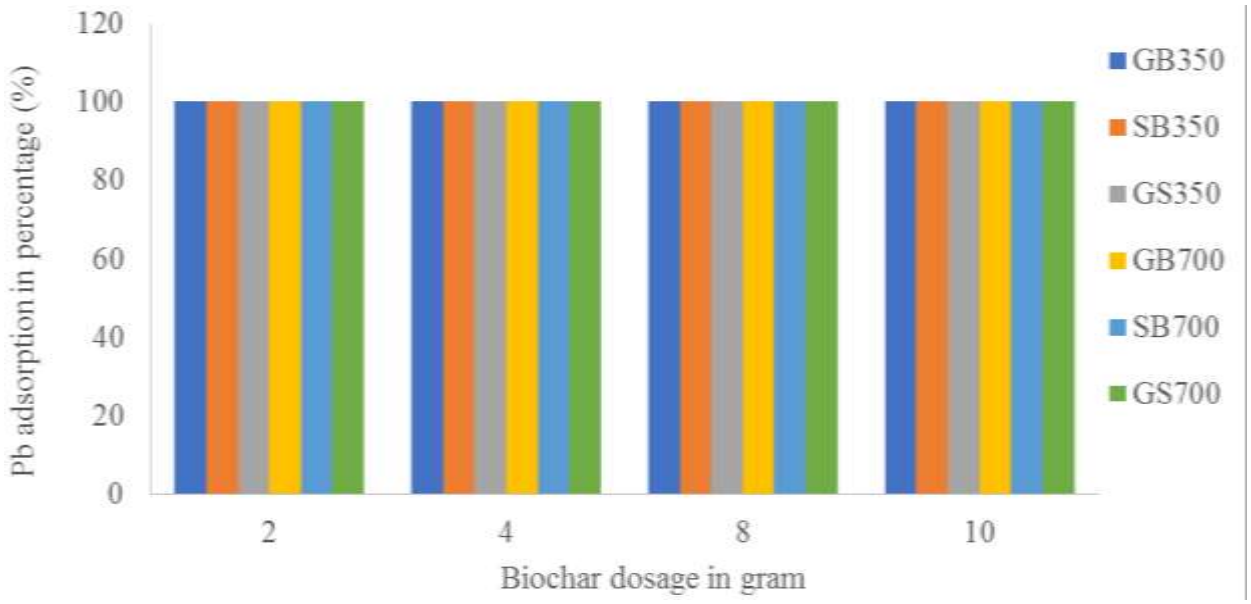

Figure 4. Effect of biochar dosage on Pb in mono system.
Source: Authors

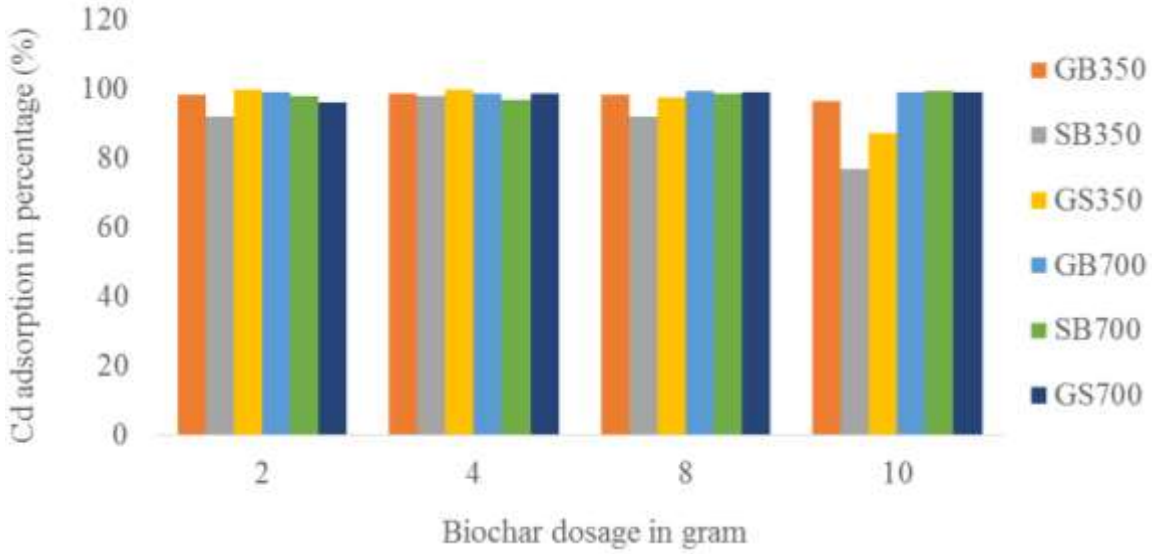

Figure 5. Effect of biochar dosage on Cd in mono system.
Source: Authors

variation of removal efficiencies was negligible with the various biochars.

Adsorption isotherms for mono metals

Langmuir model explains the quantitative monolayer formation of Cd and Hg on the biochar, outer surface and subsequently no further adsorption occurs. The specific plots of adsorption ($^{Ce}/_{Qe}$) against Ce (the equilibrium concentration) for toxic metals that did not ascertain complete adsorption in mono systems (Figure 7a to l as supplementary material). Pb adsorption was complete hence Langmuir graphs could not be plotted. The slope and intercept of the Langmuir graphs were used to compute for the linear isotherm values of Q_{max} and K_L and the R^2 (coefficient of determinations) (Table 2). The

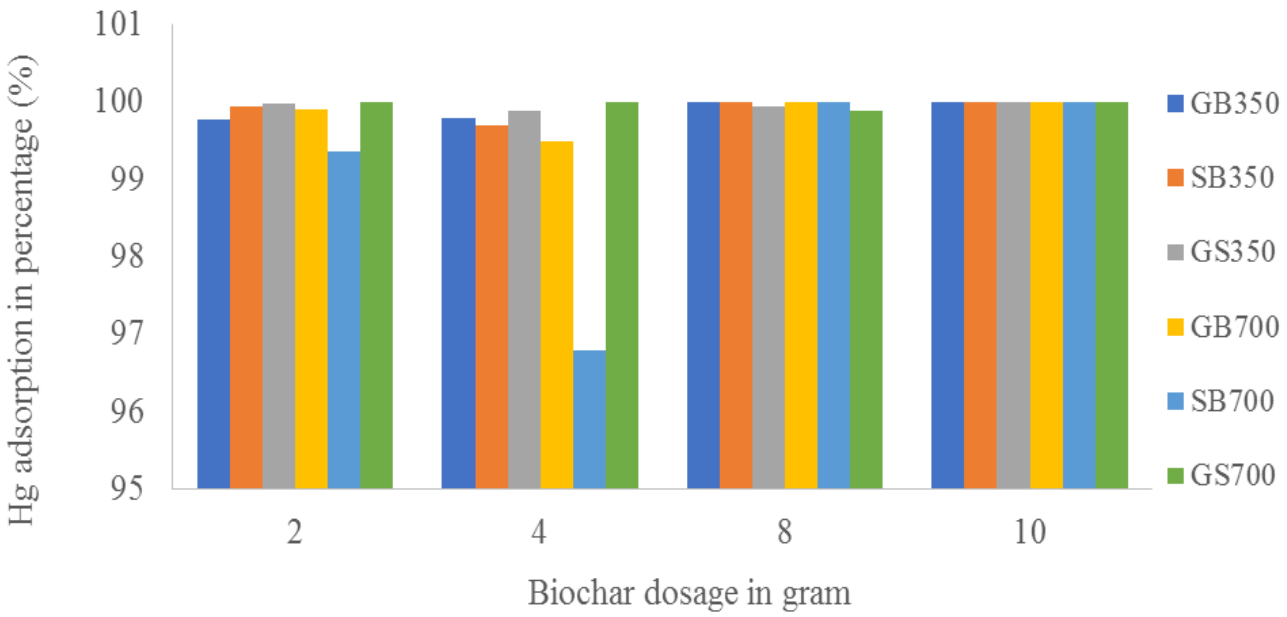

Figure 6. Effect of biochar dosage on Hg in mono system.
Source: Authors

Table 2. Modelling the results of adsorption isotherms of Cd and Hg.

Ion	Biochar	Langmuir parameter				Freundlich parameter			
		Q_{max} (mg/g)	K_L (l/mg)	R_L	R^2	$^1/_n$	n	K_F (mg/g)	R^2
Cd	GB350	526.32	0.16	8.89	0.9907	0.23	4.27	412.19	0.9711
Cd	SB350	400.00	0.36	19.00	0.9975	0.08	12.82	275.80	0.9123
Cd	GS350	434.78	0.09	5.35	0.9986	1.25	0.80	12.84	0.1500
Cd	GB700	666.67	0.13	7.67	0.8354	0.37	2.71	657.81	0.7673
Cd	SB700	416.67	0.08	5.17	0.4672	0.31	3.26	484.28	0.2543
Cd	GS700	2000.00	1.20	61.00	0.2855	0.78	1.29	1017.42	0.8942
Hg	GB350	232.56	0.00	0.94	0.9982	-0.30	-3.35	73.05	0.7202
Hg	SB350	243.90	0.00	0.93	0.9803	-0.02	-58.82	288.00	0.0039
Hg	GS350	294.12	0.00	1.01	0.9748	-0.87	-1.15	2.29	0.7929
Hg	GB700	250.00	0.00	1.00	1.00	-1.15	-0.87	1.46	0.8642
Hg	SB700	243.90	0.00	0.99	1.00	-1.60	-0.63	2.51	0.6666
Hg	GS700	312.50	0.00	0.99	0.9999	-0.97	-1.03	1.62	0.8578

Source: Authors

maximum Langmuir capacity ranged from 400 to 526.32 mg/g for Cd and 232.56 to 294.12 mg/g for Hg by GB350, SB350 and GS350 and 416.67 to 2000 mg/g for Cd and 243.90 to 312.50 mg/g for Hg by GB700, SB700 and GS700 (Table 2). The maximum Langmuir capacity was in the order of Cd ion > Hg ion and was larger for biochars produced during fast pyrolysis than in slow pyrolysis. The Langmuir constant (K_L) reflects how many the toxic metals interact with the biochar. The bigger constant value implies strong association between the toxic metal and the biochar and, small constant value indicates weak interaction (Tran et al., 2019). The K_L ranged from 0.09 to 0.36 l/mg for Cd and 0.00 l/mg for Hg by GB350, SB350 and GS350 and 0.08 to 1.20 l/mg for Cd and 0.00 l/mg for Hg by GB700, SB700 and GS700 (Table 2). The K_L (l/mg) was in the order of Cd ion > Hg ion for biochars produced at both 350 ± 5 and 700 ± 5°C. From the results, the value of K_L for Cd and Hg indicates weak interaction. The separation factor R_L was found to be in order of Cd ion > Hg ion. The R_L was found to have

ranged from 5.17 to 61.00 for Cd and 0.93 to 1.01. The R_L > 1 for Cd ions indicates unfavourable adsorption process. However, GB350, SB350, SB700 and GS700 for Hg were less than 1 indicating favourable adsorption.

Freundlich isotherm was used to explore the data fitness in terms of the biochars surface heterogeneous nature in toxic metal uptake. The specific plots of Log Q_e against Log C_e, give a slope with the value of $^1/_n$ and an intercept magnitude of log K_F (Figure 8a to l as supplementary material). Pb adsorption was complete hence Freundlich graphs could not be plotted. The adsorption intensity (n) values were found to have ranged from -58.82 to 12.82 and 1.46 to 1017.42 for K_F (adsorption capacity) (Table 2). The K_F for the metals by biochars followed the order of Cd > Hg. The $^1/_n$ and n values for the metal ions by biochars followed the order of Cd > Hg. The Cd $^1/_n$ and n values are positive, and negative for Hg ions. The $^1/_n$ < 1 for Cd and Hg implies a normal adsorption occurred. In the case of Cd, almost all n values were between one and ten, implying the process of adsorption was favourable whilst all n values for Hg were less than 1 implying the degree of nonlinearity between adsorption and solution concentration as physical process. The Langmuir model ($0.2855 \leq R^2 \leq 1.00$) was better fitted for the adsorption toxic metal ions onto biochar than the Freundlich model ($0.0039 \leq R^2 \leq 0.9711$).

Conclusion

The adsorption of Pb, Cd and Hg ions by groundnut and sheanut shells biochars produced during slow and fast pyrolysis temperatures showed similar removal efficiencies. The adsorption performance of groundnut and sheanut shells biochars was highly effective at pH range of 5.48 to 7.70. Langmuir isotherm was the model that best fit the adsorption of toxic metal ions onto the biochars except for SB700 and GS700 that recorded low R^2. Groundnut and sheanut shells biochars have proven to be good candidates for the remediation of water polluted with toxic metals.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Appendix

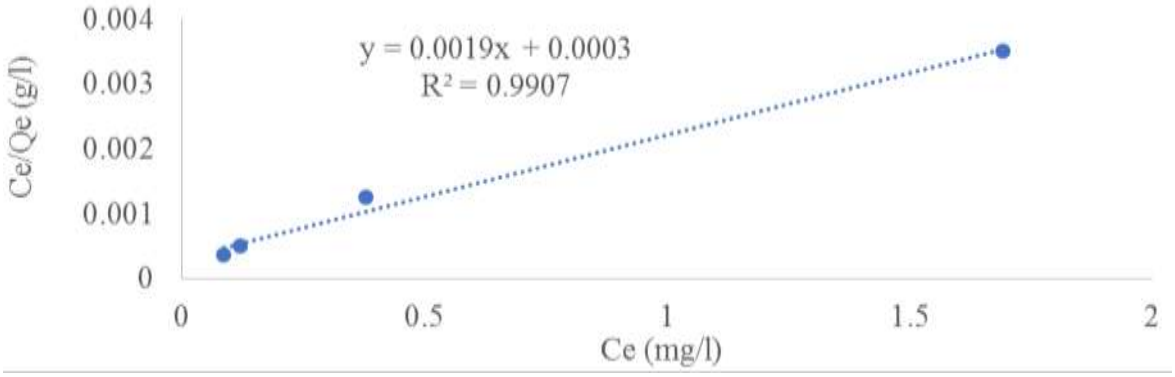

Figure 7a: Langmuir isotherm for adsorption of Cd in aqueous solution onto GB350 (solution volume: 100 ml; adsorbent dose: 2, 4, 8 and 10 g; contact time: 60 min) Source: Authors' analyses

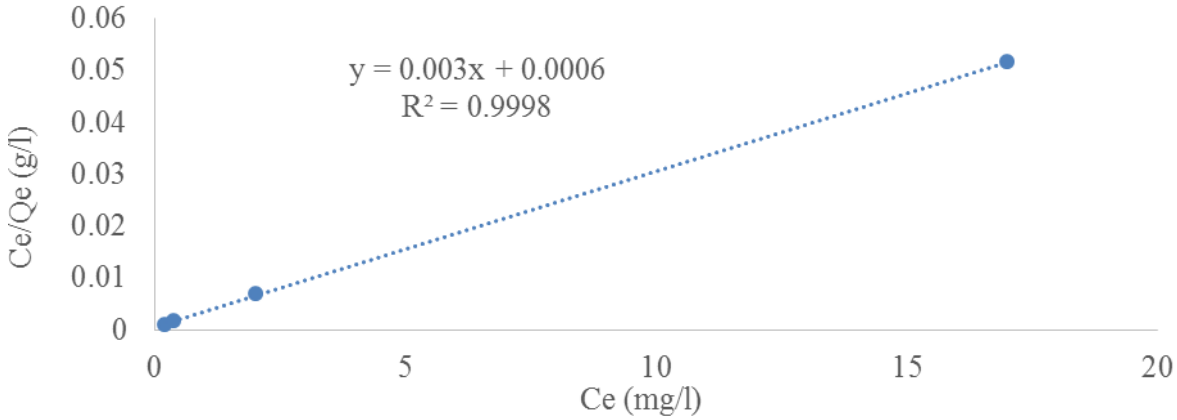

Figure 7b: Langmuir isotherm for adsorption of Cd in aqueous solution onto SB350 (solution volume: 100 ml; adsorbent dose: 2, 4, 8 and 10 g; contact time: 60 min) Source: Authors' analyses

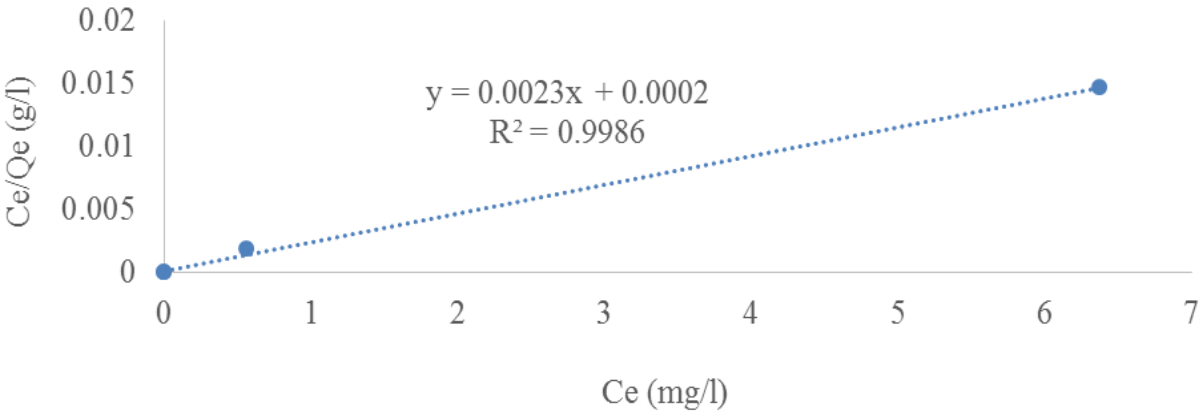

Figure 7c: Langmuir isotherm for adsorption of Cd in aqueous solution onto GS350 (solution volume: 100 ml; adsorbent dose: 2, 4, 8 and 10 g; contact time: 60 min) Source: Authors' analyses

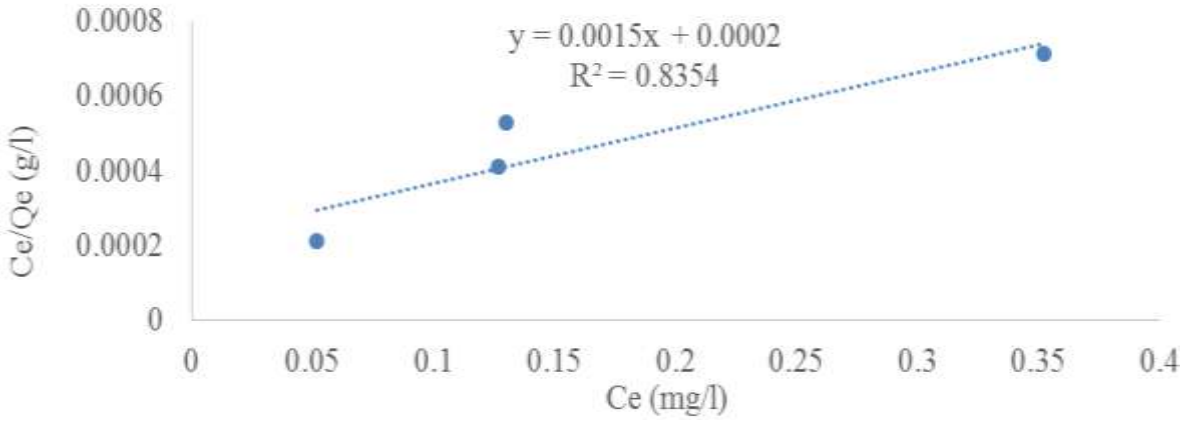

Figure 7d: Langmuir isotherm for adsorption of Cd in aqueous solution onto GB700 (solution volume: 100 ml; adsorbent dose: 2, 4, 8 and 10 g; contact time: 60 min) Source: Authors' analyses

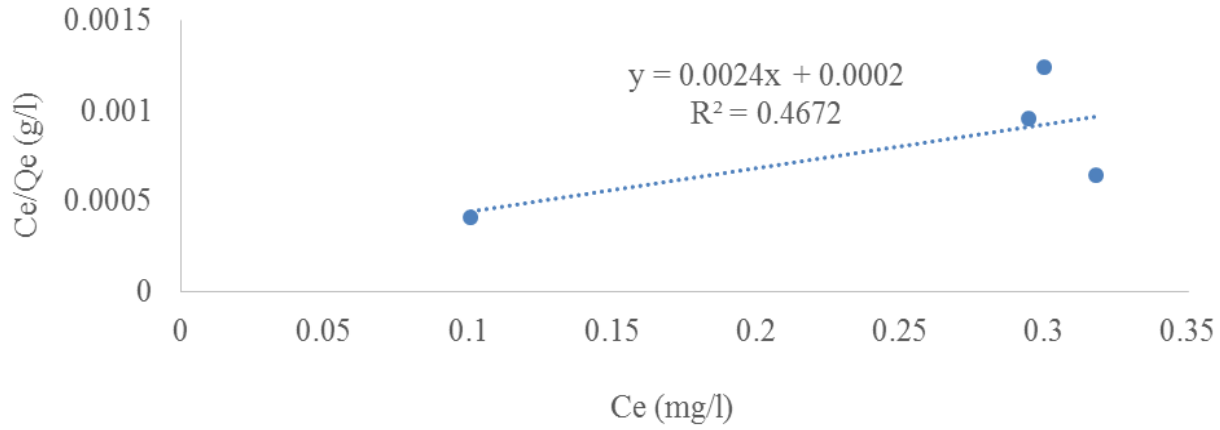

Figure 7e: Langmuir isotherm for adsorption of Cd in aqueous solution onto SB700 (solution volume: 100 ml; adsorbent dose: 2, 4, 8 and 10 g; contact time: 60 min) Source: Authors' analyses

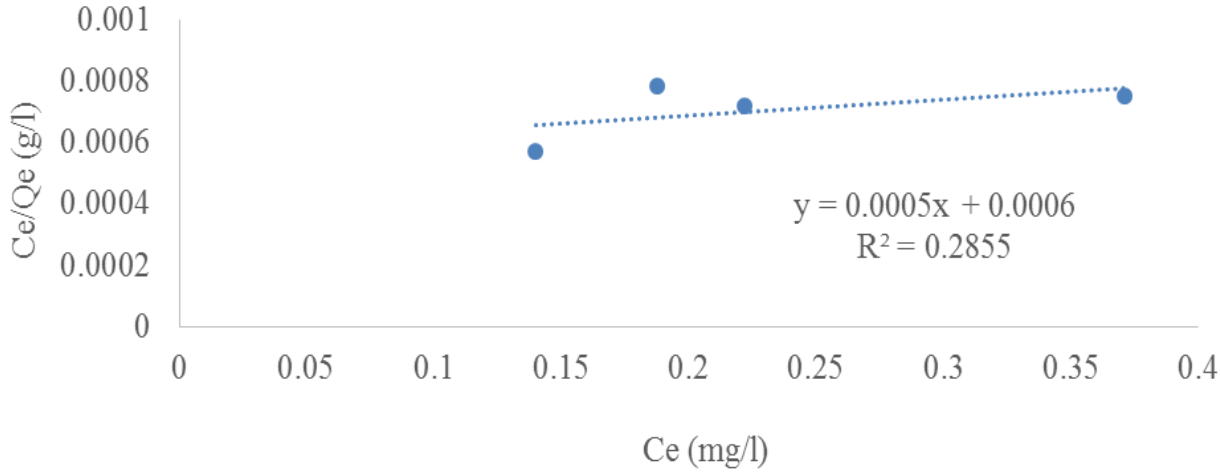

Figure 7f: Langmuir isotherm for adsorption of Cd in aqueous solution onto GS700 (solution volume: 100 ml; adsorbent dose: 2, 4, 8 and 10 g; contact time: 60 min) Source: Authors' analyses

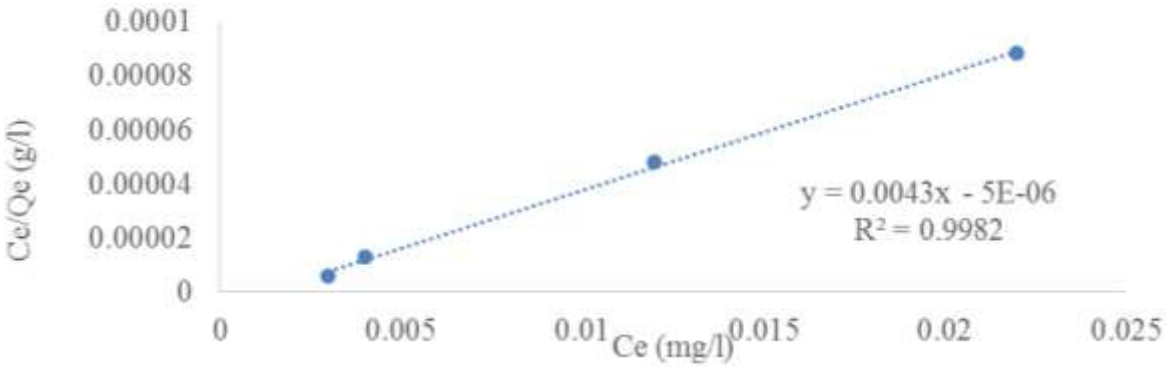

Figure 7g: Langmuir isotherm for adsorption of Hg in aqueous solution onto GB350 (solution volume: 100 ml; adsorbent dose: 2, 4, 8 and 10 g; contact time: 60 min) Source: Authors' analyses

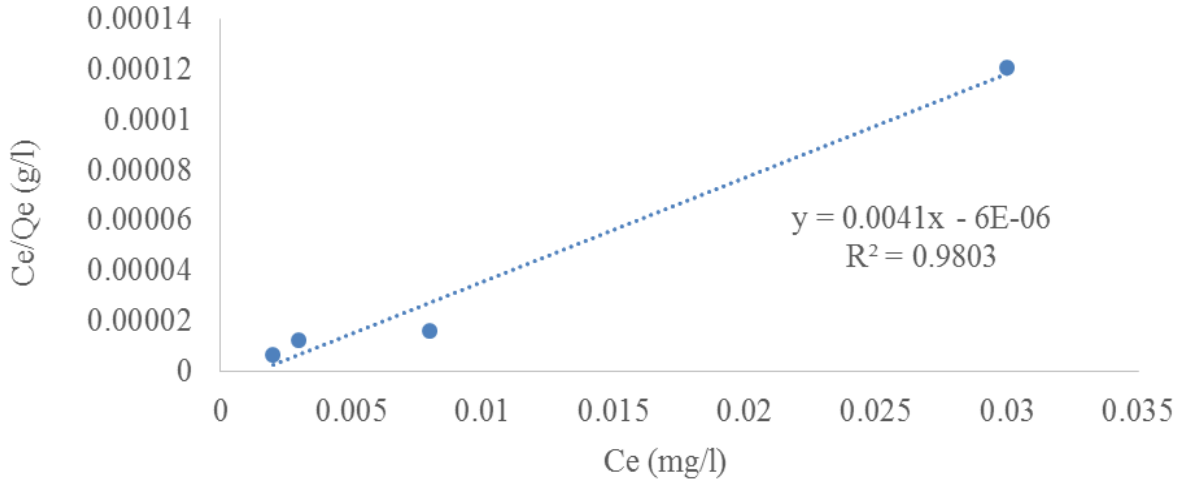

Figure 7h: Langmuir isotherm for adsorption of Hg in aqueous solution onto SB350 (solution volume: 100 ml; adsorbent dose: 2, 4, 8 and 10 g; contact time: 60 min) Source: Authors' analyses

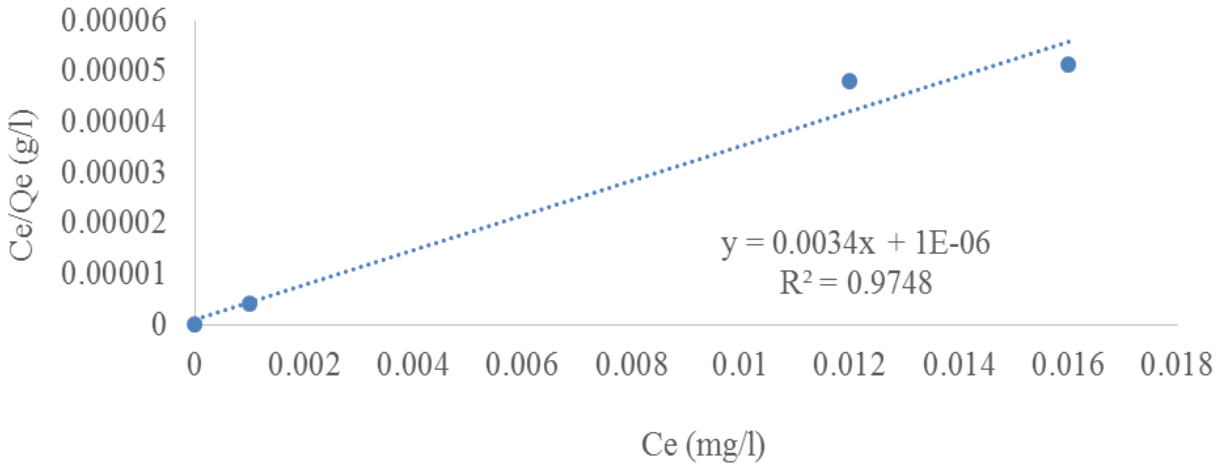

Figure 7i: Langmuir isotherm for adsorption of Hg in aqueous solution onto GS350 (solution volume: 100 ml; adsorbent dose: 2, 4, 8 and 10 g; contact time: 60 min) Source: Authors' analyses

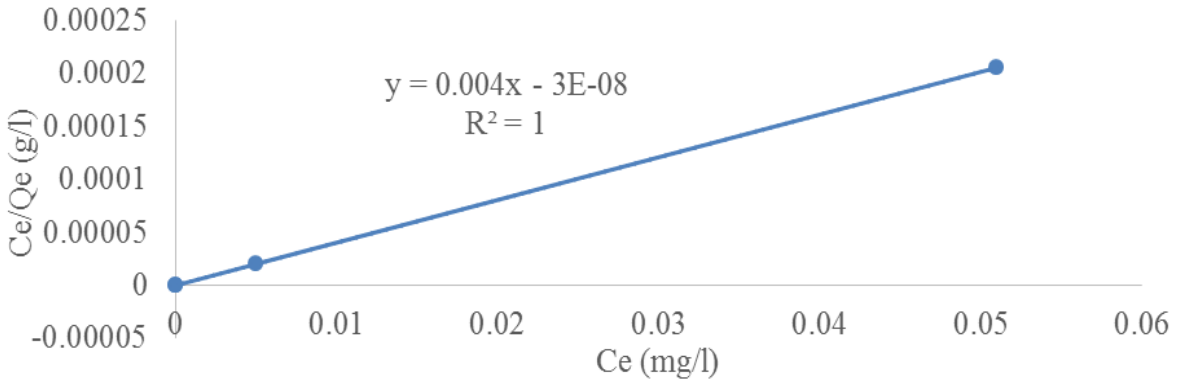

Figure 7j: Langmuir isotherm for adsorption of Hg in aqueous solution onto GB700 (solution volume: 100 ml; adsorbent dose: 2, 4, 8 and 10 g; contact time: 60 min) Source: Authors' analyses

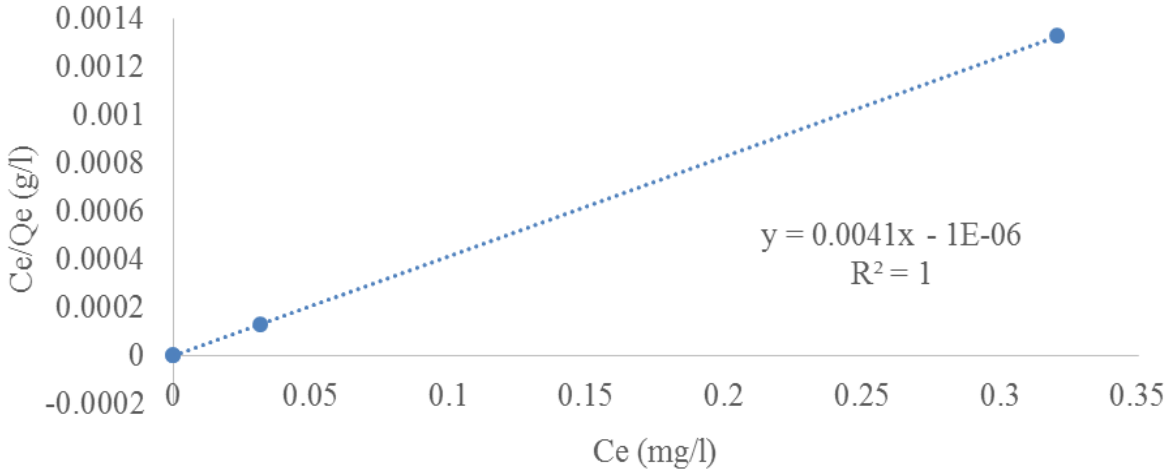

Figure 7k: Langmuir isotherm for adsorption of Hg in aqueous solution onto SB700 (solution volume: 100 ml; adsorbent dose: 2, 4, 8 and 10 g; contact time: 60 min) Source: Authors' analyses

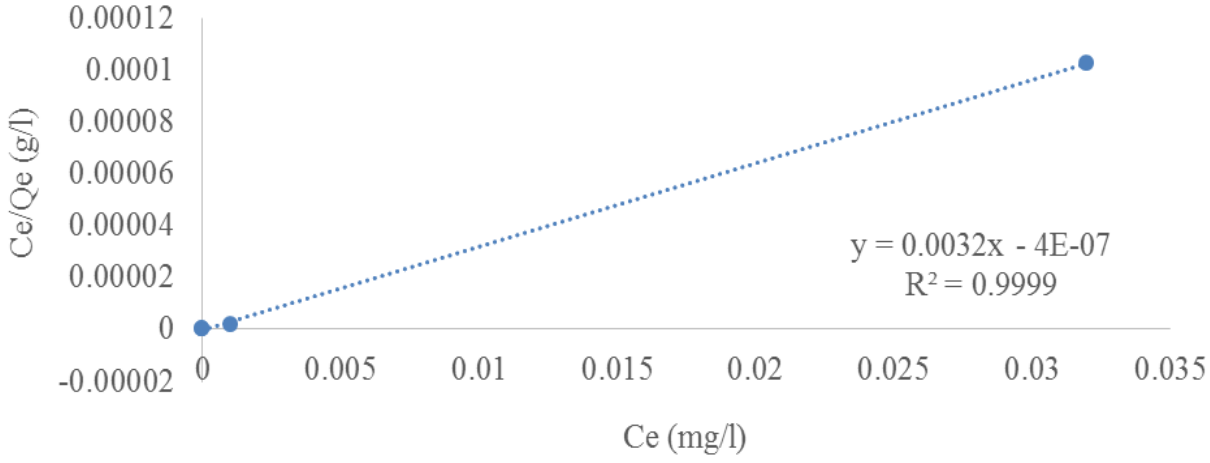

Figure 7l: Langmuir isotherm for adsorption of Hg in aqueous solution onto GS700 (solution volume: 100 ml; adsorbent dose: 2, 4, 8 and 10 g; contact time: 60 min) Source: Authors' analyses

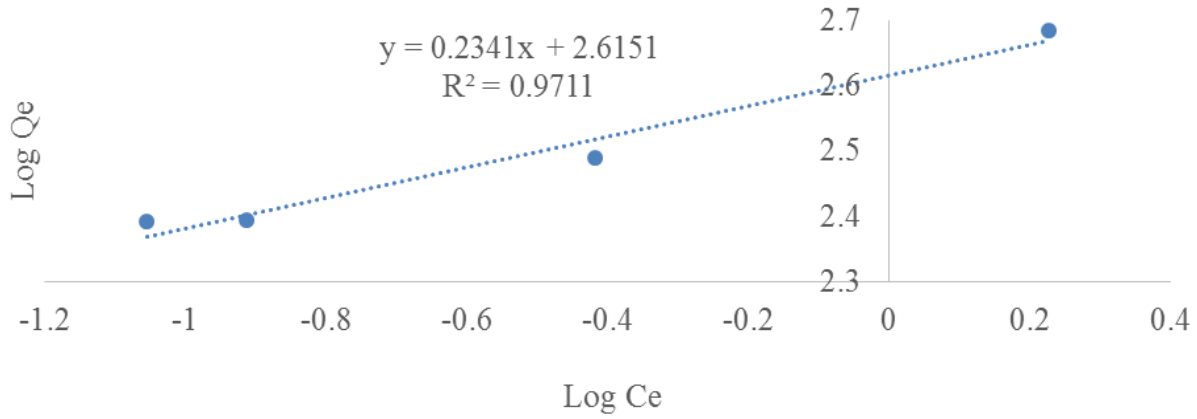

Figure 8a: Freundlich isotherm for adsorption of Cd in aqueous solution onto GB350 (solution volume: 100 ml; adsorbent dose: 2, 4, 8 and 10 g; contact time: 60 min) Source: Authors' analyses

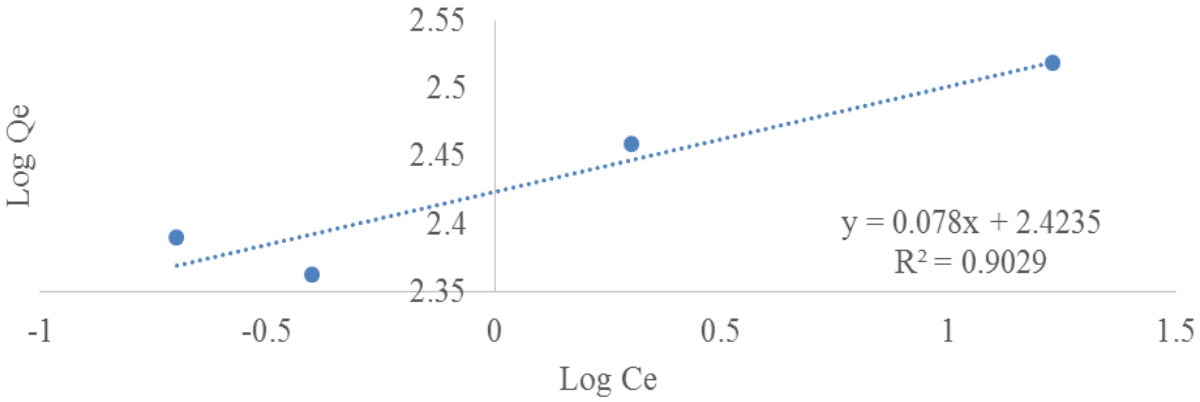

Figure 8b: Freundlich isotherm for adsorption of Cd in aqueous solution onto SB350 (solution volume: 100 ml; adsorbent dose: 2, 4, 8 and 10 g; contact time: 60 min) Source: Authors'

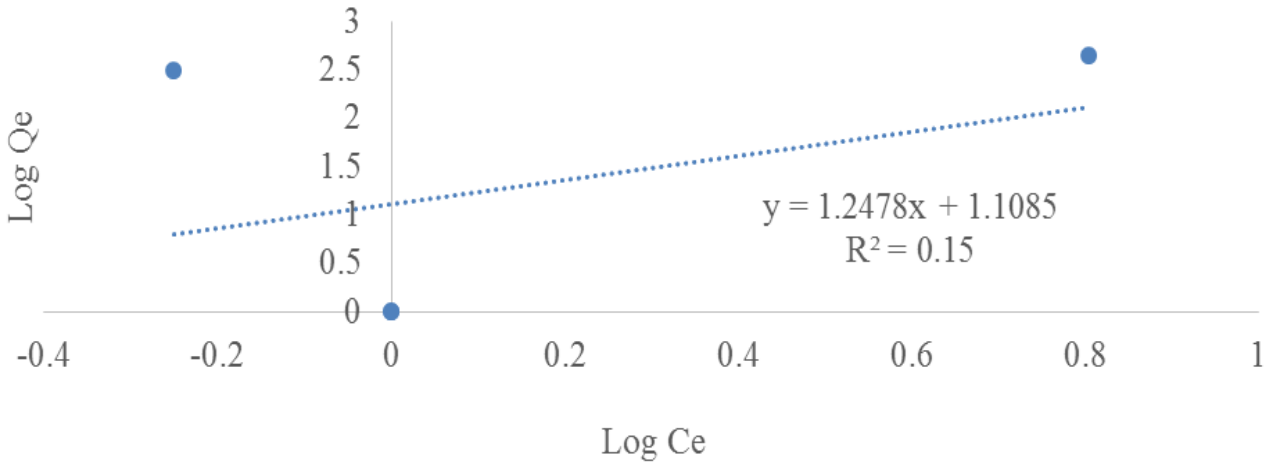

Figure 8c: Freundlich isotherm for adsorption of Cd in aqueous solution onto SB350 (solution volume: 100 ml; adsorbent dose: 2, 4, 8 and 10 g; contact time: 60 min) Source: Authors' analyses

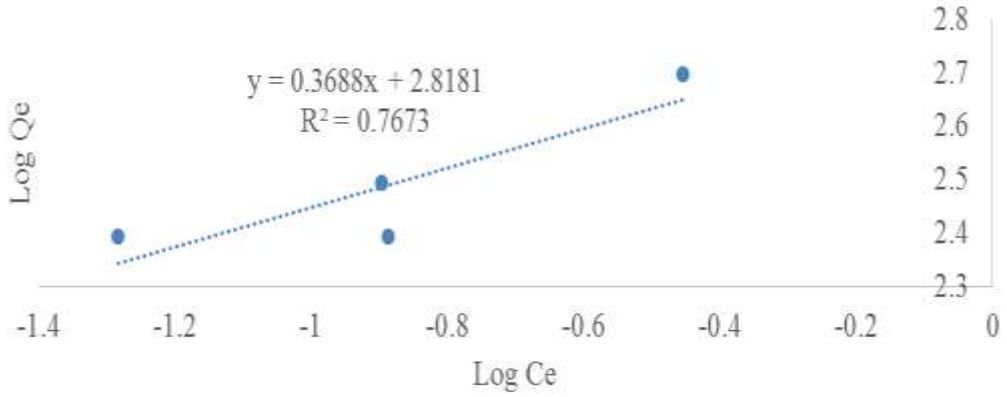

Figure 8d: Freundlich isotherm for adsorption of Cd in aqueous solution onto GB700 (solution volume: 100 ml; adsorbent dose: 2, 4, 8 and 10 g; contact time: 60 min) Source: Authors' analyses

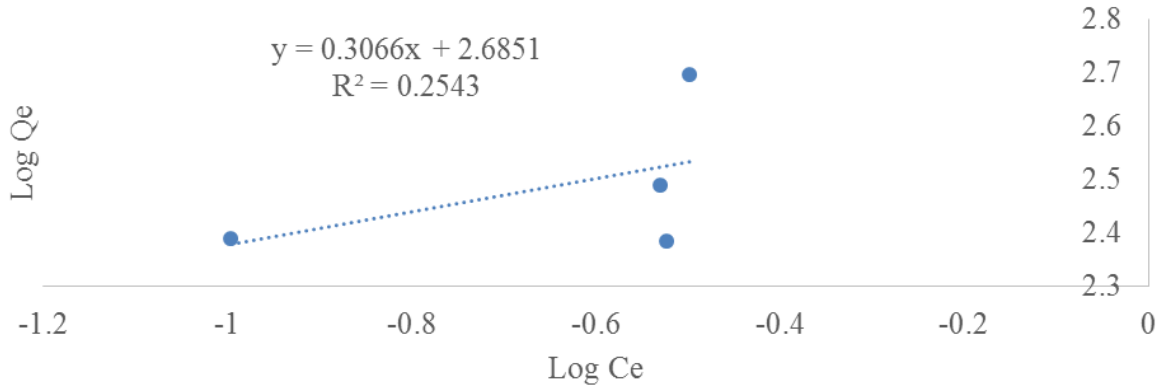

Figure 8e: Freundlich isotherm for adsorption of Cd in aqueous solution onto SB700 (solution volume: 100 ml; adsorbent dose: 2, 4, 8 and 10 g; contact time: 60 min) Source: Authors' analyses

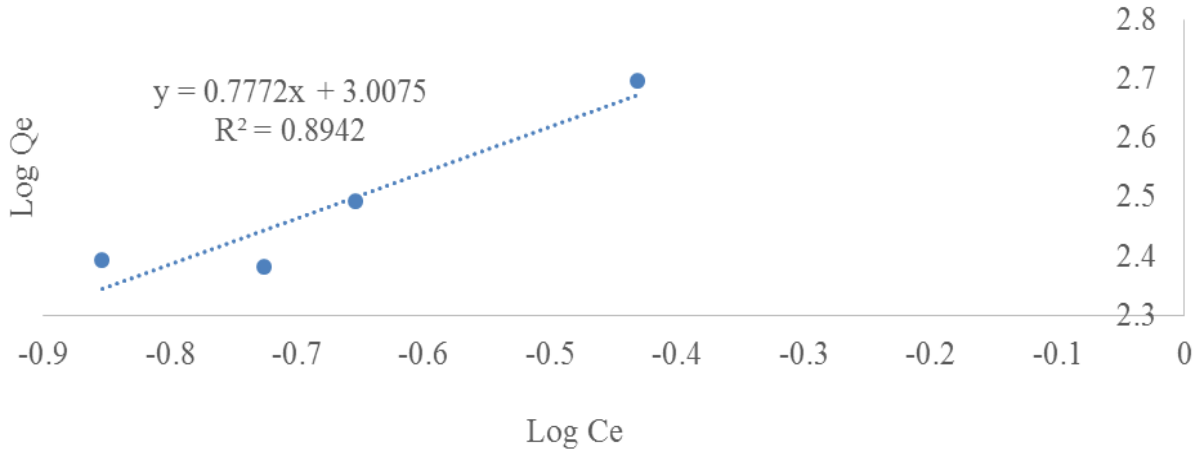

Figure 8f: Freundlich isotherm for adsorption of Cd in aqueous solution onto GS700 (solution volume: 100 ml; adsorbent dose: 2, 4, 8 and 10 g; contact time: 60 min) Source: Authors' analyses

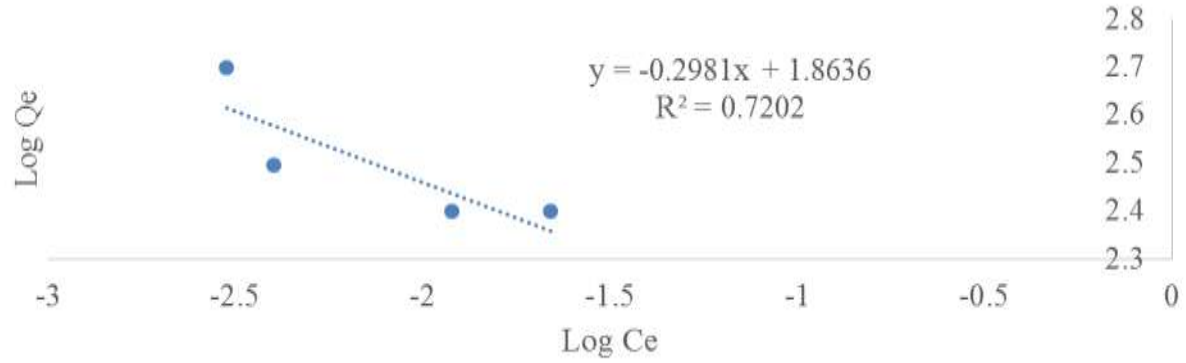

Figure 8g: Freundlich isotherm for adsorption of Hg in aqueous solution onto GB350 (solution volume: 100 ml; adsorbent dose: 2, 4, 8 and 10 g; contact time: 60 min) Source: Authors' analyses

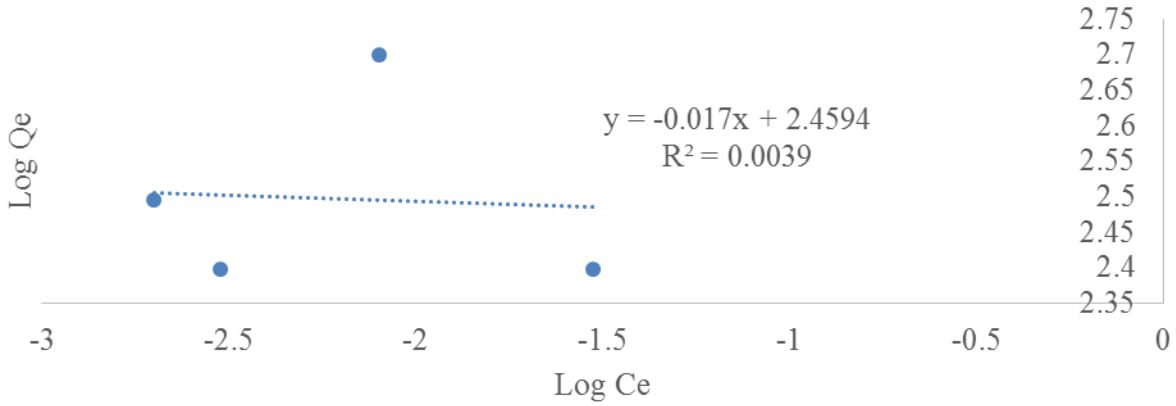

Figure 8h: Freundlich isotherm for adsorption of Hg in aqueous solution onto SB350 (solution volume: 100 ml; adsorbent dose: 2, 4, 8 and 10 g; contact time: 60 min) Source: Authors' analyses

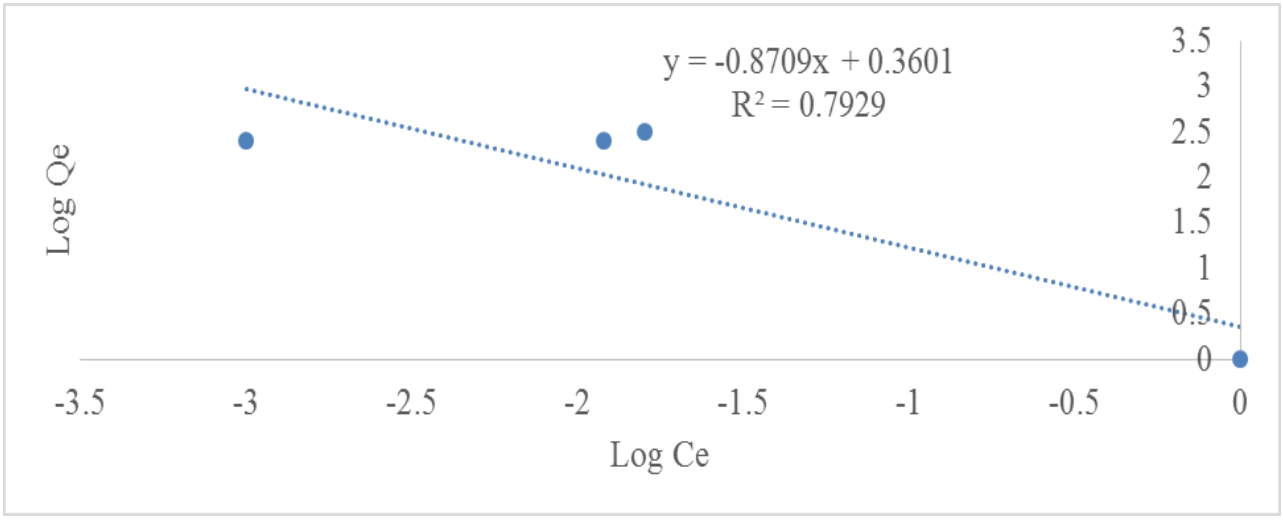

Figure 8i: Freundlich isotherm for adsorption of Hg in aqueous solution onto GS350 (solution volume: 100 ml; adsorbent dose: 2, 4, 8 and 10 g; contact time: 60 min) Source: Authors' analyses

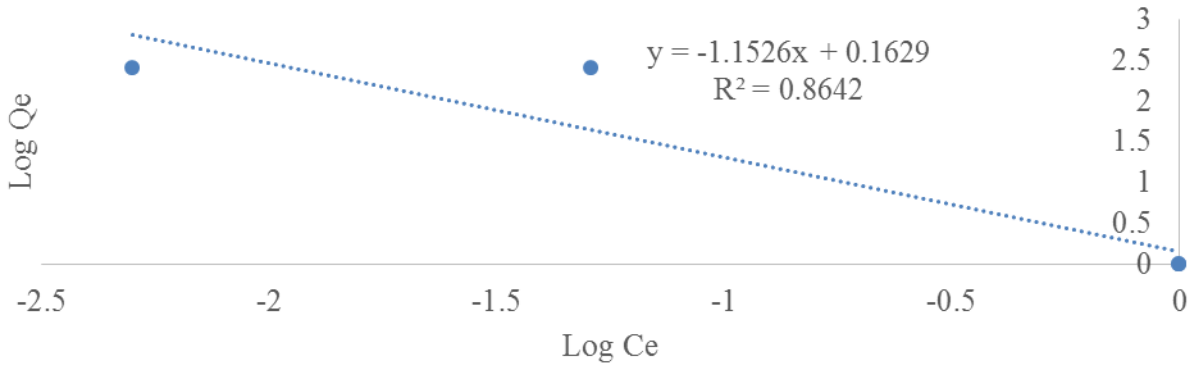

Figure 8j: Freundlich isotherm for adsorption of Hg in aqueous solution onto GB700 (solution volume: 100 ml; adsorbent dose: 2, 4, 8 and 10 g; contact time: 60 min) Source: Authors' analyses

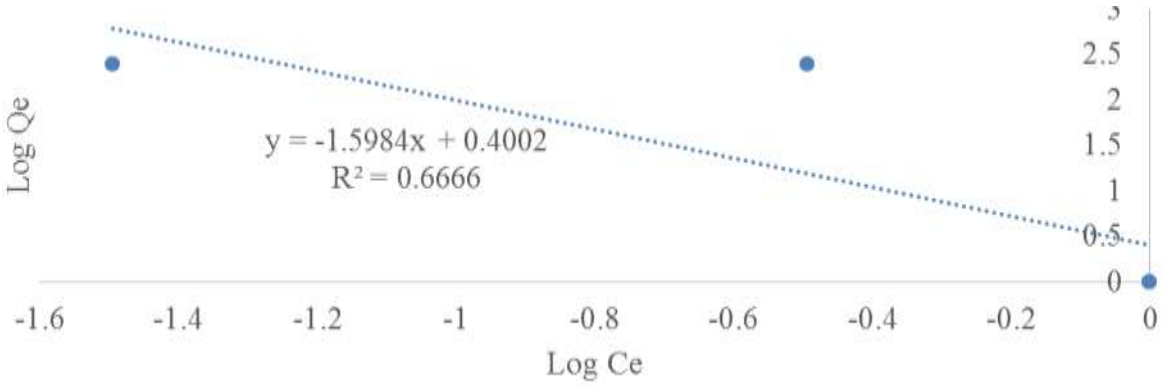

Figure 8k: Freundlich isotherm for adsorption of Hg in aqueous solution onto SB700 (solution volume: 100 ml; adsorbent dose: 2, 4, 8 and 10 g; contact time: 60 min) Source: Authors' analyses

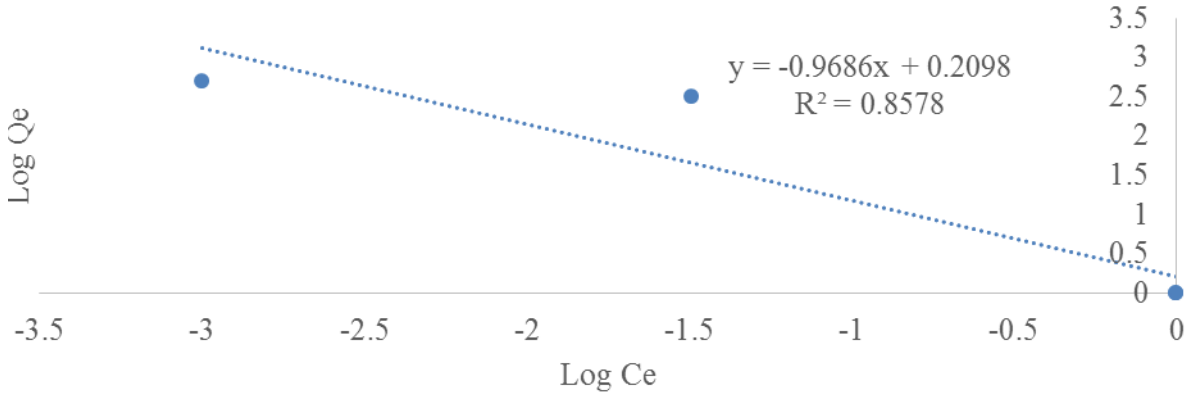

Figure 8l: Freundlich isotherm for adsorption of Hg in aqueous solution onto GS700 (solution volume: 100 ml; adsorbent dose: 2, 4, 8 and 10 g; contact time: 60 min) Source: Authors' analyses

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Assessment of anticoccidial efficacy of chitosan nanoencapsulated bromelain against coccidia in naturally infected goats in Kenya

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The current study assessed the anticoccidial efficacy of chitosan nanoencapsulated bromelain (CNB) against coccidiosis in goats. Bromelain was extracted from the pineapple peels using standard methods while oral solution of CNB was prepared using standard manufacturing methods. The *in vivo* study was done on fifteen healthy male goats naturally infected with coccidia. The goats were divided into three groups consisting of three treatment groups (90 and 270 mg/Kg CNB, Diclazuril, 5 mg/Kg). The drugs were administered orally for 3 days. Fecal Oocyst Counts (FOC) determined using the modified McMaster technique. The goats were observed for clinical signs on daily basis while body weight was recorded weekly. The level of packed cell volume (PCV), aspartate aminotransferases (AST), alanine aminotransferases (ALT), urea, and creatinine were assessed weekly. At the end of the study, goats were euthanized and gross pathology and histopathology conducted. The results showed that at day 28 post-treatment there was a significantly reduction of FOC of 98.42 and 82.30% for Diclazuril and 270 mg/Kg treatment groups, respectively. The reduction of FOC percentage was significantly higher ($p \leq 0.01$) in Diclazuril group than that in 270 mg/Kg. During the monitoring period, there was no mortality or clinical signs observed in the goats. The PCV, AST, ALT, creatinine and urea were in normal ranges for goats. There were no pathological lesions on the goat organs. In conclusion, CNB had high anticoccidial efficacy and was safe for use in goats. Strategies to improve the efficacy of this potential drug should be further investigated.

Key words: Anticoccidial efficacy, bromelain, chitosan, goat, nanoencapsulation.

INTRODUCTION

Coccidiosis is an economically menacing disease affecting livestock and poultry (Mat Yusof and Md Isa, 2016). In small ruminants, coccidiosis is mainly caused by *Eimeria* species, which develop in both small and the large intestines and mainly affects young animals (Dakpogan et al., 2019). The disease is responsible for considerable losses in husbandry, due to reduced productivity, mortality, associated clinical and sub-clinical

diseases and the cost of treatment and control measures (Etsay et al., 2020; Bawm and Htun, 2021). In Africa, coccidiosis is considered as a leading cause of mortality of young small ruminants and is compounded by the occurrence of other infectious and parasitic diseases such as helminthosis and pneumonia (Kagucia et al., 2020; Etsay et al., 2020). In Africa, prevalence of coccidiosis in goats was reported at 60% in Egypt (Mohamaden et al., 2018), 73% in Nigeria (Ikpeze et al., 2010), 85% in Ethiopia (Etsay et al., 2020) and 45% in Kenya (Maichomo et al., 2004).

Despite the aggressive use of anticoccidial drugs, coccidiosis continues to limit the productivity of livestock, especially that of small ruminants (Waller, 2006). This is due unfortunately to the rapid development of anticoccidials resistance (Hema et al., 2015), the higher cost of the latter and consumer concerns of drug residues in animal products. All these factors have led to a growing interest in alternative products to control coccidiosis. Phytochemicals from different types of botanical elements have been explored as sustainable alternatives to management of coccidiosis (Hady and Zaki, 2012). A wide variety of herbal extracts have been shown to have anti-parasitic activity, while others can enhance the immune system and growth performance, thereby helping the host to overcome coccidiosis infection (Zaman et al., 2015; Debbou-Iouknane et al., 2019). Among the plant extracts, bromelain obtained from pineapples (*Ananas comosus*) has been shown in a previous I*n vitro* study, to have high activity against *Eimeria* spp. isolated from goats (Daiba et al., 2022). In furtherance of the latter study, we assessed the *in vivo* efficacy of chitosan nano-encapsulated bromelain (CNB) against coccidiosis in goats and investigated the possible toxic effects of the extract.

MATERIALS AND METHODS

Ethical approval

The approval for goats' experiments was obtained from the Animal Ethics Committee of University of Nairobi (REF: FVM BAUEC/2020/339). The study followed the design, animal husbandry practices and protocols approved by the committee.

Experimental site

The study was carried out from January to March 2022, at the animal facility located in Jomo Kenyatta University of Agriculture and Technology (JKUAT), Juja, in Kiambu County, Kenya. The University is located at latitude 1°05 S and longitude 37°00 E, and it lies at an altitude of 1525 m above sea level with rainfall bimodal and ranges from 500 to 1,300 mm while average temperature is 19.5°C (Menge et al, 2014).

Extraction and encapsulation of bromelain in chitosan

Bromelain was extracted from the peels of pineapples (*Ananas comosus*) sold at local market in Juja Sub-county, Kenya. The enzyme was extracted using the procedure described previously (Daiba et al., 2022). Briefly, fresh ripe pineapples were ground in a blender in sodium acetate buffer (pH 7.4). The resultant crude extract was precipitated by adding 40% ammonium sulphate. Then, after 24 h of incubation at +4°C, extracted bromelain was purified using dialysis membrane (12 kDa). Encapsulation of bromelain in chitosan (Sigma Aldrich, USA) was done by ionic gelation method. The pellet obtained after encapsulation was frozen at -60°C and dried by placing in the freeze-dryer (MRC, Model FDL-10N-50-BA, Israel). The success of encapsulation of bromelain in chitosan nanoparticles was confirmed by Fourier transform infrared spectrophotometer analysis (FTIR).

Oral solution of chitosan nanoencapsulated bromelain (CNB) formulation

Oral solution of EB was made according the procedure described previously (Niazi, 2009a; Niazi, 2009b). The solvent contained in the excipients included: Tween 20 (surfactant), sucrose (to enhance solubility and stabilize against denaturation of enzymes), Potassium sorbate (antimicrobial preservative), xanthan gum (suspending and viscosity agent) and propylene glycol (humectant and stabilizing agent) (Rowe et al., 2009). Casein enzymatic assays were performed to evaluate the proteolytic activity and specific activity at diverse storage conditions (temperature, exposition to the light and pH) (Barnes, 2014).

Experimental animals

Fifteen (15) Small East African goats, which were naturally infected with coccidia were purchased from farmers in Makima Ward in Embu counties in Kenya. The average age of the goats was 15 months old and weighed between 22 and 31 Kg. Acclimatization was done for two weeks and animals were tagged with ear tags for easy identification before the start of the experiment. The goats were kept in JKUAT' goat house, where they were fed with 1.5 kg of wheat hay thrice per day, 1 Kg of concentrate made up of beet liquid molasses, maize germ, and soybean meal (Aroma Feed Suppliers, Kenya), and supplemented with feed blocks minerals (Aroma Feed Suppliers, Kenya).

Study groups and sampling of animals

The fecal samples were collected using sterile gloves from the rectum of goats and analyzed to determine the number of coccidia oocysts per gram (OPG) of feces using the McMaster method (MAFF, 1986). All the animals used in the experiments had an OPG of more than 10,000. The goats were randomly allocated into three (3) treatment groups. Groups 1 and 2 received 90 and 270 mg/Kg of CNB, respectively, while group 3 acted as the positive control where goats were administered Diclazuril at 5 mg/Kg (Diclazuril®, Sigma-Aldrich). The above dosages were chosen based on the

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results of the bromelain toxicity and efficacy tests obtained in the previous studies (Wasso et al., 2020; Daiba et al., 2022). The treatment was done orally every morning for 3 days and the goats were monitored for 28 days after last day of drug administration.

In vivo assessment of anticoccidial efficacy

Fecal samples were collected from the rectum of goats, once per week, during the 4 weeks of monitoring. The fecal samples were analyzed using a modified McMaster technique to determine the coccidia oocyst counts (FOC) under light microscopy (Optical Element Corporation, Melville, USA) at 100× magnification (Joachim et al 2018). First, the burden of oocyst was evaluated before the treatment (pre-treatment samples at day 0), followed by evaluation of treatment efficacy, by assessing post-treatment fecal samples (days 7, 14, 21 and 28). Fecal Oocyst Count Reduction (FOCR) percentage was evaluated as previously described (Odden et al., 2018).

Assessment of acute toxicity effect

Following dosing, the animals were observed during the first 30 min and then periodically during the first 24 h. Special attention was given during the first 4 h, and daily thereafter, for a total of 28 days to observe any changes in general behavior and other physiological activities (OECD Guidelines, 2002; Parasuraman, 2011). Rectal temperature of goats was measured daily, each morning (8 h 30-9 h 30 am) using a digital thermometer (Kruuse Digital Thermometer; Jorgen Kruuse). The body weight of animals was recorded weekly. Two (2 mL) of blood was sampled weekly from each animal, from the jugular vein into blood collection tube 4 mL EDTA. The PCV was determined using the micro-hematocrit method (Shamaki et al., 2017). Afterward, blood samples were centrifuged for 14,000 rpm for 10 min to obtain the plasma. The plasma samples obtained were used to determine the levels of Aspartate aminotransferases (AST), alanine aminotransferases (ALT), urea, and creatinine using standard diagnostic test kits on Automated Clinical Biochemistry analyzer (Reflotron Plus System®, model: Cobas 4800 Detection Analyzer; India) (Emma et al., 2020; Wasso et al., 2020).

At 28th day, post last day of drug administration, the goats were euthanized and gross pathology conducted according the procedure described previously (King et al., 2013). Sections of liver, kidney, spleen and heart were collected and preserved in 10% buffered formalin for 24 h before being processed for histology as described previously (Rousselle et al., 2019).

Statistical analysis

The collected data were entered into and analyzed using Graph Pad Prism 8.4.3. for data analysis. Descriptive statistics (means and standard deviations) were determined before conducting other statistical tests. The FOCR, PCV, weight, temperature and biochemical parameters of different groups were compared using Students t-test, with $p < 0.05$ indicating statistical significance.

RESULTS

Bromelain oral solution pH and activity

The oral solution of CNB was preserved for 2 weeks at various temperatures. The protease activities of oral solution of nano-encapsulated bromelain were 0.1057±0.010 and 0.0839±0.009 U/mL, respectively for drug preserved at +4°C and room temperature (25°C). The activity of CNB stored at +4°C was significant higher ($p < 0.002$) compared to that kept under room temperature. The pH of the oral solution of CNB ranged from 4.68±0.36 to 4.61±0.4 and, there were no significant differences ($p > 0.05$) between pH of CNB oral solution kept at +4°C and that kept at room temperature (25°C).

In vivo assessment of efficacy

At day 0, the mean OPG in Groups 1, 2 and 3 goats were 11,600, 12,000 and 12,075, respectively. Following treatment, the FOCR% at day 7 post treatments were 82.12, 72.69 and 49.93% for goats treated with Diclazuril, 270 mg/Kg, 90 mg/Kg CNB, respectively. At the day 28 post treatment, FOCR% were 98.42, 82.30 and 53.16% for Diclazuril, 270 mg/Kg, 90 mg/Kg CNB treatment groups, respectively. The reduction of oocyst was significantly higher ($p \leq 0.018$) for the goats treated with 270 mg/Kg than those treated with 90 mg/Kg. Further, there was a significant difference ($p < 0.01$) in FOCR% of the positive control group compared to that of 270 and 90 mg/Kg treatment groups (Table 1).

Toxicity assessment of oral solution of nanoencapsulated bromelain

Clinical observations

During the 28 days monitoring period, there were no mortalities or clinical signs reported in all treatment groups. The rectal temperature of animal body varied between 38.38 and 39.00°C and was thus within normal range for small East African goat. Following the treatment, there were no significant differences in body temperatures ($p > 0.05$) in goats from different groups. The body weights of the goats before treatment were 26.58±2.57, 27.00±1.41 and 26.75±3.40 Kg for Diclazuril, 270 mg/Kg and 90 mg/kg CNB groups, respectively. After three weeks since treatment, the weight had increased to 26.89±1.91, 28.00±1.63 and 28.00±3.56 Kg for Diclazuril, 270 mg/Kg and 90 mg/Kg CNB groups, respectively. The mean of body weights following treatments showed an increase ranging between 1 and 1.25 Kg at 28 days post treatment and the increase (compared to day 0) was statistically significant ($p < 0.05$).

Effect of treatments on PCV and biochemical parameters

The PCV levels, before treatment, were 31.23, 29.75 and 30.75% for Diclazuril, 270 mg/ Kg and 90 mg/ Kg CNB groups, respectively. During the treatment, the PCV

Table 1. Fecal oocyst count reduction from goats treated with chitosan nanoencapsulated bromelain (CNB) and Diclazuril.

Treatment group	Day 7	Day 14	Day 21	Day 28
270 mg/kg	72.69±3.82[a]	77.50±2.82[a]	81.57±3.58[a]	82.30±2.18 [a]
90 mg/kg	49.43±7.01[b]	54.08±8.07[b]	54.11±7.89[b]	53.13±7.65[b]
Positive control	82.19±4.16[c]	95.71±3.34[c]	98.49±1.08[c]	97.61±1.31[c]

For the same column, values carrying the same superscript letter are not significantly different at $p \geq 0.05$ (t-test).
Source: Author

Table 2. Effect of oral solution of nanoencapsulated bromelain and Diclazuril on biochemical parameters of goats.

Group		Day 0	Day 7	Day 14	Day 21	Day 28
AST (U/L)	270 mg/kg	133.8±26.92	134.3±21.54	134.3±12.5	132.3±10.29	132.8±9.17
	90 mg/kg	121.0±16.63	122.5±17.79	121.3±5.37	123.3±13.6	122.0±8.46
	Diclazuril	120.2±17.37	117.2±14.23	119.8±10.60	115.8±20.58	115.1±16.65
ALT (U/L)	270 mg/kg	18.55±3.97	18.8±5.18	19.06±4.43	18.36±4.19	18.50±6.67
	90 mg/kg	16.73±5.29	16.90±4.41	16.58±5.01	16.61±5.33	16.63±2.43
	Diclazuril	16.58±2.86	17.40±4.82	17.54±2.30	16.68±1.11	16.52±2.40
Creatinine (mg/L)	270 mg/kg	0.842±0.25	0.842±0.28	0.953±0.18	0.841±0.134	0.852±0.15
	90 mg/kg	0.764±0.17	0.829±0.06	0.743±0.14	0.774±0.20	0.742±0.13
	Diclazuril	0.844±0.29	0.837±0.33	0.819±0.21	0.831±0.20	0.809±0.10
Urea (mg/L)	270 mg/kg	37.15±9.69	38.00±8±93	37.80±7±45	37.30±5.77	37.38±4.46
	90 mg/kg	38.88±7.29	38.18±6.44	38.60±7.82	38.65±6.32	38.40±5.94
	Diclazuril	37.56±6.22	37.26±6.83	37.59±6.39	37.23±5.20	37.69±5.47

Source: Authors

levels ranged between 28 to 31% for 270 mg/Kg group and 27 to 32% for 90 mg/ Kg CNB group and for positive control, 28.79 to 30.24%. There were no significant differences ($P > 0.05$) between the treatment groups.

The mean of biochemical parameters of AST, ALT, creatinine and Urea for each of the dosed groups were in the normal ranges (Jackson and Cockcroft, 2002) (Table 2). During the treatment, AST and ALT ranged between 121 to 134 U/L and 16.58 to 19.06 U/L, respectively for CNB treatment groups and 115 to 120 U/L and 16.52 to 17.54 U/L for positive control group of animals. The creatinine level was 0.742 to 0.953 mg/L and urea was 37.15 to 38.88 mg/L for CNB treatment groups. There were no significant differences ($p > 0.05$) in levels of AST, ALT, creatinine and urea in different days and between treatment groups.

Necropsy findings

There were no gross changes at necropsy. All the organs were normal and similar in both treatment groups to control groups. There were no histological lesions observed in the organs in both the treated and control groups (Figure 1).

DISCUSSION

The current study is a follow-up of the previous study which had assessed the *in vitro* activity of nano-encapsulated bromelain (CNB) on oocysts of *Eimeria* spp. isolated from goats in Kenya (Daiba et al., 2022). The previous study reported high anticoccidial activity of CNB comparable to that of commercial drug (Diclazuril). In the current study, CNB was been administered as an oral solution made by adding other excipients in order to increase its activity in the animal's body (Rowe et al., 2009). The current study showed that, the proteolytic activity of oral solution of CNB was higher than crude encapsulated bromelain reported in previous studies (Hunduza et al., 2020; Wasso et al., 2020; Daiba et al., 2022). This high activity of the oral solution of CNB could be due to the lower pH (pH = 4.7 to 5) which would contribute to accelerate the release of bromelain from chitosan nanocarriers during casein protease assay. As

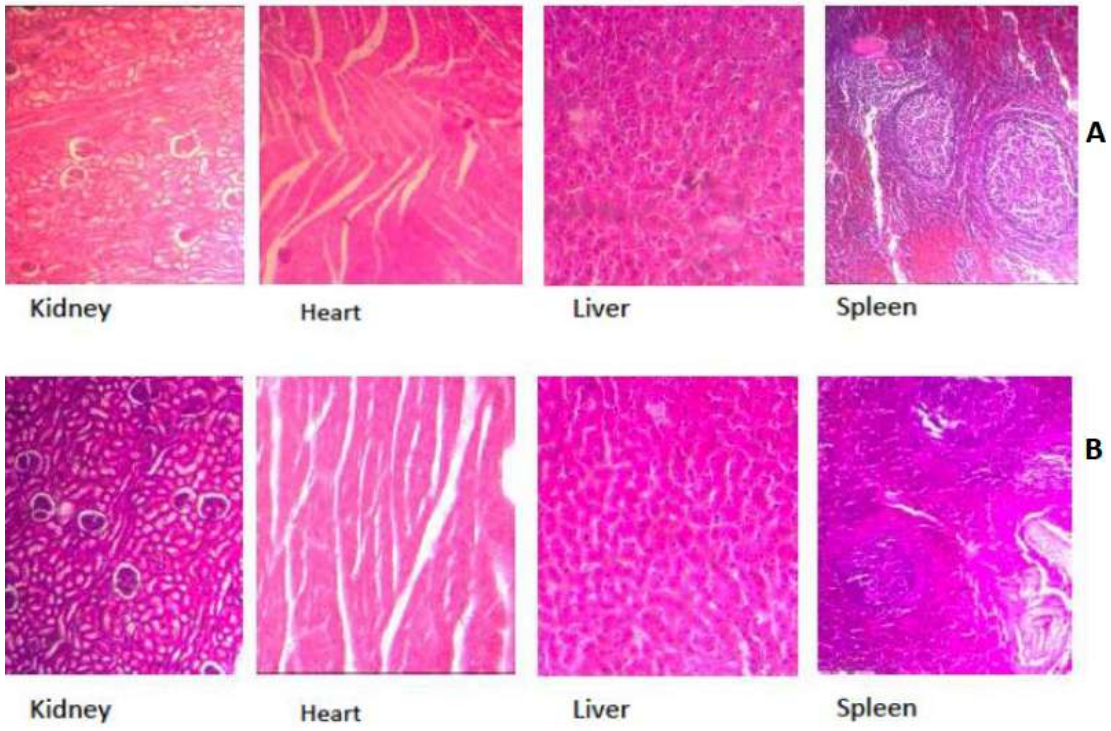

Figure 1. Histology observation of kidney, heart, liver and spleen of goats administered oral solution of CNB. A. Control group, showing no changes; and B. Treatment group, showing no changes (under the microscope X100 magnification).
Source: Authors

described by Hunduza (2018), acidic conditions chitosan nanocarriers released more bromelain into solution than at neutral pH, and thus had a higher proteolytic activity. Further, the oral solution prepared using Tween 20, sucrose, potassium sorbate, xanthan gum and propylene glycol could have enhanced the stability of CNB in the gut of the goat making it more effective against the coccidial parasites. Previous studies have used water as the solvent (Wasso et al., 2020).

The results of the present study showed that the administration of oral solution of CNB for three days, significantly reduced the excretion of coccidia oocysts during the monitoring period. Our findings are in concordance with experiments using cysteine proteinase from others plants, which show that the enzyme having an efficacy against coccidian infections (Abdel-Tawab et al., 2020; Dakpogan et al., 2019; Juasook et al., 2017; Molan et al., 2009). Juasook et al. (2017) treated *Eimeria tenella* infected broilers with pineapple (*Ananas comosus*) crude extracts, and observed that the oocyst output decreased significantly and also inhibited sporulation. In their experiment, Dakpogan et al. (2019) reported a reduction of 59% of oocyst per gram (OPG) in chicks treated with *Carica papaya* crude extracts. On the other hand, Abdel-Tawab et al. (2020) showed that *Astragalus membranaceus* extracts reduced oocyst output and sporulation of *Eimeria papillata* infection in mice by 57%. As reported in a recent study *in vitro* efficacy (Daiba et al., 2022), the activity of CNB was due to the damage it causes to coccidia oocysts. The drug causes degradation of the coccidia shell wall, softening and destruction of the central cytoplasmic mass (Daiba et al., 2022, Juasook et al., 2017).

The present study also evaluated the toxicity effect of CNB on goats. As reported in a recent study evaluating the acute toxicity (Wasso et al., 2020), during the present study, there were no mortalities and clinical signs in the experimental goats. On the other hand, a slight weight gain of the treated animals and this could show that the drug enhanced the growth performance of the goats.

During the current study, the levels of the assessed biochemical parameters were normal and were similar to the results reported by Wasso et al. (2020) where the goats were treated with EB at a dose of 30 mg/kg for 14 days, and in a single dose of 90 and 270 mg/kg. Likewise, this was confirmed by the absence of macroscopic and microscopic changes in the examined organs (Moss et al., 1963; Pavan et al., 2012). Moss et al. (1963) in rats have shown that bromelain when administered at 500 and 1500 mg/Kg/day was not toxic. Further, Pavan et al. (2012) did not observe toxic effects after 6 months administrating bromelain at 750 mg/Kg/day

to dogs. All these results show that the drug is quite safe for use in different animals.

Conclusion

The current study reported high efficacy of CNB against coccidial infections in goats. Further the oral solution of CNB had no toxic effects at 270 mg/Kg on goats. Accordingly, this formulation of bromelain can contribute to management of coccidiosis in small ruminants. Further studies should be carried out to determine dosing regimens which can increase the efficacy of CNB against coccidiosis to 100%.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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In vitro anthelmintic activities of stem and root barks extracts of *Parkia biglobosa* on infective larvae and adult of *Haemonchus contortus*

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The treatment of gastrointestinal nematode infections in the 21st century is largely through the use of modern synthetic anthelmintics. However, there is over growing drug resistance to anthelmintics in treatment of gastrointestinal nematode infections in goats and sheep. This present study was carried out to determine the in vitro anthelmintic activitities of Parkia biglobosa using Larval motility inhibition assay (LMIA) and Adult motility inhibition assay (AMIA). The stem and root barks of P. biglobosa were extracted which yielded four different extracts as Crude Aqueous Stem Bark Extract (CASBE), Crude Methanol Stem Bark Extract (CMSBE), Crude Aqueous Root Bark Extract (CARBE) and Crude Methanol Root Bark Extract (CMRBE). The infective larvae and adults of Haemonchus contortus were exposed to different concentrations each (2, 4, 8. 16 and 32 mg/ml) of plant extracts of both stem and root barks of P. biglobosa in comparable to controls (Albendazole (ABZ)-positive control and Phosphate Buffered Saline (PBS)- negative control). The result of LMIA showed that at 12 hours exposure of larvae to 32 mg/ml for four extracts, 55-75% mortality were recorded while the result for AMIA revealed that at 12 hours post exposure of all the plant extracts ranged from 2-32 mg/ml concentrations, 100% mortality of the adult worms were recorded. There was no mortality recorded in negative control (PBS) even up to 12 hours post exposure. From this result, it could be concluded that plant extracts have anthelmintic activities in comparison to ABZ with CMSBE ranked highest among the extracts. However, the potency of plant extracts was dependent on the time of exposure and concentration of the extracts as well as the solvent used.

Key words: Adulticidal, Anthelmintic, Drug resistance, Extracts, in vitro, Larvicidal, Parkia biglobosa

INTRODUCTION

Among the helminth parasites that infect small ruminants, *Haemonchus contortus* is said to be considered the most devastating and prevalent species (Dey et al., 2015). This has led to a major constraint in the production and profitability of small ruminant in Nigeria and African at large (Chiejina, 2001). The devastating and debilitating nature of *H. contortus* is as a result of disease called haemonchosis, it is considered to be the major culprit

responsible for hypoproteinemia and anaemia in ruminant animals with heavier worm burdens. However, clinical signs such as weight loss, diarrhoea, anaemia, or sub-mandibular oedema (bottle jaw) may develop (Sissay, 2007).

It has been estimated that a single worm of *H. contortus* sucks about 0.05 ml of blood per day by seepage or ingestion from the lesions (Urquhart et al., 2000). Studies conducted in many countries around the world indicated that among the domesticated animals, goats and sheep suffer mostly from haemonchosis (Nwosu et al., 2007; Tariq et al., 2008). It is important to note that, the infection caused by *H. contortus* ranks highest in global index and capable of causing acute disease and high mortality in all classes of livestock. Death rate due to acute haemonchosis is very high and may go up to 50% in small ruminants (Itty et al., 1997; Perry et al., 2002; Tariq et al., 2010).

Therefore, to minimize the infection caused by *H. contortus* to small ruminants in order to increase their production for protein and economic gain, there is need to develop sustainable control strategies that will reduce or cure the helminthic infections. The management control of nematodes in livestock is basically through systemic synthetic anthelmintics. However, treatments with conventional drugs have their own disadvantages which range from the development of drugs resistance by these parasites, unaffordability of these drugs to low-income farmers as well as accumulation of drug residue in food chain and the environment (Zajac and Gipson, 2000; Vaele, 2002; Schoenian, 2005; Athanasiadou et al., 2008; Sawleha et al., 2010). Thus, alternative methods for controlling helminth infection need to be developed. One of such alternatives is through the knowledge of ethno veterinary medicines which are available for the treatment of internal parasites but are often neglected in favour of the conventional drugs (Hammond et al., 1997).

The knowledge of medicinal plants and their collective roles in promoting health is increasing. One of these medicinal plants is *Parkia biglobosa* which have revealed several phytochemical constituents that have the potential of treating several diseases (Soetan and Aiyelaagbe, 2009). However, to ascertain this claim, further investigation needs to be conducted before proceeding to invivo test (Asase et al., 2005).

The screening of anthelmintic activity is mainly through *in vitro* tests including larval and adult paralysis/death, egg hatch assays and biochemical tests (Bachaya et al., 2009). *In vitro* tests using the infective larvae of *H. contortus* is considered to be one of the best means of screening drugs for anthelmintic activity before proceeding to *in vivo* test (Asase et al., 2005). Therefore, the present study was carried out to investigate the comparative effect of crude methanolic extracts of stem and root bark of *P. biglobosa* on infective larvae and adult stages of *H. contortus*.

MATERIALS AND METHODS

Source of plant materials and authentication

The fresh stem and root barks of *P. biglobosa* were collected in the month of March 2016 in Tsaragi district of Edu Local Government Area of Kwara State, Nigeria (Figure1). The plant samples were identified and authenticated by a plant taxonomist Mr Namadi Sanusi of the Department of Botany, Ahmadu Bello University Zaria, Kaduna State, Nigeria. The plant samples were collectively given a voucher number ABU/7064 which was deposited in the herbarium for reference purposes.

Preparation of plant extracts

The fresh stem and root barks of *P. biglobosa* were separately washed with water and air dried in the shade at room temperature for one month and thereafter crushed with a mortar and pestle or blender into powder form. These were stored in air tight container for later use as described by Soetan et al. (2011) and Meraiyebu et al. (2013).

Methods of aqueous and methanol extraction

The method of Soetan et al. (2011) was used for aqueous extraction. 200 g of the milled stem bark of *P. biglobosa* was weighed using a sensitive weighing balance ranging from 0.01 to 500 g with Model No. SHP1100313194 2011-07 and poured inside a bowl with cover. Then 2 L of distilled water was added and stirred immediately. Stirring was done every 30 min and after 24 h, the supernatant was first filtered with muslin cloth and later through Whatman filter paper No 1. The filtrate was evaporated using the water bath at 65°C for 6 h. The weight by weight (w/w) yield of the aqueous extract was stored in a capped bottle and preserved inside the refrigerator at 4°C. The same procedures were done for the root bark of *P. biglobosa*.

For the methanol extraction, the stem and root barks of *P. biglobosa* were extracted separately using Soxhlet's apparatus. 100 g of each extract was extracted with 600 ml of methanol for 4 h until all the required grams of extracts were exhausted as described by Asuzu and Onu (1994) and Builders et al. (2012). All the filtrate was evaporated using water bath at 65°C and the w/w yield of the extract was stored in an airtight container at 4°C until use.

Yield of percentage determination of aqueous and methanol extracts

The determination of each of percentage yield of aqueous and methanol extract was calculated using the formula of Anokwuru et

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Figure 1. Sample of *Parkia biglobosa* tree for sourcing of stem bark and root bark.
Source: Author

al. (2011) and Ezekwe et al. (2013) as follows:

$$\% \text{ Yield} = \frac{\text{Weight of each extract}}{\text{Weight of pulverized of each part of } Parkia\ biglobosa} X100$$

Qualitative phytochemical screening of aqueous and methanol extracts

The phytochemical screening of the extracts was carried out to identify the constituents using standard phytochemical methods. The screening was carried out on each of crude aqueous and methanol extracts of stem and root bark extracts of *P. biglobosa* to determine the possible presence of alkaloids, flavonoids, saponins, tannins, terpenoids, anthraquinones, glycosides, cardiac glycoside/ cardenolides, phlobatannins, sterols and steroids, carbohydrates, starch, proteins, and oils (Sofowora, 1993; Evans, 2002).

Sourcing of infective larvae and adult stage of *H. contortus* for *in vitro* studies

Adult worms of *H. contortus* were obtained from the abomasums of slaughtered goats purchased in Zaria abattoir Kaduna State, Nigeria. These abomasums were transported on ice block container to Parasitilogy laboratory in the Department of Parasitology and Entomology, Ahmadu Bello University (ABU) Zaria, Kaduna State, Nigeria. The worms were recovered using the method of Hansen and Perry (1994). These worms were later washed in distilled water and then suspended in phosphate buffered saline (PBS) made by dissolving 0.85 g of sodium chloride (Nacl) and 1 g glucose in 1 L distilled water and allowed for 2 h to acclimatize (Kareru et al., 2012). The adult worms processed were divided into two portions for adult motility inhibition assay (AMIA) and larval motility inhibition assay (LMIA). For the latter portion, the female worms were separated from male worms and the female worms were crushed in a mortar and pestle to liberate the eggs. The eggs were recovered from suspension by the method described by Coles et al. (1992). These eggs were cultured at room temperature in damp heat-sterilized bovine faeces for 7 days to provide development using the method of Makun et al. (2008) and Dey et al. (2015). After 7 days, the culture was baermannized to harvest the L_3 stages and stored in distilled water at 4°C in the laboratory.

Larval motility inhibition assay (LMIA)

For larval motility inhibition assay, a total of 20 ml of L_3 larvae (infective larvae) suspension in water were gotten and 0.1 ml was taken on microscope slide and counted. Approximately 20 L_3 were counted in 0.1 ml. Then, 0.1 ml suspension containing approximately 20 L_3 were pipetted into 96- flat-bottomed microtitre plate and mixed with the same volume of different concentrations in triplicate as follows:

(1) For plant extracts: 2, 4, 8, 16 and 32 mg/ml
(2) For albendazole (the positive control wells): 2, 4, 8, 16 and 32 mg/ml.
(3) Negative control plates received only PBS.

The motility was recorded after 0, 1, 3, 6, 9 and 12 h intervals under microscope. The nonmotile (dead) L_3 was identified and the percentage calculated (Dey et al., 2015).

Adult motility inhibition assay (AMIA)

Adult motility assay was conducted on mature live *H. contortus* following the methods of Iqbal et al. (2006), Muhammad et al. (2011) and Zaman et al. (2012). Ten worms were exposed in triplicate at each of the following treatment in separate Petri-dishes at room temperature (25 to 30°C):

(1) Plant extracts: 2, 4, 8, 16, and 32 mg/ml
(2) Albendazole: 2, 4, 8, 16, and 32 mg/ml
(3) Control (PBS)

The inhibition of motility and/or mortality of the worms were subjected to the aforementioned treatments and were used as the criteria for anthelmintic activity. The motility was recorded after 0, 1, 3, 6, 9 and 12 h intervals. Finally, the treated worms were kept for 30 min in the lukewarm fresh PBS to observe the revival of motility. The numbers of live and dead worms were recorded in all the Petri-dishes.

Data analysis

The data gotten were presented in tables and charts. The percentage yields of all the extracts were calculated as well as percentages of larva mortality and adult mortality of *H. contortus*. For larval motility inhibition and adult motility inhibition assays, probit transformation was performed to transform a typical sigmoid dose response curve to linear function (Hubert and Kerboeuf, 1992). The linear regression (for y = 0 on the probit scale) using Microsoft Excel Widow 2007 were used to calculate the extract concentration required to prevent 50%, that is, lethal concentration (LC_{50}) of adult and larval from motility.

RESULTS

The percentage yielded for four different extracts are

Table 1. Percentage yield of aqueous and methanol extracts from pulverized form of stem bark and root bark of *Parkia biglobosa.*

Extracts	Initial weight of pulverized (g)	Final weight of the extracts (g)	W/W yield (%)
CASBE	200	31.1	15.6
CMSBE	200	50.29	25.15
CARBE	200	32.47	16.26
CMRBE	200	27.98	13.9

CASBE: Crude aqueous stem bark extract, CMSBE: crude methanol stem bark extract, CARBE: crude aqueous root bark extract, CMRBE: crude methanol root bark extract.
Source: Authors

Table 2. Qualitative phytochemical screening of stem and root bark extracts of *P. biglobosa.*

Constituents	Test methods	CASBE	CMSBE	CARBE	CMRBE
Alkaloids	Mayer's test	+	+++	-	-
Anthraquinones	Bontrager's test	++	+++	++	+
Cardiac Glycosides	Keller-Kiliani test	+	+	+	+
Flavonoids	NaoH test	-	++	-	+
Glycosides	Benedict's test,	+	+++	-	-
Oil	Filter paper test	+	++	-	-
Protein	Millon reagent test	-	-	+	-
Phlobatannins	Hcl test	+++	++	-	+++
Reducing Sugar	Fehling test	+	++	-	+
Saponins	Frothing test	+	+++	++	++
Starch	Iodine test	+	-	-	-
Sterols and Steroids	Conc H_2So_4 test	-	++	-	+
Tannin (Condensed)	Ferric chloride test	+++	+++	+++	++
Tannin (Hydrolysable)	Ferric chloride test	++	++	+	+
Terpenoid	Salkowski test	-	++	-	+
Triterpenoids	Salkowski test	-	-	-	-

- Absent, + present, ++ very present, +++ much present.
Source: Authors

shown in Table 1. The Crude Methanol Stem Bark Extract (CMSBE) yielded the highest percentage while the least percentage was Crude Methanol Root Bark Extract (CMRBE). The qualitative phytochemical constituents of aqueous and methanol extracts of stem and root bark of *P. biglobosa* are shown in Table 2. All the extracts tested were positive with the presence of at least ten phytochemical constituents with various degrees. More phytochemical constituents were present in CMSBE. The anthelmintic activity present in the extracts might be due to the presence of these compounds.

The percentage mortality of larvae (L_3) of *H. contortus* when exposed to ABZ and different concentrations of CASBE, CMSBE, CARBE and CMRBE of *P. biglobosa* at different hours are shown in Table 3. There was no larvae (L_3) mortality after 1 to 3 h exposure to ABZ at different concentrations (2, 4, 8, 16 and 32 mg/ml) and CASBE, CMSBE, CARBE and CMRBE of *P. biglobosa*, but at 6 h exposure, 50, 50, 43 and 25% mortality of larvae were recorded for ABZ, CASBE, CMSBE and CMRBE at 32 mg/ml. At 12 h exposure, 88% mortality was recorded when L_3 larvae were exposed to 32 mg/ml concentration of ABZ. Similarly, mortality of 55, 87, 63 and 75% were also recorded when L_3 larvae were exposed to 32 mg/ml of CASBE, CMSBE, CARBE and CMRBE, respectively at 12 h. No mortality of the larvae was recorded in PBS up to 12 h post exposure.

The LC_{50} was determined graphically from the regression equation at different hours of exposure using the probit analysis. The values of $LC_{50,}$ coefficient of determination (R^2) and regression equation of the effect of CASBE, CMSBE, CARBE and CMRBE of *P. biglobosa* as well as standard drug (ABZ) on larvae (L_3) mortality are shown in Tables 4, 5 and 6. At 6 h exposure, the value of LC_{50} for ABZ, CMSBE and CMRBE was 25.70, 144.54 and 58.88 mg/ml, respectively. The regression and coefficient of determination (correlation of regression

Table 3. Percentage mortality of larvae (L_3) of *H. contortus* exposed to stem and root bark extracts of *P. biglobosa* in comparison with albendazole.

Treatment (mg/ml)	% Mortality of L_3 at different hours					
	0 h	1 h	3 h	6 h	9 h	12 h
	Phosphate Buffer Saline (PBS)					
	0	0	0	0	0	0
Albendazole (ABZ)						
2	0	0	0	0	0	10
4	0	0	0	0	0	25
8	0	0	0	10	23	52
16	0	0	0	13	53	68
32	0	0	0	50	68	88
Crude Aqueous Stem Bark Extract (CASBE) of *P. biglobosa*						
2	0	0	0	0	0	3
4	0	0	0	0	0	12
8	0	0	0	10	8	40
16	0	0	0	13	38	50
32	0	0	0	50	50	55
Crude Methanol Stem Bark Extract (CMSBE) of *P. biglobosa*						
2	0	0	0	0	0	8
4	0	0	0	0	10	33
8	0	0	0	0	35	53
16	0	0	0	0	68	73
32	0	0	0	43	70	87
Crude Aqueous Root Bark Extract (CARBE) of *P. biglobosa*						
2	0	0	0	0	0	0
4	0	0	0	0	5	10
8	0	0	0	0	25	30
16	0	0	0	0	42	52
32	0	0	0	0	53	63
Crude Methanol Roots Bark Extract (CMRBE) of *P biglobosa*						
2	0	0	0	0	0	5
4	0	0	0	0	5	10
8	0	0	0	0	17	23
16	0	0	0	5	55	60
32	0	0	0	25	70	75

Each treatment group had three replicates having 20 L_3 larvae each.
Source: Authors

"R^2") were Y= 4.716x - 1.658, R^2 = 0.871; Y= 3.213x 1.934, R^2 = 0.506; and Y= 3.984x - 2.055, R^2 =0.795, respectively while the LC_{50} and R^2 were not recorded for CASBE and CARBE (Table 4). At 9 h, the lowest concentration that resulted to 50% mortality of larvae of *H. contortus* was 14.79 mg/ml of CMSBE and the highest concentration was 23.99 mg/ml of CASBE (Table 6). The CMSBE resulted to 50% mortality of L_3 of with 14.79 mg/ml concentration while the standard drug (ABZ) resulted to 50% mortality at concentration of 19.50 mg/ml. At 12 h exposure to PBS, ABZ and different extracts shown in Table 6. The lowest concentration of extracts that resulted to 50% mortality was CMSBE at 7.94 mg/ml which was even lower than the standard drug (ABZ) which was 8.51 mg/ml for 50% mortality. While the highest concentration was CARBE at 16.98 mg/ml for 50% mortality. The ranking of potency of CASBE, CMSBE, CARBE and CMRBE of *P. biglobosa* and standard drug (ABZ) based on their LC_{50} and dose dependant effect (R^2) at different hours of exposure are shown in Table 7. It was evident from the result that the effect of CMSBE was prominent and was ranked first

Table 4. $LC_{50,}$ coefficient of determination (R^2) and regression equation of the effect of stem and root bark extracts of *P. biglobosa* as well as standard drug (ABZ) on larvae (L_3) *H. contortus* motility and/or mortality at 6 h.

Treatments (mg/ml)	LC_{50} (mg/ml)	Coefficient of Determination (R^2)	Regression Equation
PBS (-Ve Control)	-	-	-
ABZ (+Ve Control)	25.70	0.871	Y= 4.716x - 1.658
CASBE	-	-	-
CMSBE	144.54	0.506	Y= 3.213x - 1.934
CARBE	-	-	-
CMRBE	58.88	0.795	Y= 3.984x - 2.055

PBS: Phosphate buffer saline; ABZ: albendazole; CASBE: crude aqueous stem bark extract; CMSBE: crude methanol stem bark extract; CARBE: crude aqueous root bark extract; CMRBE: Crude methanol root bark extract.
Source: Authors

Table 5. $LC_{50,}$ coefficient of determination (R^2) and regression equation of the effect of stem and root bark extracts of *P. biglobosa* as well as standard drug (ABZ) on larvae (L_3) *H. contortus* motility and/or mortality at 9 h.

Treatments (mg/ml)	LC_{50} (mg/mL)	Coefficient of Determination (R^2)	Regression Equation
PBS (-Ve Control)	-	-	-
ABZ (+Ve Control)	19.50	0.852	Y= 5.296x - 1.815
CASBE	23.99	0.874	Y= 4.857x - 1.725
CMSBE	14.79	0.781	Y=4.225x + 0.052
CARBE	19.50	0.781	Y = 3.832x + 0.057
CMRBE	16.98	0.849	Y= 4.234x - 0.207

Source: Authors

Table 6. $LC_{50,}$ coefficient of determination (R^2) and regression equation of the effect of stem and root bark extracts of *P. biglobosa* as well as standard drug (ABZ) on larvae (L_3) *H. contortus* motility and/or mortality at 12 h.

Treatments (mg/ml)	LC_{50} (mg/ml)	Coefficient of Determination (R^2)	Regression Equation
PBS (-Ve Control)	-	-	-
ABZ (+Ve Control)	8.51	0.995	Y= 2.006x + 3.139
CASBE	18.62	0.903	Y= 1.718x + 2.813
CMSBE	7.94	0.981	Y= 2.062x + 3.144
CARBE	16.98	0.762	Y= 3.961x + 0.142
CMRBE	14.79	0.973	Y= 2.036x + 2.615

Source: Authors

Table 7. Ranking of stem and root bark extracts and standard drug (ABZ) based on LC_{50} values and coefficient of determination on larvae (L_3) *H. contortus* motility and/or mortality.

Treatments	Ranking of potency based on LC_{50}			Ranking of potency based on dose dependent effect (R^2 - values)		
Duration of exposure	**6 h**	**9 h**	**12 h**	**6 h**	**9 h**	**12 h**
ABZ (Control)	01	03	02	01	02	01
CASBE	-	04	05	-	01	04
CMSBE	03	01	01	03	04	02
CARBE	-	03	04	-	04	05
CMRBE	02	02	03	02	03	03

Source: Authors

Table 8. Percentage mortality of adult *H. contortus* exposed to stem and root bark extracts of *P. biglobosa* in comparison with albendazole (ABZ).

Treatment (mg/ml)	% number of dead worms at different hours					
	0 h	1 h	3 h	6 h	9 h	12 h
Phosphate Buffer Saline (PBS)						
	0	0	0	0	0	0
Albendazole (ABZ)						
2	0	0	50	60	100	100
4	0	0	60	80	100	100
8	0	0	63	83	100	100
16	0	0	100	100	100	100
32	0	0	100	100	100	100
Crude Aqueous Stem Bark Extract (CASBE) of *P. biglobosa*						
2	0	0	0	0	3	100
4	0	0	57	67	73	100
8	0	0	80	90	90	100
16	0	0	100	100	100	100
32	0	0	100	100	100	100
Crude Methanol Stem Bark Extract (CMSBE) of *P. biglobosa*						
2	0	0	43	50	100	100
4	0	0	50	80	100	100
8	0	0	77	83	100	100
16	0	0	80	100	100	100
32	0	0	83	100	100	100
Crude Aqueous Root Bark Extract (CARBE) of *P. biglobosa*						
2	0	0	0	0	100	100
4	0	0	40	50	100	100
8	0	0	50	57	100	100
16	0	0	57	93	100	100
32	0	0	87	100	100	100
Crude Methanol Root Bark Extract (CMRBE) of *P. biglobosa*						
2	0	0	0	20	100	100
4	0	0	0	30	100	100
8	0	0	20	53	100	100
16	0	0	40	80	100	100
32	0	0	70	100	100	100

Source: Authors

based on the LC_{50}, followed by CMRBE and lastly ABZ. At 12 h, the top most effective extract/treatment based on LC_{50} was CMSBE, followed by ABZ and CMRBE, CARBE, and CASBE, respectively in decreasing order while based on dose dependant effect were ABZ, CMSBE, CMRBE, CASBE and CARBE, respectively in decreasing order.

Table 8 shows the percentage mortality of adult *H. contortus* exposed to different concentrations of CASBE, CMSBE, CARBE and CMRBE of *P. biglobosa* in comparison to the standard drug (ABZ) at different hours. There was no mortality when adult worms were exposed to different concentrations of the extracts for 2 h including the positive and negative controls. The onset killing of 50% of the worm at 3 h began with ABZ at 2 mg/ml followed by CMSBE which killed 43% of the adult worms at the same concentration with that of ABZ. But at 4 mg/ml concentration of CMSBE, 50% of adult of *H. contortus* were killed. At 12 h post exposure of all the plant extracts and ABZ ranged from 2 to 32 mg/ml

Table 9. $LC_{50,}$ coefficient of determination (R^2) and regression equation of the effect of stem and root bark extracts of *P. biglobosa* as well as standard drug (ABZ) on adult *H. contortus* motility and/or mortality at 3 h.

Treatment (mg/mL)	LC_{50} (mg/mL)	Coefficient of Determination (R^2)	Regression Equation
PBS (-Ve Control)	-	-	-
ABZ (+Ve Control)	2.69	0.818	Y=2.270x + 4.015
CASBE	10.47	0.629	Y= 4.224x + 0.697
CMSBE	2.24	0.900	Y= 0.942x + 4.670
CARBE	12.30	0.689	Y= 4.195x + 0.428
CMRBE	20.41	0.867	Y= 5.222x - 1.824

Source: Authors

Table 10. $LC_{50,}$ coefficient of determination (R^2) and regression equation of the effect of stem and root bark extracts of *P. biglobosa* as well as standard drug (ABZ) on adult *H. contortus* motility and/or mortality at 6 h.

Treatment (mg/mL)	LC_{50} (mg/mL)	Coefficient of Determination (R^2)	Regression Equation
PBS (-Ve Control)	-	-	-
ABZ (+Ve Control)	1.45	0.888	Y= 1.856x + 4.697
CASBE	7.41	0.749	Y= 5.506x + 0.236
CMSBE	1.86	0.909	Y=2.074x 4.435
CARBE	8.71	0.803	Y= 5.363x - 0.031
CMRBE	5.62	0.926	Y= 2.580x +3.058

Source: Authors

Table 11. $LC_{50,}$ coefficient of determination (R^2) and regression equation of the effect of stem and root bark extracts of *P. biglobosa* as well as standard drug (ABZ) on adult *H. contortus* motility and/or mortality at 9 h.

Treatments (mg/mL)	LC_{50} (mg/mL)	Coefficient of Determination (R^2)	Regression Equation
PBS(-Ve Control)	-	-	-
ABZ (+Ve Control)	-	1E-1	Y=7.37
CASBE	4.17	0.854	Y=3.390x + 2.892
CMSBE	-	1E-1	Y= 7.37
CARBE	"	"	"
CMRBE	"	"	"

Source: Authors

concentration, 100% mortality of the adult worms were recorded while no mortality was recorded in negative control (PBS) even up to 12 h post exposure.

The LC_{50} and correlation of regression (R^2) at 3 h of adult *H. contortus* exposure to CASBE, CMSBE, CARSE and CMRBE as well as positive and negative control are shown in Table 9. All the four extracts including ABZ at varying concentrations resulted to 50% mortality. At 6 h, 50% mortality of the adult worm was recorded for all the plant extracts and the standard drug at various concentrations with the exception of negative control (Table 10). Furthermore, at 9 h post exposure of standard drug and all the extracts, only CASBE at 4.17 mg/ml concentration resulted to 50% mortality of adult *H. contortus*, the remaining extracts and ABZ resulted to 100% mortality of all adult *H. contortus* shown in Table 11. Also, in Table 12, at 12 h post exposure of all the extracts and ABZ to adult *H. contortus*, 100% mortality was recorded. The LC_{50} could not be determined.

The ranking of potency of CASBE, CMSBE, CARBE and CMRBE of *P. biglobosa* and ABZ based on LC_{50} and dose dependant effect (R^2 values) are shown in Table 13. At 3 h of exposure, CMSBE was ranked the highest for LC_{50} and R^2, followed by ABZ. But at 6 h exposure, LC_{50} was ranked first for ABZ, followed by CMSBE while for R^2, CMRBE was ranked first, followed by CMSBE and ABZ, respectively. It is interesting to know that at 12 h exposure, LC_{50} and R^2 were all ranked first for all plant extracts and ABZ. It was also evident from the data that all the extracts from different parts of the plant have dose dependant anthelmintic activity despite the varied changes.

Table 12. $LC_{50,}$ coefficient of determination (R^2) and regression equation of the effect of stem and root bark extracts of *P. biglobosa* as well as standard drug (ABZ) on adult *H. contortus* motility and/or mortality at 12 h.

Treatment (mg/mL)	LC_{50} (mg/mL)	Coefficient of Determination (R^2)	Regression Equation
PBS (-Ve Control)	-	-	-
ABZ (+Ve Control)	-	1E-1	Y= 7.37
CASBE	-	1E-1	Y= 7.37
CMEBE	-	1E-1	Y= 7.37
CARBE	"	"	"
CMRBE	"	"	"

Source: Authors

Table 13. Ranking of stem bark extracts, root bark extracts and Standard Drug (Albendazole) based on LC_{50} values and coefficient of determination on adult *H. contortus* motility and/or mortality.

Treatment (mg/mL)	Ranking of potency based on LC_{50}				Ranking of potency based on dose dependent effect (R^2 - values)			
Duration	3 h	6 h	9 h	12 h	3 h	6 h	9 h	12 h
ABZ (Control)	02	01	01	01	02	03	01	01
CASBE	03	04	02	01	05	05	02	01
CMSBE	01	02	01	01	01	02	01	01
CARBE	04	05	01	01	04	04	01	01
CMRBE	05	03	01	01	03	01	01	01

Source: Authors

DISCUSSION

Different methods exist for the extraction and separation of plant materials for pharmacological and medicinal uses. In this study, exhaustive extraction of the dried powdered material of stem and root barks of *P. biglobosa* were extracted with water and methanol separately. Among the extraction of aqueous and methanol extracts of *P. biglobosa*, CMSBE gave the highest yield (25.15%) while the CMRBE gave the lowest yield (13.9%). The highest yield reported could possibly be as a result of stem bark of *P. biglobosa* having more phytochemical constituents whose polarity corresponded to that of methanol (Kimani et al., 2013). The percentage yield in this result is higher than that of Salit et al. (2014) who reported 14.5 and 4.0% of yield extracts of seed-husk and stem bark of *P. biglobosa* plant, respectively.

Right from the time immemorial, plants formed part of therapy against parasitic infections of both humans and animals (Priya et al., 2015). Therefore, the basic phytochemicals investigations of extracts of different components of *P. biglobosa* for their major phyto-constituents are important in order to know the secondary metabolites present in this plant.

In this study, the phytochemical constituents present in water and methanol stem bark extracts of *P. biglobosa* were alkaloid, anthraquinones, cardiac glycosides, glycosides, flavonoids, oils, phlobatannins, reducing sugar, saponins, starch, sterols/steroids, tannin (condensed and hydrolysable), and terpenoids. The presence and/or absence of these phytochemical constituents vary in each extract. The results in this study were similar with the results of Ezekwe et al. (2013) in methanol extracts of stem bark of *P. biglobosa*. Millogo Kone et al. (2006) also reported the presence of saponins, glycosides, tannins and other phenolics with trace quantity of alkaloids while Banwo et al. (2004) confirmed the same.

However, the report of Builders et al. (2012) differed slightly from the result of this study due to absence of alkaloids from the methanol stem bark extracts. Thus, the absence may not be a minus for the medicinal efficacies of stem bark of *P. biglobosa* but could be the methods of processing and geographical location of this plant that might have led to differences in phytochemical constituents in the two works.

Similarly, in aqueous and methanol extracts of root bark of *P. biglobosa*, the phytochemical constituents present were anthraquinones, cardiac glycosides, flavonoids, phlobatannins, protein, reducing sugar, saponins, sterols/steroids, tannin (condensed and hydrolysable), and terpenoids. These results coincided with the report of Udobi and Onaolapo (2009), who used aqueous and petroleum ether solvents for extraction *P. biglobosa*, although, anthraquinone was absent in their result. It is therefore, important to note that from the four

different extracts extracted from different plant parts of *P. biglobosa,* CMSBE contained more secondary metabolites when compared with CASBE, CARBE and CMRBE. *In vitro* tests using the infective larvae of *H. contortus* is considered to be one of the best means of screening drugs for anthelmintic activity (Asase et al., 2005). Several researchers (Ademola et al., 2005; Bizimenyera et al., 2006; Soetan et al., 2011) have reported the activities of *in vitro* anthelmintic study of plant extracts for the treatment of gastro-intestinal helminths of animals. Therefore, perturbation induced by anthelmintic plants on infective larvae and adult worm survival or their prolificacy that constitute the pathogenic stage could be an important element in parasites struggle (Josiah et al., 2018).

The *in vitro* screening of CASBE, CMSBE, CARBE and CMRBE of *P. biglobosa* showed a significant anthelmintic activity against larvae (L_3) of *H. contortus*. In this study, there was no mortality or inhibition of motility of larvae when exposed to PBS, ABZ and all the aqueous and methanol extracts of *P. biglobosa* at 3 h post exposure. These results contradicted the findings of Dey et al. (2015) who worked on *in vitro* anthelmintic effect of some medicinal plants but not *P. biglobosa* against *H. contortus* and reported varied degree of mortality with different concentration of plant extracts in less than 3 h post exposure.

In this study, the highest efficacy was observed in positive control (ABZ) at 32 mg/ml with 88% mortality in 12 h post exposure. This was followed by CMSBE, CMRBE, CARBE and CASBE with 87, 75, 63 and 55% mortality, respectively at 32 mg/ml in 12 h post exposure. There was no mortality in negative control for up to 12 h post-exposure. It is therefore evident that, the positive control (ABZ) and all the extracts are dose and time dependent.

The LC_{50} determination of larva motility suggested that 50% of L_3 larvae were inhibited at concentration of 25.70, 58.88 and 144.54 mg/ml for ABZ, CMRBE and CMSBE, respectively at 6 h post-exposure. As ascertained, this is the first scientific evidence of the anthelmintic activity of CASBE, CMSBE, CARBE and CMRBE of *P. biglobosa* against larvae (L_3) of *H. contortus* when exposed below or above 3 h. At 12 h post exposure, 50% mortality was recorded for ABZ, CASBE, CMSBE, CARBE and CMRBE of *P. biglobosa* at 8.51, 18.62, 7.94, 16.98 and 14.79, respectively. This suggested a wide difference in the anthelmintic effect among the different extracts as far as the time and dose dependent effects are concerned. Therefore, CMSBE has high antihelmintic efficacy when compared with other extracts and even ABZ.

Larvae (L_3) represent an important stage of the parasitic cycle of *H. contortus*. They are the infective stage and could be a source of losses of production in the host (Paolini et al., 2003; Brunet et al., 2007). The decrease in larval migration induced by plant extracts could be due either to larval mortality or to larval paralysis caused by bioactive compounds present in the *P. biglobosa* especially in CMSBE and CMRBE.

Many researchers such as Molan et al. (2000, 2003), Brunet et al. (2007) and Olounlade et al. (2011), have shown different extracts from plant rich in tannins and terpenoids are responsible for the inhibition of larval migration of *H. contortus* as well as affected the kinetics of unsheathing of strongyle L_3 and consequently reduced the migration ability of ovine nematode larvae.

Additionally, this plant extracts (especially stem bark of *P. biglobosa*) contained others major metabolites affecting the migration of L_3 larvae of *H. contortus.* The larval migration was also inhibited either by saponins (Lukhoba et al., 2006) and triterpens, or by flavonoids and glycosides (Ademola et al., 2005; Barrau et al., 2005; Azando et al., 2011). Furthermore, Ayers et al. (2008) reported the contribution of phenols and flavonoïds with anthelmintic activity of *Struthiola argentea*. Thus, the higher flavonoids and saponins present in the extracts of *P. biglobosa* especially in methanol stem bark could be actively associated to anthelmintic activity observed.

The mortality induced by CASBE, CMSBE, CARBE and CMRBE of *P. biglobosa* on adult *H. contortus* on this study could be an important element in parasites struggle. The concentration range of 2 to 32 mg/ml of CASBE, CMSBE, CARBE and CMRBE of *P. biglobosa* was used in comparison with positive control - ABZ (2, 4, 8, 16 and 32 mg/ml) and negative control (PBS) on mature live *H. contortus* of goats. The results of this study indicated that exposure of adult worms to at least 2 mg/ml concentration of ABZ, CASBE CMSBE, CARBE and CMRBE of *P. biglobosa* for 12 h post exposure, lead to 100% mortality or inhibition of adult worms. These results were quite similar with that of Dedehou et al. (2014) who reported that the extracts of pods fruit of *P. biglobosa* and leaves of *P. erinaceus* inhibited 100% adult worm motility after 36 h of incubation. However, the result contradicted that of Bogning et al. (2016) who reported 16.67% of inhibition of the parasite motility when exposed to highest concentration of the aqueous extract (2400 µg/ml) of *Crassocephalum crepidioides* for 12 h and 100% inhibition after exposure to 30 h of incubation.

It is important to note that all the extracts resulted in paralysis and mortality of the tested worms at 12 h post exposure. All the worms exposed to ABZ (a standard anthelmintic drug) were found death at 9 h, whereas none of the worms was dead or paralysed in PBS up to 12 h post exposure. The higher concentrations resulted in early onset of activity and higher number of dead worms compared with lower concentrations. This suggested that the extracts response were time and concentration dependent. In this study, CMSBE resulted to onset killing of adult worm when compared with ABZ. This is evidence from the 3 h post exposure where CMSBE was ranked first on potency based on LC_{50}. The tannins contained in plants have been reported to possess anthelmintic (Paolini et al., 2003, 2005; Ademola et al., 2004, 2005)

activities. It is postulated that condense tannin may impair vital processes such as feeding and reproduction of the parasite or may bind and disrupt the integrity of the parasites' cuticle (Niezen et al., 1995). In general, it is important to note that the literature is scarce on the *in vitro* study of stem and root barks extracts of *P. biglobosa* on infective larvae and adult of *H. contortus.*

In vitro evaluation for anthelmintic activity of CASBE, CMSBE, CARBE and CMRBE of *P. biglobosa* showed that all extracts exhibited anthelmintic activity against *H. contortus* as evident from larva motility inhibition assay and adult motility assay of the worms. A wide difference, however, was recorded in the anthelmintic effects among different extracts as far as the intensity, time and dose dependent effects were concerned. The larvicidal and adulticidal properties of these extracts may be due to active compounds present in the extracts that penetrate across the cuticle of the parasites on one hand or the absorption of the active compounds by the parasites through the mouth on the other hand. Active compounds could penetrate through the cuticle of nematodes and prevent the absorption of glucose or block the post-synaptic receptors, thus, paralyzing the parasites as mentioned by Enriquez et al. (1993).

Conclusion

The overall findings of the study showed that CASBE, CMSBE, CMRBE and CARBE exhibited *in vitro* anthelmintic of 55, 87, 63 and 75% mortality, respectively against infective larvae of *H. contortus* when exposed to 32 mg/ml concentration for 12 h while 100% mortality was recorded against adult *H. contortus* when exposed to 32 mg/ml concentration of CASBE, CMSBE, CMRBE and CARBE for 6 h with CMSBE ranked the highest in LC_{50} and R^2. The *in vitro* anthelmintic activity against infective larvae of *H. contortus* was less efficacious in both the aqueous and methanol extracts when compared with adult *H. contortus*. However, the potency of plant extracts was dependent on the time of exposure and concentration of the extracts as well as the solvent used to extract the active ingredients. It is therefore, concluded that, 32 mg/ml of aqueous and methanol extracts of stem and root barks of *P. biglobosa* have higher adulticidal activity at 6 h post exposure but lower larvicidal activity against *H. contortus* and this justifies their traditional ethno-veterinary use. However, further studies are needed to carry out the *in vivo* study to assess the toxicological effect on animal model.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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