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Removal of Multi-Class Antibiotic Drugs from Wastewater Using Water-Soluble Protein of *Moringa stenopetala* Seeds

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Abstract: The removal of ten selected antibiotic drugs belonging to different classes (sulphonamides, fluoroquinolones, macrolides, and tetracycline) was investigated using water-soluble proteins from the seeds of *Moringa stenopetala*. The surface functional groups of water-soluble protein powder before and after removal of antibiotics were characterized using Fourier transform infrared (FTIR). Processing parameters that could affect the removal efficiency, such as initial analyte concentration, protein dosage, and pH were studied. An optimized method was applied to a real wastewater sample collected from Daspoort Wastewater Treatment Plant (WWTP) located in Pretoria, South Africa. Under optimal conditions, the results indicated good agreement between the efficiency of water-soluble proteins to remove antibiotics from the real wastewater sample and from the synthetic wastewater sample prepared in the laboratory using standard solutions with known concentrations. The percentage of removal under optimum conditions (protein dosage of 40 mg, initial analyte concentration of 0.1 mg L^{-1}, and pH 7) was between 85.2 ± 0.01% and 96.3 ± 0.03% for standard mixture solution and from 72.4 ± 0.32% to 92.5 ± 0.84% and 70.4 ± 0.82% to 91.5 ± 0.71% for the real wastewater (effluent and influent) sample.

Keywords: *Moringa stenopetala*; water-soluble proteins; antibiotics removal efficiency

1. Introduction

Antibiotics have saved countless lives since their discovery, and large quantities of these drugs are widely administered and used as antimicrobial drugs throughout the world. Antibiotic drugs are predominantly used to treat bacterial diseases in human therapy and as veterinary medicines to prevent diseases in animal husbandry, and also function as growth promoters, mainly in livestock [1,2]. Excessive usage of antibiotics increases the amount of antibiotic residue discharged into the environment. However, the extent to which antibiotics contaminate the environment has only received attention in recent decades, which could be attributed to the widespread use of and concentration of antibiotics in the aquatic environment, development of advanced and sensitive analytical instruments, and the toxic and chronic effect of antibiotic residues [3]. The reason for the increase in antibiotic concentration levels in the aquatic environment is that they are not completely metabolized in the human/animal body, but rather excreted via urine, animal manure, and/or feces. They are excreted as the parent compounds, metabolites, or water-soluble conjugate compounds and are thus released into the aquatic environment [1,4]. Other sources of antibiotic drugs in the environment include agricultural runoff and the disposal of unused antibiotic drugs from manufacturing industries [5]. As has been reported by different studies, antibiotic drugs have been detected in the influents and effluents of wastewater treatment plants, hospital wastewater, industrial effluent, surface water, groundwater, drinking water, and sediments [1,6–9].

The discharge of antibiotic drugs to the aquatic environment increases the possibility for bacteria to acquire antibiotic resistance genes (ARGs), which are easily transferred to other bacteria through horizontal gene transfer [10]. The development of ARGs in bacteria causes the microbes to become resistant to conventional antibiotic drugs, which had hitherto been effective [11]. The presence of antibiotic resistant bacteria has been observed in wastewater treatment plants, effluents, and surface water in Europe. These drug-resistant bacteria are mainly found in hospital effluents where antibiotics are frequently used.

Amongst the antibiotics drugs, sulphonamides, fluoroquinolones, macrolides, and tetracycline are some of the most frequently detected antibiotic drugs in the aquatic environment [12,13]. These drugs have been detected in municipal wastewater, surface water, ground water, and overland water systems [14,15]. Several recent studies confirm that conventional wastewater treatment plants only partially remove antibiotic drugs from wastewater [6,16].

A number of treatments methods, such as ozonation, chlorination, ultraviolet (UV) irradiation, nanofiltration, reverse osmosis, flocculation, filtration, and adsorption via activated carbons and other materials have been used hitherto. However, most of these methods were developed with the intention of removing heavy metals, hydrophobic drugs, and to treat microbial contaminants rather than pharmaceutically active compounds such as antibiotics [5,17]. Shortcomings have also been identified in some of the methods developed specifically for the purpose of removing antibiotic drugs including photodegradation with UV/catalysts, adsorption by carbon nanotubes, clays, and ion exchange [18–21]. Various materials have been used for the removal of antibiotics, such as zeolite, alumina, silica, mesoporous silica, functionalized mesoporous silica activated carbon, and metal–organic frameworks, biosorbents, agricultural waste, and others [22,23]. It is clearly observed that indeed there is still a need for inexpensive and environmentally friendly yet effective materials for the removal of antibiotics from the various contaminated aquatic environments. In order to reduce the high investments, rigorous research has been carried out to find innovative and cost-effective methods. Amongst the adsorbents, bio-materials have received much attention and have recently become an extensive area of interest due to their effectiveness and versatility in the removal of different kinds of pollutants from the aquatic environment. Moringa has previously been used for water treatment such as flocculation, coagulation, and removal of heavy metals [24,25]. The seeds of Moringa are rich in the water-soluble protein, which has coagulation properties similar to those of alum and synthetic cationic polymers.

The objective of this study was to investigate the removal of multi-class antibiotic drugs, such as sulphonamides, fluoroquinolones, macrolides, and tetracycline, from wastewater by using water-soluble proteins extracted from *Moringa stenopetala* seeds.

2. Materials and Methods

2.1. Chemicals

All standards used were of the highest purity available (≥98%). Sulphanilamide, marbofloxacin, ciprofloxacin, danofloxacin, oxytetracycline, sulphadimethoxine, sulphacetamide, sulphamonomethoxine, sulphamethoxazole, tylosin, and sulphamerazine were obtained from Sigma–Aldrich, (Schnelldorf, Germany). Acetonitrile of high purity HPLC grade (>99.9%) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and a Milli-Q® Integral Water Purification System (Molsheim, France) was used to produce ultra-pure water (18.2 mΩ). The physicochemical properties of the selected compounds are presented in Table 1.

Table 1. Molecular structure and physicochemical properties of selected analytes.

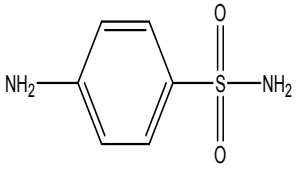
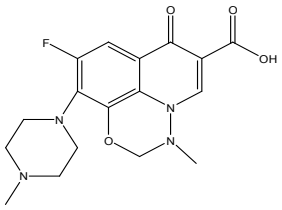
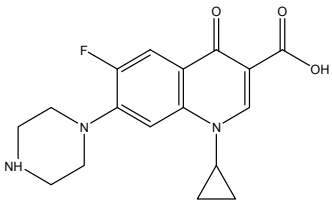
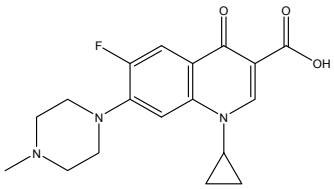
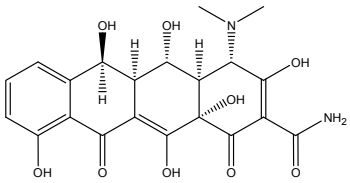
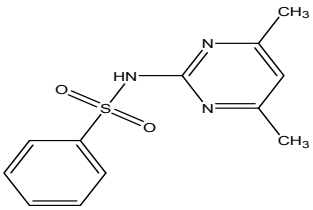
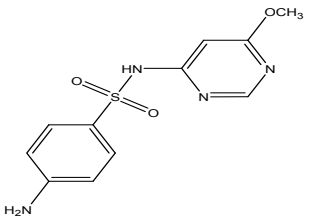
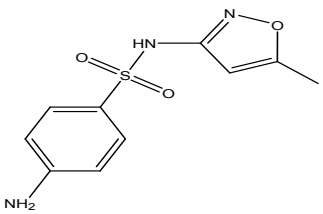
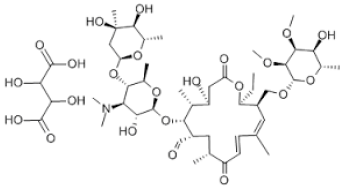
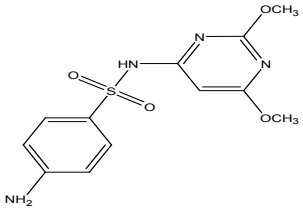
Name of Pharmaceuticals	Structure	pK_{a1} and pK_{a2} (pK_{a3})	Log K_o	Reference
Sulphanilamide		1.94/10.67	−0.62	[26]
Marbofloxacin		5.77/8.22	0.07 ± 0.01	[27]
Ciprofloxacin		1.84 (0.08)/7.63 (0.01)	0.35	[28,29]
Danofloxacin		2.06/6.90	0.44	[4]
Oxytetracycline		3.3/7.55/9.7	−1.12	[30,31]
Sulphamerazine		2.06/6.90	0.44	[31]
Sulphamonomethoxine		2.07(0.05)/6.91 (0.01)	0.70	[28,32]
Sulphamethoxazole		1.6/5.7 (7.40 (0.04))	0.89	[4,28]

Table 1. *Cont.*

Name of Pharmaceuticals	Structure	pK_{a1} and pK_{a2} (pK_{a3})	Log K_o	Reference
Tylosin tartrate		3.31/7.5	-	[31]
Sulphadimethoxine		2.1/6.3	1.63	[32]

2.2. Instrumentation

An Agilent 1220 High-Performance Liquid Chromatography with Diode-Array Detection HPLC-DAD (Agilent Technologies, Waldbronn, Germany) system consisting of a binary high-pressure pump, autosampler, a thermostat column compartment, a fluorescence detector, and refractive index detector was used. ChemStation (version 1.9.0) software (Agilent Technologies, Waldbronn, Germany) was used to process the data. An XTerra MS C18 column (Agilent Technologies, Waldbronn, Germany) was used (4.6 × 100 mm, 3.5 µm), with mobile phase solvent A: 0.1% aqueous formic acid solution, and solvent B: acetonitrile. The column temperature was maintained at 40 °C. The separation was done under gradient elution with the organic phase increasing linearly from 5 to 30% in 7 min and further increasing to 60% within 5 min. The mobile phase was pumped at a flow rate of 1.2 mL·min^{-1} and the detection wavelength was 260 nm, with a post-run time of 1 min before the next injection to equilibrate the column. The chromatographic separation of antibiotic compounds is presented in Figure 1.

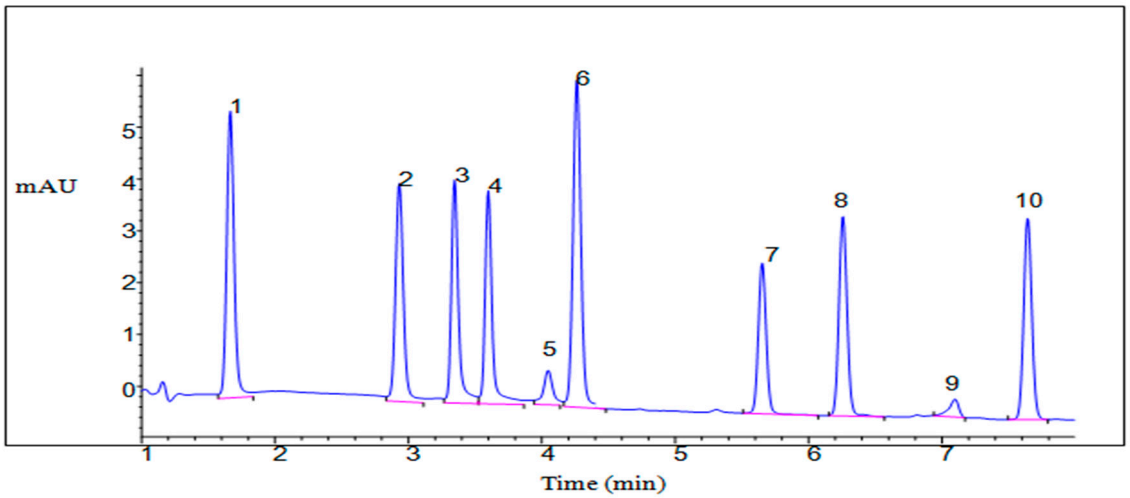


Figure 1. Chromatographic separation of 1 mg L^{-1} standard mixture of ten multiclass antibiotic drugs (sulphanilamide, marbofloxacin, ciprofloxacin, danofloxacin, oxytetracycline, sulphadimethoxine, sulphacetamide, sulphamonomethoxine, sulphamethoxazole, tylosin, and sulphamerazine).

2.3. FTIR Spectroscopy

Fourier transform infrared (FTIR) spectra of freeze-dried, water-soluble proteins before and after antibiotics drug removal were obtained using a Vertex series FTIR spectrophotometer (Bruker Optic GmbH, Hamburg, Germany) with a diamond ATR fitting. The spectra were obtained in transmittance mode with 32 scans at a resolution of 2 cm^{-1} in the 4000–400 cm^{-1}spectral regions. The data were processed using Opus 7.3.139.1294 software (Bruker Optic GmbH, Hamburg, Germany)).

2.4. Preparation of Standard Solutions

All stock solutions for the standards were prepared by weighing 1.5 mg standards and dissolving in acetonitrile/water (50:50) to give a concentration of 1 mg mL^{-1}. All working solutions were prepared by diluting the stock solution in 50:50 (v/v) acetonitrile and water. The calibration curve was prepared using six concentration levels of standard solutions within the concentration range of 0.01 mg mL^{-1} to 2.5 mg mL^{-1}. All calibration standards were prepared from a stock solution of 1 mg mL^{-1}.

2.5. Extraction of Water-Soluble Proteins

The preparation of the *Moringa stenopetala* seeds for protein extraction was done according to the method described by Kebede et al. [33]. The water-soluble proteins were extracted according to the method adopted from Ndabigengesere et al. [34,35], with a minor modification made as explained in our previous work [33]. Petroleum ether (37% w/v) was added to the powder and the mixture was stirred for 30 min on a magnetic stirrer to dissolve any fats, oils, or waxes. The undissolved material was then separated via filtration through a Whatman paper No. 1. The residue was dissolved in ultra-high purity water and stirred for 30 min to extract the water-soluble protein. This step was followed by filtration through a Whatman paper No. 3 in order to remove the water-insoluble substances. The filtrate was treated with ammonium sulfate to precipitate proteins from the aqueous extract. The precipitated protein was filtered, re-dissolved in water, and then re-filtered to remove insoluble material. The protein solution was then dialyzed through a cellulose membrane with a molecular cut-off of between 3.5 and 14 kDa. After dialysis, the pure protein was freeze-dried and a white powder was obtained, which was stored at room temperature until ready for use.

2.6. Preparation of Pure Protein for the Removal of Antibiotics

Removal studies were carried out by adding 25 mL aliquots of the standard mixture solution (concentration range 0.1–1.5 mg L^{-1}) in 50 mL Erlenmeyer flasks. The desired amount of protein powder (10–50 mg) was mixed with the standard mixture solution. These solutions were vortexed for 1 min at room temperature to allow the interaction to take place between the protein and analytes of interest, followed by filtration using a 0.45 µm Polyvinylidene fluoride (PVDF) (Sigma-Aldrich, St. Louis, MO, USA). The concentrations of the pharmaceutical drugs were determined in terms of absorbance measurements using HPLC-DAD. The effects of the major experimental parameters (protein dosage, analyte concentration, and pH) on the removal of pharmaceutical drugs by proteins were investigated. Each sample was measured five times.

2.7. Data Analysis

The percentage removal of each of the pharmaceutical drugs was calculated based on the difference between the initial analyte concentration (C_0) (before removal) and the final analyte concentration (C_f) (after removal), which was obtained from the calibration curve for each of the analytes in the sample solutions, using the following formula:

$$\% \; removal = \frac{C_0 - C_f}{C_0} \times 100 \quad (1)$$

2.8. Sample Collection

Wastewater samples (effluent and influent) were collected from Daspoort, a WWTP located in Pretoria, one of the major cities in Gauteng Province, South Africa. The Daspoort WWTP discharges into a neighboring Apies River in its surroundings. The grab sampling method was used and the samples were collected in 2.5 L amber glass bottles, which had previously been washed and rinsed with ultra high pure UHP water and then flushed at least thrice with wastewater before collection. Each sample (influent and effluent) was collected in duplicate. All water samples were transported to the laboratory in cooler boxes packed with ice. Upon arrival, the samples were filtered through a Whatman (120 mm) filter paper using vacuum filtration and extracted immediately to avoid degradation. Thesamples were then stored at <4 °C in the dark and analyzed within 24 h.

3. Results and Discussion

3.1. Characterisation of Moringa Seed Protein Powder

The characterization of protein powder assists in gaining an understanding of features such as composition, structure, and various properties like physical and chemical properties. Moringa seed protein powder was characterized using FTIR to identify the functional groups of the active sites.

3.2. FTIR Characterisation of Moringa Protein before and after Removal of Antibiotics

As indicated in the FTIR spectra given in Figure 2, protein powder contains amine and amide functional groups at wave numbers 1647 cm^{-1}, 1541cm^{-1}, 1515 cm^{-1},and 1412 cm^{-1}associated with C=O stretch amide I, NH amide II, NH amide I bend, and C-N stretch amide III [33], respectively, which are active and able to bind with antibiotics. After the removal of the antibiotics, the interactions between the compound and the protein powder were indicated by the appearance of new peaks or the disappearance of peaks that were previously observed and a significant shift in peaks, as shown in Figure 2. The peaks at wave numbers 1541 $cm^{-1}$and 1529 cm^{-1}associated with NH amide II and NH amide I bending vibration, respectively, disappeared after loading antibiotics, while new peaks appeared at wave numbers 1565 and 3750 cm^{-1}. The band position shift from wave number 3207 to 3270 $cm^{-1}$was also observed.

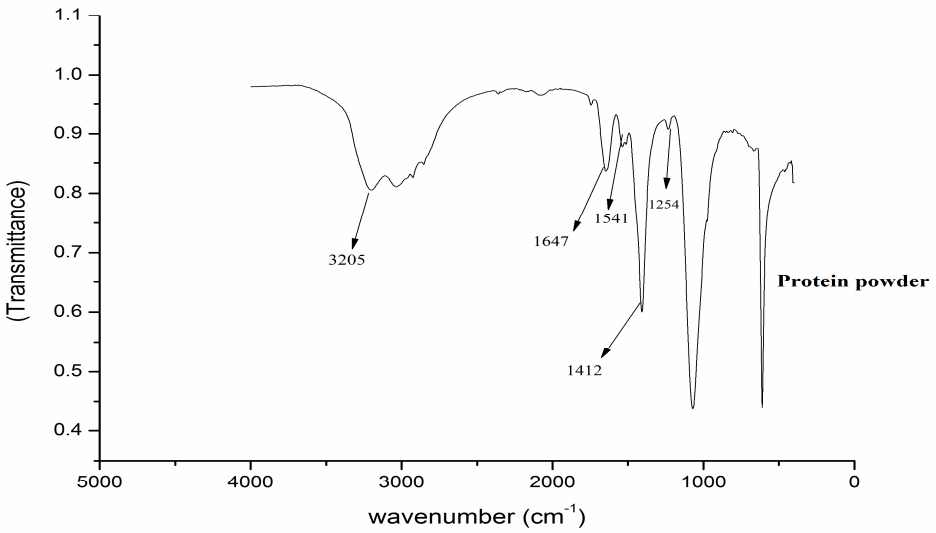


Figure 2. *Cont.*

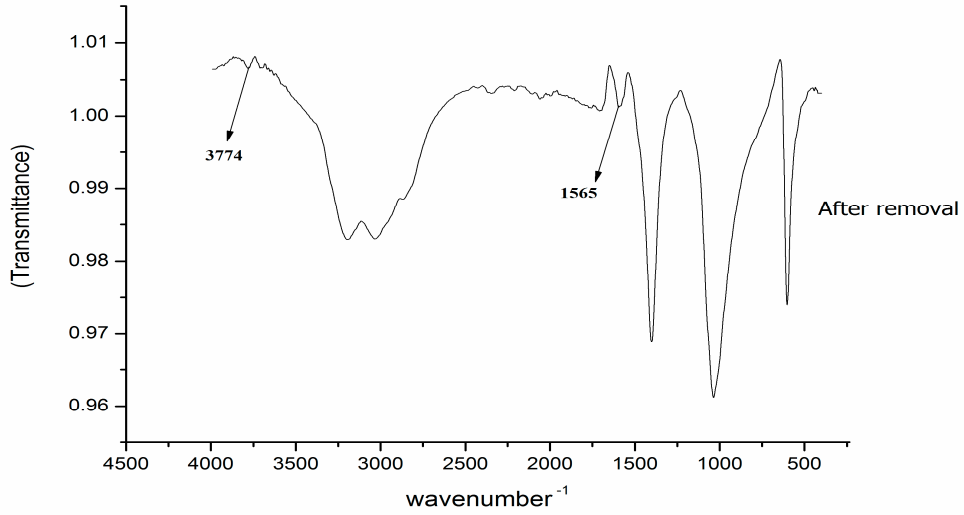


Figure 2. FTIR spectra of the water-soluble protein powder before and after the removal of multiclass antibiotics.

3.3. Removal of Antibiotics Using Moringa Protein

The water-soluble proteins extracted from *Moringa stenopetala* seed were used for the removal of selected antibiotics. Due to their complicated structure, proteins present in the seeds have a large number of functional groups; however, the amides and amines are the dominant functional groups. These functional groups were responsible for and played the major role in the removal of antibiotics. At different pH ranges, these functional groups behave differently. At pH values of between 3.5 and 10, proteins exist mainly in the zwitterionic form [33] while antibiotics are in the neutral and zwitterionic forms. Maximum removal was thus expected to occur when proteins are in the zwitterionic form and analytes in the neutral form, attributable to hydrogen bonding or electrostatic force of attraction between proteins and antibiotics. Different parameters that affect the efficiency of water-soluble proteins to remove antibiotics, such as protein dose, initial analyte concentration, and pH, were studied and the optimum conditions for each parameter were selected. For every analysis, each analyte was measured five times.

3.4. Effect of Initial Analyte Concentration

The analyte concentration in aqueous solution was one of the main factors that were found to affect the removal of antibiotics as shown in Figure 3. The concentration of antibiotics in the solution was varied from 0.1–1.5 mg L^{-1} while keeping the other parameters constant (i.e., protein dosage of 40 mg, pH at 5.5, volume 25 mL, contact time 30 min, and temperature at 23 °C). The experiment was run from the highest to the lowest concentration of antibiotics in order to determine the concentration where maximum removal of all analytes was achieved. The percentage removal for ten selected antibiotic drugs increased as the initial concentration decreased. This may have been due to the fact that at lower concentrations, there are sufficient active sites on the protein molecule for the analytes to occupy. However, the number of active sites is limited, and at higher concentrations, the active sites on proteins are rapidly occupied, and hence ions of selected analytes are left unbound in solution as a result of the saturation of binding sites. The maximum percentage removal obtained was 89.3 ± 0.05, 94.2 ± 0.02, 95.0 ± 0.02, 92.9 ± 0.05, 83.9 ± 0.06, 84.6 ± 0.04, 85.5 ± 0.06, 96.7 ± 0.01, 92.7 ± 0.07, and 84.9 ± 0.02 for sulphanilamide, marbofloxacin, ciprofloxacin, danofloxacin, oxytetracycline, sulphamerazine, sulphamonomethoxine, sulphamethoxazole, tylosin, and sulphadimethoxine, respectively.

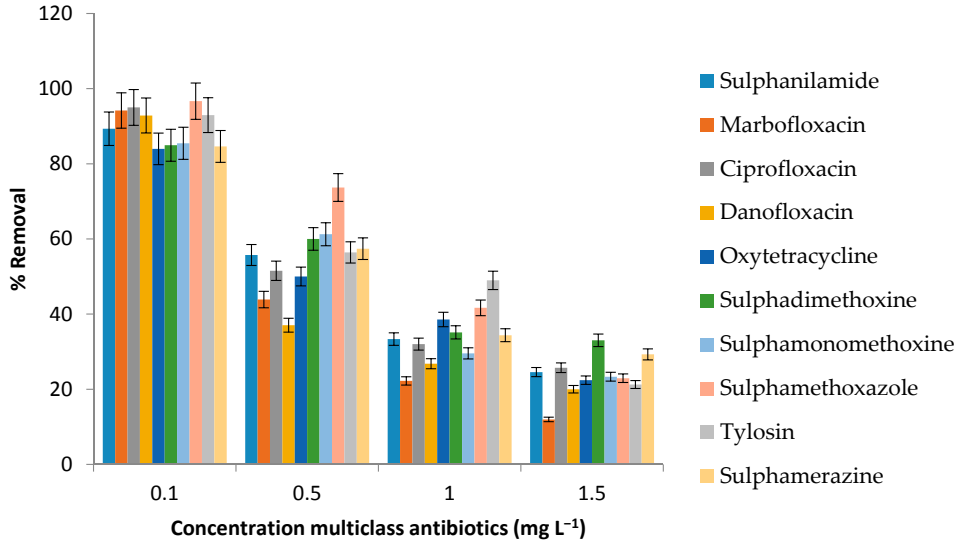


Figure 3. Effect of initial concentration on percentage removal of antibiotics using water-solubleprotein powder (n = 5).

3.5. Effect of Adsorbent Dosage

The amount of protein dosage was found to be an important parameter that affected the removal process, as shown in Figure 4. The removal was investigated by varying the dosage from 10 mg to 50 mg while keeping the other parameters constant (pH 5.5, analyte concentration of 0.1 mg L^{-1}, volume of 25 mL, contact time 30 min, and temperature at 25 °C). As the protein dosage was increased, the efficiency to remove antibiotics significantly increased, due to the increase in the number of available active sites responsible for the removal of antibiotics. The removal was also affected by the chemical structure and size of antibiotics. Maximum percentages removal of 88.8 ± 0.08, 94.2 ± 0.03, 94.2 ± 0.01, 91.9 ± 0.02, 89.7 ± 0.05, 87.6 ± 0.09, 85.2 ± 0.02, 96.3 ± 0.02, 91.5 ± 0.02, and 85.5 ± 0.02% were achieved for sulphanilamide, marbofloxacin, ciprofloxacin, danofloxacin, oxytetracycline, sulphamerazine, sulphamonomethoxine, sulphamethoxazole, tylosin, and sulphadimethoxine, respectively, at a protein dosage of 40 mg.

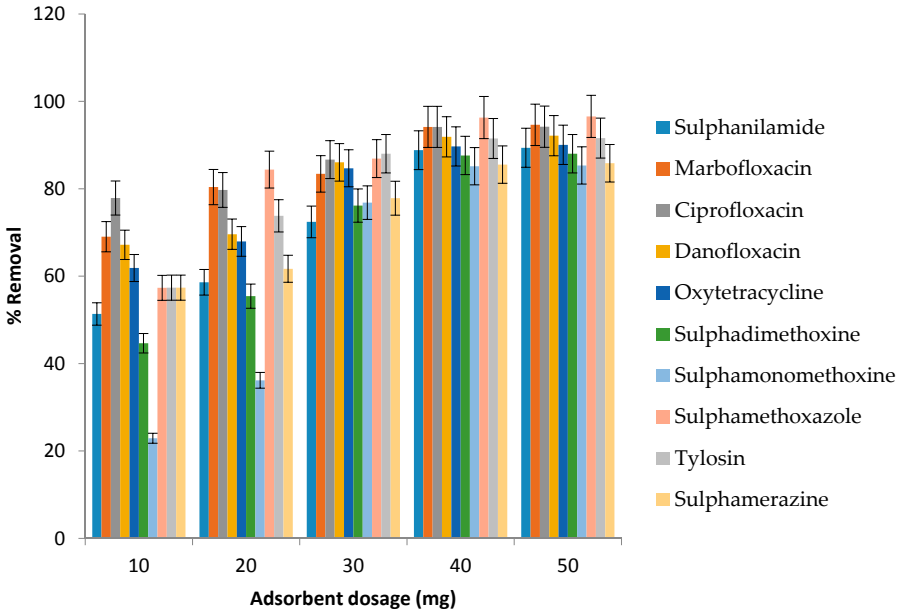


Figure 4. Effect of adsorbent dosage on percentage removal of antibiotics using water-soluble protein powder (n = 5).

3.6. Effect of pH

The percentage removal of selected antibiotics using water-soluble protein as a function of pH was studied in acidic, neutral, and basic media, as shown in Figure 5, while keeping other parameters constant (protein dosage 40 mg, analyte concentration of 0.1 mg L^{-1}, volume of 25 mL, contact time 30 min, and temperature at 25 °C. From the results, it was observed that the removal of antibiotics was strongly pH dependent. The pH does not only affect the property of analytes to be removed, but also affects the removal efficiency of the material as the shape and charge properties of active sites are affected by pH changes. The removal of ten selected antibiotics (sulphanilamide, marbofloxacin, ciprofloxacin, danofloxacin, oxytetracycline, sulphamerazine, sulphacetamide, sulphamonomethoxine, sulphamethoxazole, tylosin, and sulphadimethoxine) was significantly affected as the pH changed. The amine groups of fluoroquinolones (marbofloxacin, ciprofloxacin, and danofloxacin) at pH values below their pK_{a1} value ($\leq$2.5) were protonated and positive charges were predominated. Protein also shows similar properties at pH values below their pK_{a1} value ($\leq$2.5) [33], which may cause electrostatic repulsion between the protein and fluoroquinolones, resulting in a lower removal of fluoroquinolones. As the pH was increased to a range betweenpK_{a1} and pK_{a2}, both fluoroquinolones and proteins existed as zwitterions and maximum removal was observed due to hydrogen and electrostatic interaction. Further increases in pH to above 9 created a dominance of negative ions in both fluoroquinolones and protein molecules which might cause an electrostatic repulsion and would result in the poor removal of fluoroquinolones. The removal of sulphonamides (sulphanilamide, sulphadimethoxine, sulphacetamide, sulphamonomethoxine, sulphamethoxazole, and sulphamerazine) using water-soluble protein also proceeded in a similar way as that of fluoroquinolones due to the protonation of the amide group of sulphonamides at pH values below their pK_{a1} value ($\leq$2.5). On the other hand, at pH values of between 2.5 and 6, sulphonamides mainly exist as neutral compounds. Maximum removal was observed for all sulphonamides when proteins were in the zwitterionic form and sulphonamides were in the neutral form. These findings are in agreement with the results reported by Yang et al. [32]; according to their studies, the maximum removal of sulphonamides using activated sludge was observed when sulphonamides were in the neutral form. Further increases in pH resulted in a decrease in the removal of sulphonamides, because at pH values above their pK_{a2}value, sulphonamides as well as proteins were negatively charged, which would create a stronger repulsion between the analyte and the adsorbent.

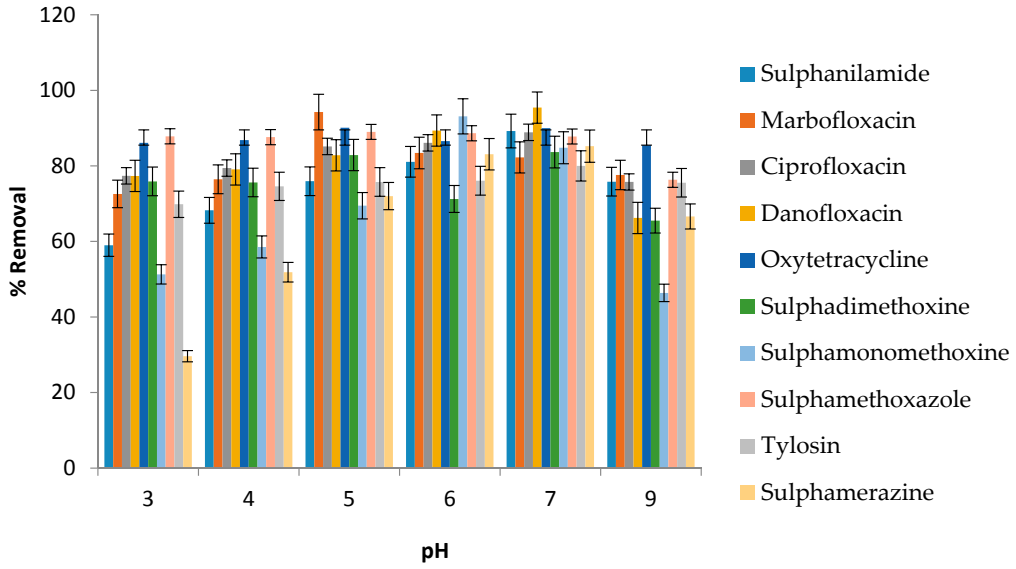


Figure 5. Effect of pH on the percentage removal of antibiotics using water-soluble protein powder (n = 5).

Oxytetracycline showed the same trend as sulphonamides and fluoroquinolones; however, it has three pK_a values. It exists as $X^0Y^+Z^0$ at a low pK_a value (3.3) and dissociates into $X^-Y^+Z^0$. At pK_{a2} its dissociation is from $X^-Y^+Z^0$ to either $X^-Y^+Z^-$ or $X^-Y^0Z^0$, and then it dissociates into$X^-Y^0Z^-$ [36]. At a pH value below the pK_{a1}, quaternary amine was protonated, and cationic species became dominant. At pH values between pK_{a1} and pK_{a2}, the solution was mainly governed by neutral or zwitterionic species because of the protonation of tricarbonyl amide and deprotonation of phenolic diketone. With pH values higher than 10, anionic charges were predominant in oxytetracycline due to the deprotonation of all the functional groups. The maximum removal was observed in the neutral or zwitterionic state as a result ofhydrogen bonding and electrostatic interaction.

Tylosin tartrate which is group of macrilide, has two pK_a values (pK_{a1} = 3.3 and pK_{a2} = 7.7) and its protonation, deprotonation, and removal efficiency were the same as those of the other groups. At a pH value below pK_{a1}, the tartaric acid and tertiary amine were protonated, and as a result, the positive ions predominated; however, negative ions predominated at pH values above pK_{a2}.At a pH value below pK_{a2} and above pK_{a1}, zwitterions predominated and that is where the maximum removal was observed.

3.7. Application of Method on Real Wastewater Sample

The optimized method (protein dosage 40 mg, pH 6, analyte concentration of 0.1 mg L^{-1}, volume of 25 mL, contact time 30 min, and temperature at 25 °C) developed to evaluate water-soluble protein for the removal of selected antibiotics from a standard mixture solution was applied to wastewater after spiking 25 mL of the real wastewater sample with 2 mg L^{-1} standard solutions in order to obtain an effective concentration of 0.1 mg L^{-1}. The effluent and influent samples were spiked as the analytes of interest could not be detected using HPLC-DAD without preconcentration. As shown in Table 2, the results revealed that the developed method was found applicable for the removal of antibiotic drugs from the real wastewater sample. The maximum percentage removal obtained was in the range of 72.4 ± 0.32–92.5 ± 0.84 and 70.4 ± 0.82–91.5 ± 0.71 for effluent and influent, respectively. A slight decrease in the removal of the antibiotics in the real wastewater sample was observed compared to the removal obtained when using a standard mixture solution. The decrease in removal could be due to the presence of different competing ions in the real wastewater sample, which would compete for the available active sites on the protein. The results, however, confirmed the removal of antibiotic drugs from wastewater using water-soluble proteins extracted from *Moringa* seeds.

Table 2. Removal efficiency of water-soluble proteins in a real wastewater sample spiked with 2 mg L^{-1} of the standard solution (effective concentration was 0.1 mg L^{-1}) (n = 5).

Analytes	Retention Time	Concentration (mg L^{-1}) before Removal	Percentage Removal in the Ultrahigh Purity Water (%)	Percentage Removal in Effluent (%)	Percentage Removal of Influent (%)
Sulphanilamide	1.62	0.1	88.8 ± 0.05	72.4 ± 0.32	71.3 ± 0.56
Marbofloxacin	2.99	0.1	94.2 ± 0.05	88.2 ± 0.45	85.2 ± 0.66
Ciprofloxacin	3.35	0.1	94.2 ± 0.02	88.3 ± 0.56	83.5 ± 0.45
Danofloxacin	3.60	0.1	95.4 ± 0.12	89.5 ± 0.44	87.2 ± 0.85
Oxytetracycline	4.09	0.1	89.7 ± 0.04	92.5 ± 0.84	91.5 ± 0.71
Sulphamerazine	4.32	0.1	87.6 ± 0.05	74.4 ± 0.52	76.2 ± 0.32
Sulphamonomethoxine	5.69	0.1	85.2 ± 0.01	74.2 ± 0.32	70.1 ± 0.51
Sulfamethoxazole	6.28	0.1	96.3 ± 0.03	89.0 ± 0.56	83.7 ± 0.61
Tylosin tartrate	6.99	0.1	91.5 ± 0.01	86.8 ± 0.84	81.9 ± 0.55
Sulphadimethoxine	7.64	0.1	85.5 ± 0.01	74.7 ± 0.56	70.4 ± 0.82

4. Conclusions

In this study, the water-soluble protein powder extracted from the *Moringa stenopetala* seeds was characterized and the removal behavior was studied for ten selected antibiotic drugs (sulphanilamide, marbofloxacin, ciprofloxacin, danofloxacin, oxytetracycline, sulphamerazine, sulphamonomethoxine,

sulphamethoxazole, tylosin tartrate, and sulphadimethoxine) using a known concentration of standard solution mixture prepared in the laboratory.

Different parameters that affected the removal efficiency of water-soluble proteins, such as protein dosage, initial analyte concentration, and pH, were studied and the optimum conditions for each parameter were selected. The optimum conditions for the removal of ten selected antibiotics usingwater-soluble proteins were a protein dosage of 40 mg, an initial antibiotic concentration of 0.1 mg L^{-1}, pH at 7, contact time 30 min, and volume 25 mL. The developed and optimized method was applied on wastewater. The simultaneous removal of selected antibiotics was investigated, and the results obtained confirmed that the water-soluble proteins extracted from *Moringa stenopetala* seeds are potentially useful to remove antibiotics from synthetic wastewater and real wastewater. The maximum percentage removal obtained using the developed method was in the range of 85.2–96.3% for sulphanilamide, marbofloxacin, ciprofloxacin, danofloxacin, oxytetracycline, sulphadimethoxine, sulphamonomethoxine, sulphamethoxazole, and tylosin tartrate. The percentage removal in the range of 72.4–92.5% and 70.4–91.5% was observed when the developed method was applied to the real wastewater sample (effluent and influent) collected from the wastewater treatment plant. Therefore, the developed method for the removal of selected antibiotics using water-soluble proteins from *Moringa stenopetala* seeds was simple, cost-effective, environmentally friendly, and easily applicable to monitor environmental pollution. Thus, *Moringa* can be regarded as a multipurpose tree (for medicinal purposes, food, and wastewater treatment), and as the results of this study indicate, its products can assist in keeping the environment free from pollution. Cultivating and managing these useful trees could also make a contribution towards reducing deforestation.

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