


ORIGINAL CONTRIBUTION

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# Bioactive constituents with antibacterial, resistance modulation, anti-biofilm formation and efflux pump inhibition properties from *Aidia genipiflora* stem bark

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## Abstract

**Background:** Antimicrobial resistance is a global health challenge. The involvement of bacterial biofilms and efflux pumps in the development of multidrug resistance (MDR) is well established. Medicinal plants have been proposed as alternatives for combating MDR focusing on their bioactive constituents with resistance modulatory activities. This study was aimed at investigating the stem bark of *Aidia genipiflora* for bioactive constituents with anti-biofilm, efflux pump inhibition and resistance modulatory activities.

**Method:** The crude methanol extract was purified by column chromatography and isolated compounds characterized by mass and nuclear magnetic resonance spectrometry. Antibacterial activity was determined by the High-throughput spot culture growth inhibition and the broth micro-dilution assay. The ethidium bromide accumulation assay was used to determine efflux pump inhibition property. Biofilm inhibition was determined in a microplate crystal violet retention assay.

**Results:** Purification of the ethyl acetate fraction led to the isolation of oleanonic acid (1), 4-hydroxy cinnamic acid docosyl ester (2),  $\beta$ -stigmasterol/ $\beta$ -sitosterol (mixture 3a/b) and D-mannitol (4). The minimum inhibitory concentrations (MICs) ranged from 250 to > 500  $\mu$ g/mL for extracts and fractions and from 15 to 250  $\mu$ g/mL for compounds. In the presence of sub-inhibitory concentrations of the compounds, the MIC of amoxicillin against *E. coli* (20  $\mu$ g/mL) and *P. aeruginosa* (320  $\mu$ g/mL) was reduced by 32 and 10 folds respectively. The whole extract demonstrated anti-biofilm formation and efflux pump inhibition in *E. coli*, *S. aureus* and *P. aeruginosa*. The sterol mixture (3a/b) at concentration of 100  $\mu$ g/mL caused the highest inhibition (73%) of biofilm formation in *S. aureus*. Oleanonic acid (1) demonstrated remarkable efflux pump inhibition at MIC of 7.8  $\mu$ g/mL in *E. coli* better than the standard drugs verapamil and chlorpromazine.

**Conclusion:** This study confirms the prospects of *A. genipiflora* as a source of new antibacterial agents and adjuvants that could interact with some resistance mechanisms in bacteria to enhance the activity of hitherto ineffective antibiotics. "A small portion of the study has been presented in a conference in the form of poster".

**Keywords:** *Aidia genipiflora*, Biofilm, Modulation, Efflux pump, Oleanonic, Antibacterial

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## Introduction

The increasing frequency of antimicrobial resistance (AMR) has resulted in lower rates of antibiotic efficacy and hence therapeutic failure [1]. Today common infections that were hitherto easy to cure have become increasingly difficult and sometimes impossible to treat resulting in an increased morbidity and mortality from infectious diseases [2]. Coupled with this problem is the depleting pipeline of new antibiotics which has necessitated the search for new potent antimicrobial agents [3].

Among the various mechanisms of antimicrobial resistance, bacteria biofilms and efflux pumps have been shown as main contributors to AMR [4]. The over expression of efflux pumps enable bacteria to extrude broad spectrum of antibiotics to their exterior rendering the drugs ineffective [5]. Biofilm formation is a vital microbial survival strategy through which they exhibit higher resistance than planktonic forms. An enlarged gene pool, more efficient quorum sensing systems, passive resistance and metabolic cooperation are ways by which biofilm forming bacteria protect themselves from antimicrobial agents and host immune responses [6]. Research has shown that efflux pumps are highly active in bacterial biofilms making these two attractive targets for the pharmacological development of new antibacterial agents against resistant pathogens [4].

Medicinal plants are constantly faced with attack from pathogenic microorganisms in the environment, prompting them to produce a wide range of metabolites to protect themselves [7]. Studies have shown that these plant metabolites may be of clinical significance in combatting resistant bacteria due to their highly diversified chemical structures and mechanisms of action [8, 9]. The objective of this study was to investigate the crude extract, fractions and some constituents of the stem bark of *Aidia genipiflora* for antimicrobial, anti-biofilm formation, efflux pump inhibition and resistance modulation activities as part of a continuing effort to identify

bioactive constituents of tropical medicinal plants [10, 11].

*Aidia genipiflora* (DC.) Dandy (Rubiaceae) also referred to as *Randia genipiflora* is distributed in the wild forests of African countries including Ghana, Sierra Leone, Liberia, Nigeria, Ivory Coast, Guinea-Bissau, Sudan and Cameroon. Its leaves and stem bark are used for treating gout, oedema and boils in traditional medicine [12]. A search in literature however revealed little or no scientific investigations on its biological activity and phytochemistry, thus prompting this research.

## Materials and methods

### Drugs and chemicals

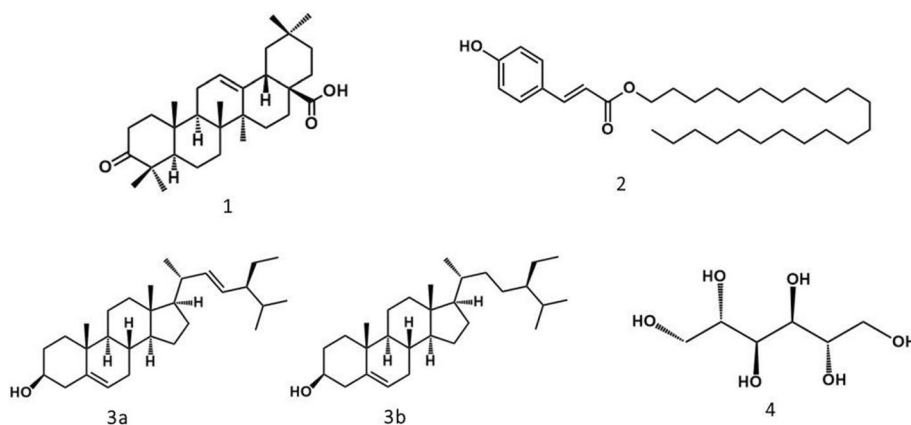
Amoxicillin (Phyto-Riker, Accra, Ghana); organic solvents were purchased from BDH laboratory supplies, England. Silica gel 60 (70–230 mesh, AppliChem, GmbH, Darmstadt, Germany) and pre-coated silica gel F<sub>254</sub> aluminium sheets (Merck kGaA, Germany).

### Plant material collection

The stem bark of *A. genipiflora* was collected from Kwahu Asakraka, a town in the Eastern Region of Ghana (06° 36.704'N/ 000° 42.659'W) in November, 2018. The plant material was authenticated by a botanist, Dr. George Henry Sam of the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. A voucher specimen (KNUST/HM/2017/SB016) was kept at the herbarium of the Faculty.

### Preliminary phytochemical screening

The presence of major secondary metabolites in the powdered stem bark of *A. genipiflora* was determined by simple qualitative phytochemical screening methods [13].



**Fig. 1** Isolated compounds from the stem bark of *Aidia genipiflora*

**Table 1** Phytochemical screening of the stem bark of *A. genipiflora*

Secondary metabolite	Result
Tannins	+
Reducing sugars	+
Alkaloids	+
Saponins	-
Triterpenoids	+
Phytosterols	+
Flavonoids	+
Coumarins	+

+: detected; -: not detected

**Preparation of extracts and fractions**

The stem bark was washed under running water, chopped into pieces, air dried for a week and pulverized into coarse powder. Three kilograms of the powdered stem bark was extracted by Soxhlet extraction with methanol/chloroform (4:1) for 6 h. The extract obtained was concentrated on a rotary evaporator under reduced pressure and temperature to obtain a brown solid extract subsequently referred to as AG or 'the whole extract' in this report. A yield of 5.1% <sup>w</sup>/<sub>w</sub> (153.2 g) was obtained. The whole extract (100 g) was adsorbed onto silica gel 60, packed onto a glass column and eluted successively with petroleum ether (200 mL×3), ethyl acetate (200 mL×3) and methanol (200 mL×3) to afford pet ether (AGPE, 4.3 g), EtOAc (AGEt, 38.8 g) and MeOH (AGM, 53.2 g) fractions. The extract and fractions were kept in a dessicator until required for use.

**Isolation and characterization of bioactive constituents**

The ethyl acetate fraction (AGEt, 28.3 g) was subjected to purification by column chromatography (CC). CC was performed using silica gel 60 (70–230 mesh). Elution was done using mixtures of pet-ether, EtOAc and

**Table 2** MICs of *A. genipiflora* stem bark extract and fractions against clinically significant bacteria in HTSPOTi assay

Microorganism	Minimum inhibitory concentration (μg/mL)				
	AG	AGPE	AGEt	AGM	Amoxicillin
<i>S. aureus</i>	> 500	> 500	> 500	250	3.91
<i>S. pyogenes</i>	500	> 500	> 500	> 500	1.95
<i>E. faecalis</i>	500	> 500	> 500	> 500	0.49
<i>P. aeruginosa</i>	> 500	> 500	500	> 500	500
<i>P. mirabilis</i>	250	250	250	> 500	31.25
<i>K. pneumoniae</i>	> 500	> 500	250	> 500	31.25
<i>S. typhi</i>	> 500	> 500	500	> 500	62.50
<i>E. coli</i>	250	> 500	> 500	> 500	125
<i>V. cholerae</i>	500	500	500	500	125

AG-whole extract, AGPE- pet ether fraction, AGET - EtOAc fraction, AGM - MeOH fraction

**Table 3** MICs of isolated compounds from *A. genipiflora* stem bark extract

Microorganism	Minimum inhibitory concentration (μg/mL)			
	1	2	3	Amoxicillin
<i>S. aureus</i>	15.63	31.25	31.25	10
<i>E. faecalis</i>	15.63	31.25	31.25	10
<i>E. coli</i>	15.63	125	125	20
<i>P. aeruginosa</i>	15.63	250	125	> 320

MeOH by gradient elution. Thin layer chromatography (TLC) was done using pre-coated silica gel 60 TLC plates (GF254 0.25 mm, Alpha laboratories, UK). Three pure compounds and one phytosterol mixture were isolated (Fig. 1). Characterization of the compounds was achieved by comparing their <sup>1</sup>H, <sup>13</sup>C NMR and mass spectral data with published data. Details of the isolation procedure are presented in supplementary material (See Additional File 1).

**Antimicrobial testing****Bacterial strains and inoculum standardization**

Clinical and American type culture collection (ATCC) bacteria strains were provided by the cell culture laboratory of the Department of Pharmacology, KNUST. They included Gram-positive bacteria: *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Streptococcus pyogenes*- clinical strain and Gram-negative bacteria: *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 12453, *Klebsiella pneumoniae*-clinical strain, *Salmonella typhi*-clinical strain, *Escherichia coli* ATCC 25922 and *Vibrio cholerae*-clinical strain.

A standardized bacteria culture was prepared from overnight cultures (prepared by inoculating sterile nutrient broth with test organism and incubating at 37 °C for 24 h). The organisms were then collected and standardized by serial dilution in sterile normal saline to achieve an initial cell count of approximately 1 × 10<sup>5</sup> CFU/mL.

**Evaluation of antibacterial activity of crude extracts and fractions**

The antibacterial activity of the whole extract and major fractions was tested by the high-throughput spot culture growth inhibition assay (HT-SPOTi) as previously described [14]. Amoxicillin was included as the positive control and 2% DMSO, negative control. The experiment was performed in triplicate.

**Determination of the antibacterial activity and antibiotic modulation effect of compounds**

The MIC of isolated compounds against two WHO high priority (*S. aureus* and *E. faecalis*) and WHO critical priority organisms (*P. aeruginosa* and *E. coli*) was

**Table 4** Minimum inhibitory concentration (MIC) of Amoxicillin in the absence or presence of compounds at ¼ MIC concentration

Minimum Inhibitory Concentration (µg/mL)							
<i>P. aeruginosa</i>				<i>E. coli</i>			
MIC	MIC combined			MIC	MIC combined		
Amox	1	2	3	Amox	1	2	3
> 320	< 31.25	< 31.25	< 31.25	20	< 0.625	0.625	0.625
MF	< 10	< 10	< 10		< 32	32	32

MF: Modulation factor, Amox-Amoxicillin

determined by the broth dilution method as previously described by Cos, 2006 [15]. Amoxicillin was included as positive control and 2% DMSO as negative control.

For the modulation test, the MIC of amoxicillin was determined in the absence or presence of the isolated compounds against *P. aeruginosa* and *E. coli* using the broth dilution assay as previously described [16]. The modulation factor (MF) was used to express the modulation effect of the compound on the MIC of amoxicillin and was calculated as the ratio of the MIC of the antibiotic alone and the MIC of antibiotic in the presence of compounds.

#### Biofilm inhibition assay

The effect of the crude extract and isolated compounds on biofilm formation by *S. aureus*, *E. coli* and *P. aeruginosa* was investigated using the microplate crystal violet stain retention method as previously described [17]. As controls, wells containing only medium without test samples were included. The experiment was performed in triplicate.

#### Ethidium bromide (EtBr) accumulation assay (efflux pump inhibition assay)

The crude extract and isolated compounds were investigated for their ability to enhance the accumulation of EtBr (a substrate for efflux pumps) into bacterial cells according to a previously described method [16]. The

known efflux pump inhibitors (EPI) verapamil and chlorpromazine were included as comparative probes and DMSO as the negative control. The experiment was performed in triplicate.

#### Data management and analysis

All experimental results were analyzed using Graph Pad Prism (Version 5 for windows, San Diego, USA).

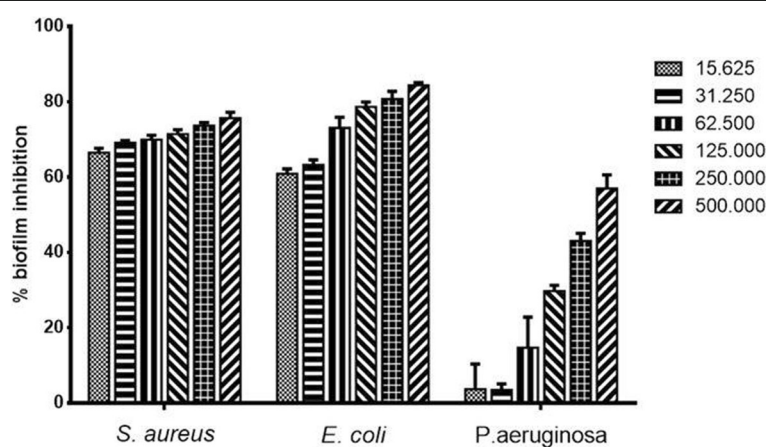
## Results

### Preliminary phytochemical investigation of the stem bark of *A. genipiflora*

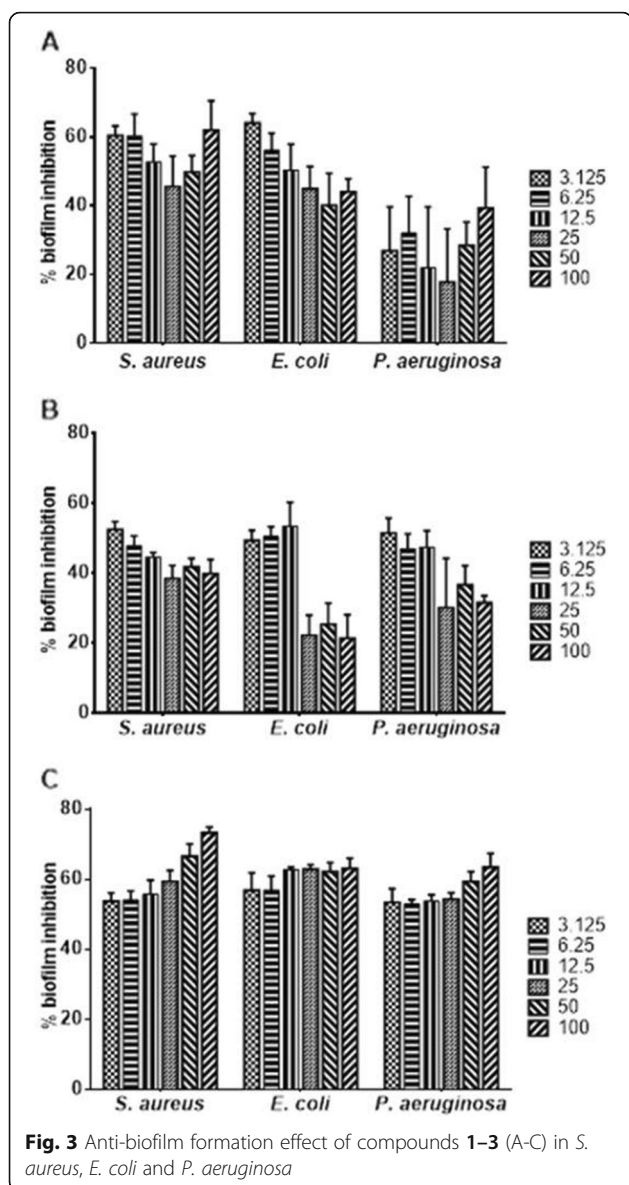
The result of preliminary phytochemical screening performed on the dried powdered stem bark of *A. genipiflora* revealed the presence of some classes of plant secondary metabolites as presented on Table 1.

### Isolated compounds from the stem bark of *A. genipiflora*

Chromatographic fractionation and purification of the bioactive EtOAc fraction resulted in the isolation of five known compounds identified based on their <sup>1</sup>H and <sup>13</sup>C NMR data. All spectral and physicochemical data obtained for the compounds matched those reported in literature for oleanonic acid (1) [18], 4-hydroxy cinnamic acid docosyl ester (2) [19], β-stigmasterol and β-sitosterol (3a/3b; 2:3) [20] and D-mannitol (4) [21] (Fig. 1). These compounds are known to occur in several

**Fig. 2** Anti-biofilm formation effect of AG in *S. aureus*, *E. coli* and *P. aeruginosa*





plant species but are being reported for the first time from *A. genipiflora*. The spectral data of the compounds have been provided in the supplementary material (See Additional File 1).

#### Antibacterial activity of *A. genipiflora* stem bark extract and major fractions

The whole extract, MeOH, EtOAc and pet-ether fractions exhibited varying degrees of growth inhibition towards Gram-positive and Gram-negative bacteria in the HTSPOTi assay. For susceptible bacteria, the MIC ranged between 250 and 500 µg/mL and varied with respect to the different bacteria and solvent extract (Table 2). *P. mirabilis* was the most susceptible organism being inhibited at 250 µg/mL by most extracts. Amoxicillin had variable inhibitory activities depending

on the organisms tested with the highest inhibitory effect (MIC < 10 µg/mL) against the Gram positive bacteria i.e. *S. aureus*, *S. pyogenes* and *E. faecalis*. *P. aeruginosa* was the least susceptible to amoxicillin.

#### Antimicrobial activity and resistance modulation effect of isolated compounds

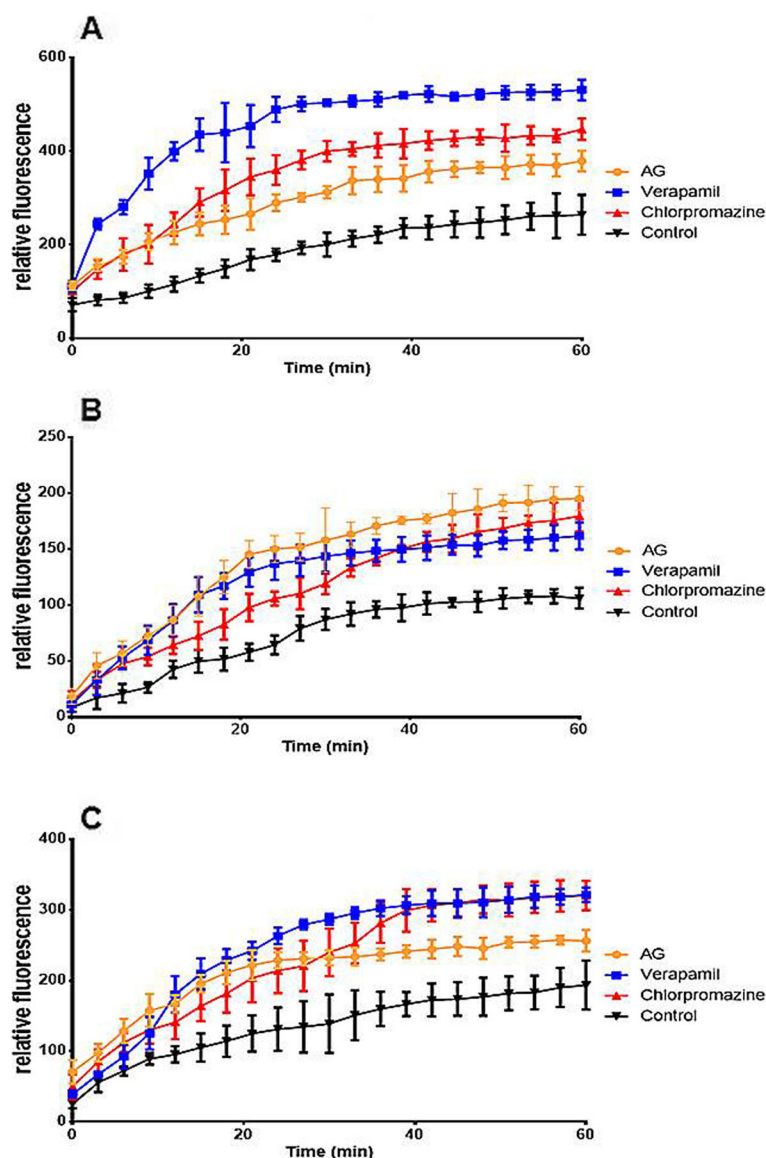
The antimicrobial activity of the isolated compounds was investigated against *S. aureus*, *P. aeruginosa*, *E. coli* and *E. faecalis* (WHO high priority and critical priority organisms, WHO, 2019) in the broth dilution assay. The minimum inhibitory concentrations are presented on Table 3. All compounds demonstrated antibacterial activity at MIC range between 15 and 250 µg/mL (Table 3). Compound 1 demonstrated the highest inhibitory effect against the test organisms at MIC 15.6 µg/mL. Compounds 2 and 3 showed varying inhibitory effects against the organisms with the Gram negative bacteria being less susceptible (MIC: 125–250 µg/mL) than the Gram positive (MIC: 31.25 µg/mL).

The isolated compounds at sub-inhibitory concentrations (1/4 MIC) were investigated for antibacterial resistance modulatory effect in amoxicillin against *E. coli* and *P. aeruginosa*. From the results, all compounds when co-administered with amoxicillin notably potentiated its antibacterial activity against *P. aeruginosa* and *E. coli* (Table 4). The MIC of amoxicillin in the presence of the isolated compounds was reduced from 320 to 31.25 µg/mL i.e. modulation factor (MF) of 10 for *P. aeruginosa*. In *E. coli*, the MIC of amoxicillin was reduced from 20 to 0.625 µg/mL i.e. MF = 32.

#### Biofilm formation inhibitory effect of *A. genipiflora* stem bark extract and isolated compounds

The whole extract of *A. genipiflora* stem bark (AG) was investigated for biofilm formation inhibitory effect against *S. aureus*, *E. coli* and *P. aeruginosa* in the crystal violet retention assay. The extract exhibited remarkable concentration-dependent inhibition of biofilm formation between 15.6–500 µg/mL. The highest anti-biofilm formation effect was expressed against *E. coli* followed by *S. aureus* then *P. aeruginosa* (Fig. 2). The percentage biofilm inhibition against *E. coli* at its sub-inhibitory concentration (15–125 µg/mL) ranged between 60 and 79%. For *S. aureus* and *P. aeruginosa*, the percentage biofilm inhibition ranged between 60 and 76% and 3–57% respectively at sub-inhibitory concentration of 15–500 µg/mL.

The effect of compounds 1, 2 and 3a/b (3.1–100 µg/mL) on biofilm formation in *S. aureus*, *E. coli* and *P. aeruginosa* is presented on Fig. 3. The highest inhibition of biofilm formation was given by compound 3a/b against *S. aureus* (73% inhibition) in a concentration-dependent manner. The percentage biofilm inhibition for the other



**Fig. 4** Efflux pump inhibition effect of AG ( $\frac{1}{2}$  MIC) in *S. aureus* (A), *E. coli* (B), *P. aeruginosa* (C)

compounds against the test organisms ranged between 17 and 66% and was not concentration dependent. *P. aeruginosa* was the least susceptible to all test compounds.

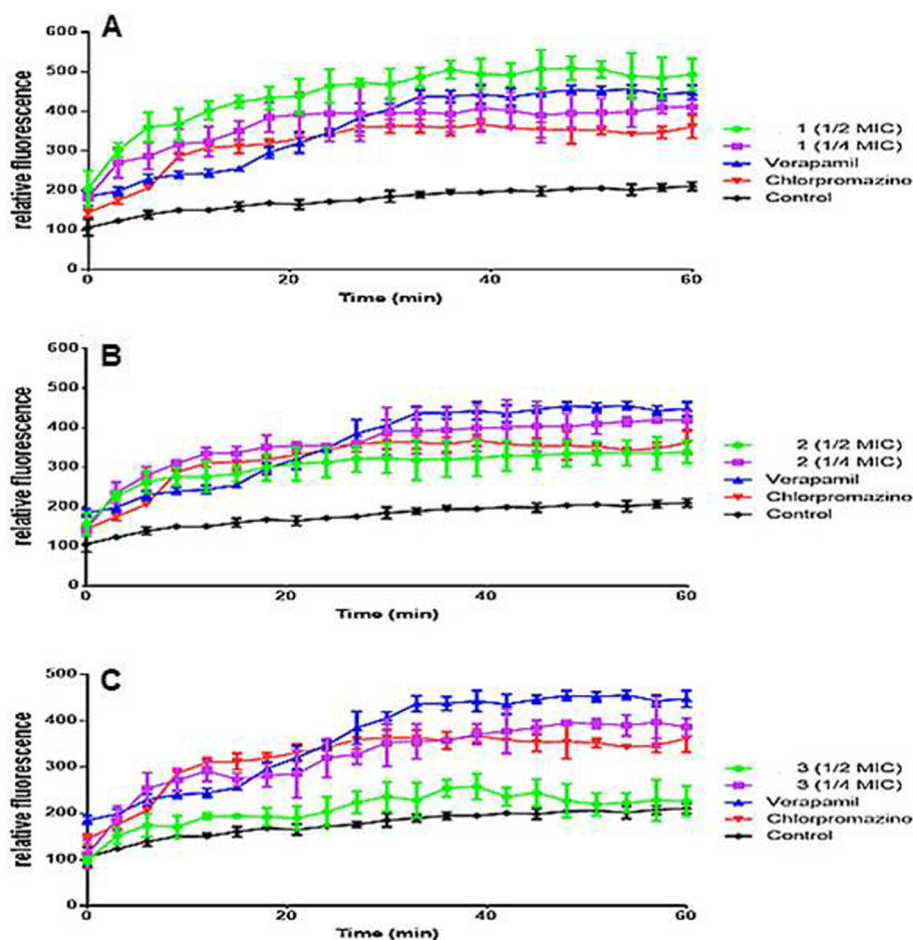
#### Effect of *A. genipiflora* stem bark extract and isolated compounds on ethidium bromide (EtBr) accumulation in bacterial cells

The potential of the crude extract (AG) and isolated compounds (1, 2, 3) to act as efflux pump inhibitors was investigated by the EtBr accumulation assay. EtBr, a substrate for several multidrug resistant efflux pumps, emits a strong fluorescent signal when bound to DNA intracellularly and only has a weak signal when present extracellularly. Thus, the activity of putative EPIs can be

measured fluorometrically due to the retention of fluorescence overtime if efflux is reduced [22].

Figure 4 shows the EtBr accumulation behaviour in *S. aureus*, *E. coli* and *P. aeruginosa* in the presence of the extract (AG) compared to the action of two standard EPIs, verapamil (VP) and chlorpromazine (CP) measured over 60 mins. From the results it can be inferred that in *E. coli*, the crude extract (AG) exhibited remarkable efflux pump inhibition resulting in much higher EtBr fluorescence than both standard EPIs (Fig. 4b). However, in *S. aureus* and *P. aeruginosa*, the standard EPIs proved much effective in causing higher accumulation of EtBr in the bacterial cells than the test extract (Fig. 4a and c respectively).

Figure 5 shows the EtBr accumulation behaviour in *E. coli* in the presence of isolated compounds. Compound



**Fig. 5** Efflux pump inhibition effect of compounds 1–3 (A–C) in *E. coli*

1 at  $\frac{1}{2}$  MIC showed a superior EtBr accumulation than both VP and CP. At  $\frac{1}{4}$  MIC however, its effect was higher than CP but lower than VP (Fig. 5a). For compound 2, EtBr accumulation was higher at  $\frac{1}{4}$  MIC than  $\frac{1}{2}$  MIC. VP was superior to compound 2 at all concentrations tested. At  $\frac{1}{4}$  MIC, compound 2 showed a higher effect than CP but lower than VP. At  $\frac{1}{2}$  MIC however its effect was similar to that of CP (Fig. 5b). Compound 3 at  $\frac{1}{2}$  MIC showed little or no effect on EtBr accumulation activity as its effect was similar to the negative control. At  $\frac{1}{4}$  MIC however, it showed a better potential to accumulate EtBr than CP but lower than VP (Fig. 5c).

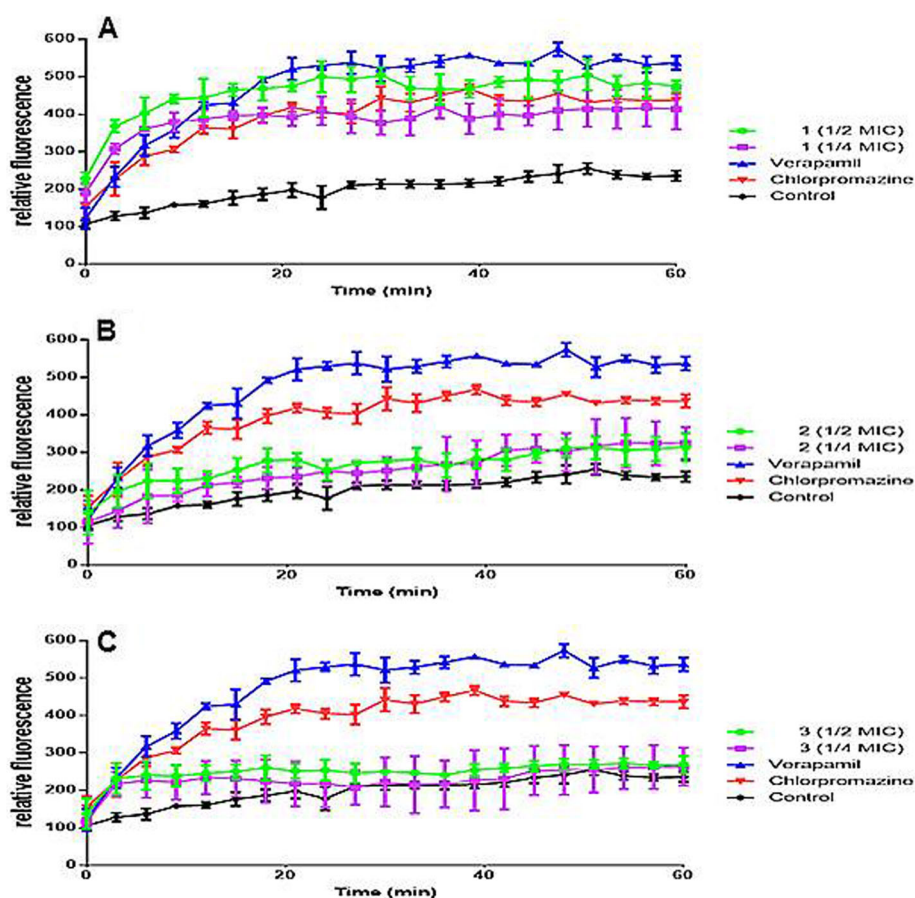
The EtBr accumulation enhancement behaviour of compounds 1, 2, and 3 in *P. aeruginosa* is demonstrated in Fig. 6. Compound 1 at  $\frac{1}{2}$  MIC enhanced accumulation of EtBr slightly higher than CP but lower than VP. At  $\frac{1}{4}$  MIC, its effect was lower than both VP and CP (Fig. 6a). Compounds 2 and 3 at the test concentrations showed no obvious increase on the accumulation of EtBr in *P. aeruginosa* over 60 min (Fig. 6b and c).

## Discussion

This study investigated the antimicrobial activity of *A. genipiflora* stem bark extract (MeOH/ $\text{CHCl}_3$ ), three solvent fractions (i.e. pet-ether, EtOAc and MeOH fractions) and some isolated constituents. The effect of the whole extract and isolated compounds on bacteria bio-film formation and efflux-pump inhibition as well as resistance modulation potential of isolated compounds on the antibacterial activity of amoxicillin was also evaluated.

Preliminary phytochemical studies revealed the presence of classes of secondary metabolites (Table 1) that exhibit a wide range of biological activities including antimicrobial, anti-inflammatory and antioxidant effects [23] supporting the use of the plant in traditional medicine. Other *Aidia* species were reported to contain coumarins, phenolic acids (catechol) and fatty acid esters [24]. From antibacterial screening of the whole extract and fractions, the MIC ranged from 250 to  $> 500$   $\mu\text{g/mL}$ . In general, the extract and fractions had quite high MICs for most of the organisms tested (MIC  $\geq 500$ ).





**Fig. 6** Efflux pump inhibition effect of compounds 1–3 (A–C) in *P. aeruginosa*

Nevertheless, the antibacterial activity observed for AG, AGPE and AGEt towards *P. mirabilis* (MIC = 250 µg/mL), AG towards *E. coli* (MIC = 250 µg/mL), AGET towards *K. pneumonia* (MIC = 250 µg/mL) and AGM towards *S. aureus* (MIC = 250 µg/mL) are notable (Table 2). The antimicrobial activity of plant extracts can be classified as significant (MIC < 100 µg/mL), moderate (100 ≤ MIC ≤ 625 µg/mL) or weak (MIC > 625 µg/mL) [25]. By this criterion, the whole extract and fractions can be said to have demonstrated moderate antibacterial activity. In the genus *Aidia*, antibacterial activity against *B. subtilis*, *E. coli*, *P. aeruginosa* and *S. aureus* was reported for the MeOH and aqueous extracts of the leaf of *Aidia boorneensis* [24].

Detailed phytochemical investigation led to the isolation of oleanonic acid (1) [18], 4-hydroxy cinnamic acid docosyl ester (2) [19], β-stigmasterol and β-sitosterol (3a/3b; 2:1) [20] and D-mannitol (4) [21] (Fig. 1). These compounds are known to occur in several plant species but are being reported for the first time from *A. genipiflora*. All compounds demonstrated antibacterial activity at MIC range between 15 and 250 µg/mL (Table 3). The MICs of isolated compounds being lower than the

parent fraction implies that fractionation led to more active samples. For pure compounds, antimicrobial activity may be classified as significant (MIC < 10 µg/mL), moderate (10 ≤ MIC ≤ 100 µg/mL) or weak (MIC > 100 µg/mL) [25, 26]. Though none of the compounds had MIC < 10 µg/mL, the antibacterial activity of compound 1 against all organisms (MIC 15.6 µg/mL) and compounds 2 and 3a/b against *S. aureus* and *E. faecalis* (MIC = 31.25 µg/mL) are remarkable. In previous studies, oleanonic acid (1) demonstrated significant antibacterial activity against pathogenic Gram positive and Gram negative bacteria at MIC range between 10 and 50 µg/mL [27] and against methicillin resistant *S. aureus* (MRSA) at 10 µg/mL [28]. The anti-tubercular activity of oleanonic acid (1) against *Mycobacterium tuberculosis* was previously reported [18]. β-stigmasterol and β-sitosterol (3a/b) have demonstrated broad spectrum antibacterial activity against several pathogenic bacteria in previous studies [29–32]. This is the first report on the antibacterial activity of 4-hydroxy cinnamic acid docosyl ester (2) though some cinnamic acid derivatives have been shown to possess very potent antibacterial activity [33]. The presence of these compounds in *A.*



*genipiflora* may thus contribute to its antibacterial activity. In modulatory tests, the MIC of amoxicillin in the presence of the isolated compounds was reduced by 10 folds for *P. aeruginosa* and 32 folds in *E. coli*. This suggests that the isolated compounds have the ability to potentiate the antibacterial effect of amoxicillin against these Gram negative bacteria. In previous studies, oleanolic acid, the parent derivative of compound **1** was found to synergistically potentiate the antibacterial activity of ampicillin and oxacillin towards *S. aureus*, *P. aeruginosa*, *L. monocytogenes*, *S. epidermidis* [34]. A combination of stigmasterol (**3a**) and ampicillin resulted in a significant increase in the effect of ampicillin against clinical isolates of *S. aureus*, *E. coli*, *P. aeruginosa* and *S. pyogenes* [35]. The synergistic action of plant-derived compounds and antibiotics has been proposed to be by the interaction of these agents with bacterial resistance mechanisms [36] such as destruction of the bacterial membrane structure, increasing the influx of antibiotics into the bacteria cell [37] inhibition of bacterial efflux pumps, inhibition of quorum sensing and some gene expression modulations [22, 38].

The whole extract and isolated compounds further showed remarkable inhibition of biofilm formation in *S. aureus*, *E. coli* and *P. aeruginosa*. Several plant extracts and plant derived compounds have been shown to prevent the adhesion or implantation of planktonic forms of bacterial cells on abiotic surfaces thus inhibiting the formation of biofilms [39–41]. Though this is the first report on the anti-biofilm formation potential of *A. genipiflora* and its constituents, previous studies have reported the biofilm inhibitory activity of similar bioactive constituents. Cinnamic acid derivatives of natural origin exhibited significant quorum sensing and biofilm inhibition in *Chromobacterium violaceum* increasing its susceptibility to tobramycin [42]. Pentacyclic triterpenes and some sterols including  $\beta$ -sitosterol (**3b**) also demonstrated remarkable anti-biofilm activities [43, 44]. Extract and plant derived compounds may inhibit biofilm formation by damaging microbial membrane structures, inhibiting peptidoglycan synthesis [45], inhibition of nucleic acid synthesis [46], quorum sensing inhibition or disruption [47] and anti-cell-adhesion properties [48].

The whole extract demonstrated potential to inhibit efflux pumps in *E. coli* and to a lesser extent in *S. aureus* and *P. aeruginosa*. For pure compounds as well, efflux pump inhibition was higher in *E. coli* than *P. aeruginosa*. It was also interesting to note that lower concentrations (1/4 MIC) of compounds **2** and **3** exhibited far greater EPI activity in *E. coli* than more concentrated samples (1/2 MIC) (Figs. 5 and 6). This observation is consistent with a previous reports on the EPI activity of some *Berberis* spp [49] which was attributed to the possibility of putative EPIs binding at only high affinity sites of the

efflux pump causing greater inhibition at only low concentrations whereas higher concentrations would result in low affinity binding sites being occupied.

The antibacterial activity demonstrated by *A. genipiflora* and its constituents in this study has revealed the prospects of this plant as a potential source of new antibacterial agents and adjuvants that could possibly interact with some resistance mechanisms in bacteria to enhance the activity of antibiotics that have been rendered inactive by resistant organisms.

## Conclusion

This study has provided the first evidence of the antimicrobial, resistance modulation, anti-biofilm formation and efflux pump inhibitory potentials of *A. genipiflora* stem bark and its constituents. This gives scientific credence to the traditional uses of the stem bark of *A. genipiflora* for managing infections. Although oleanonic acid,  $\beta$ -stigmasterol,  $\beta$ -sitosterol, 4-hydroxy cinnamic acid docosyl ester and D-mannitol are known plant bioactive compounds, this is the first report of their isolation from *A. genipiflora* as well as the genus, *Aidia*.

*“A small portion of the study has been presented in a conference in the form of poster [50]”*

## Abbreviations

AG: *A. genipiflora* stem bark whole extract; AGPE: *A. genipiflora* stem bark pet ether fraction; AGEt: *A. genipiflora* stem bark ethyl acetate fraction; AGM: *A. genipiflora* stem bark methanol fraction; AMR: Antimicrobial resistance; AMOX: Amoxicillin; ATCC: American type culture collection; CC: Column chromatography; CFU: Colony forming unit; CHCl<sub>3</sub>: Chloroform; CP: Chlpromazine; CV: Crystal violet; DMSO: Dimethyl sulfoxide; HT-SPOTi: High throughput spot culture growth inhibition; EtBr: Ethidium bromide; EtOAc: Ethyl acetate; MeOH: Methanol; MIC: Minimum inhibitory concentration; MF: Modulation factor; MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; NMR: Nuclear magnetic resonance; PCR: Polymerase chain reaction; TLC: Thin layer chromatography; VP: Verapamil

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40816-021-00266-4>.

**Additional file 1.** Methods of isolation of compounds and spectroscopic data of isolated compounds from *Aidia genipiflora* stem bark.

## Acknowledgements

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## Authors' contributions

DA and AYM developed the idea and designed the study. DA, CAD and IKA performed antimicrobial studies and analysis of results. DA and EAK carried out chromatographic studies and were major contributors in writing

manuscript. BKH and LO were responsible for the structural elucidation of the compounds. All authors read and approved the manuscript.

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#### Availability of data and materials

The dataset supporting the conclusions of this article are included within the article and its additional files. Raw data sets used and analysed during the study are available from the corresponding author on reasonable request.

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Competing interests

Authors have no conflict of interest to declare.

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