IJISET - International Journal of Innovative Science, Engineering & Technology, Vol. 6 Issue 9, September 2019
ISSN (Online) 2348 – 7968 | Impact Factor (2019) – 6.248
www.ijiset.com

The Susceptibility of *Escherichia Coli* Biofilms to Various Extracts of Senna Siamea Plant

Usman, Nasiru Adoyiza[a] , Prof. Adeshina, G.O[b] , Dr Tytler, Babajide A[c] ,Abdulrahim, Umar[d]

[a] Department of pharmaceutics & pharmaceutical microbiology, Faculty of pharmacy, University of Maiduguri, Nigeria.
[b] Department of pharmaceutics & pharmaceutical microbiology, Faculty of pharmaceutical science. Ahmadu Bello University, Zaria, Nigeria.
[c] Department of pharmaceutics & pharmaceutical microbiology, Faculty of pharmaceutical science. Ahmadu Bello University, Zaria, Nigeria.
[d] Department of pharmaceutics & pharmaceutical microbiology, Faculty of pharmaceutical science, University of Maiduguri, Nigeria
Corresponding author : Usman Nasiru Adoyiza, Email address : nastradoy@gmail.com

ABSTRACT

Escherichia coli biofilm has remained an albatross in the treatment of many urinary tract infections throughout the world. Recently developed antibiofilm agents include ajoene from garlic, flavonoids like phloretin and proanthocyanidins from cranberry. In this work, our aim was to evaluate the antiadhesive and antibiofilm activities of the leaf, stem bark and root extracts of *Senna siamea* plant. Crude extracts of this plant parts were prepared using ethanol as the solvent for the cold extraction. Phytochemical screening of the various extracts were performed using standard methods. Isolates from urine, stool and higher vaginal swab samples were collected from the Medical Microbiology Department of Ahmadu Bello University Teaching Hospital (ABUTH), Zaria. The isolates were identified and confirmed using the microgen GN, A+B kit. Congo red assay was used to determine their biofilm production. Antimicrobial susceptibility testing of the crude extracts against the isolates was carried out using the agar diffusion method. The Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) were determined for the stem bark extract of the plant using micro broth dilution and agar diffusion methods respectively. In order to establish the antiadhesive and antibiofilm activities of the stem bark extract of the plant, sub-inhibitory concentrations (sub-MIC) were used against the test isolates in the remaining assays in the work. The stem bark extract of the plant was further partitioned using n-hexane, ethyl acetate and n-butanol in order to determine the effect of solvent extraction on its antibiofilm activities. Eight of the isolates collected were

confirmed to be *Escherichia coli*. Five of the confirmed isolates were found to be biofilm-formers. The Phytochemical screening showed that all the extracts contained steroid and terpenes, cardiac glycoside, tannins, flavonoids and alkaloids. The stem bark extract had the highest zones of inhibition ranging from 10-18 mm in diameter against the bacterial isolates. The MIC and MBC of the stem bark extract were 250 mg/ml and 500 mg/ml respectively. The highest percentage biofilm inhibition observed was 90.6% from the antiadhesive assay and 88.7% from the antibiofilm assay. The n-butanol extract showed the best activity with percentage biofilm inhibition between 92.7% to 84.0%. against the *Escherichia coli* isolates. From this work, *Senna siamea* stem bark ethanol extract showed antibiofilm activity against test from urine, stool and higher vaginal swab samples from Ahmadu Bello University Teaching Hospital, Zaria, Nigeria.

KEYWORDS : *Senna siamea*, *Escherichia coli*, biofilm, isolates, extracts

INTRODUCTION

Biofilm is a community of microorganism adhering to biotic or abiotic surfaces embedded by a self-produced extra-polymeric matrix facilitating the survival in an adverse environment. A biofilm is formed and maintained via cell-to-cell communication. A biofilm first forms when one or a few cells attach to a surface. These first cells produce proteins that act as signals to nearby cells. The signals are detected by neighboring cells and essentially recruit new cells into the colony. As the nearby cells detect the chemical cues they aggregate and begin to form the biofilm. These cells then send out additional signals, recruiting more cells to the colony and growing the biofilm. The proteins also signal the development of polysaccharides that will form the slime layer. This slime layer forms over and around the growing colony (Madigan *et al.*, 2006).

The formation of biofilms markedly impedes the treatment of urinary tract infections by protecting encased bacteria from both the host immune response and antimicrobial therapy. Bacteria are in close proximity within the biofilm, facilitating the exchange of genetic material, such as antimicrobial resistance plasmids and transposomes (Flemming *et al*., 2010 ; Aminov *et*

al., 2011). Once attached to the surface, *Escherichia coli* change from a planktonic form to a sessile form which replicate while producing extracellular matrix (ECM). As the biofilm colony grows and matures, *Esherichia coli* within the ECM respond to signals from their surrounding environment, eventually leading to a portion of the encased bacteria dispersing from the biofilm colony (Kostakioti *et al*., 2013 ; Kaplan *et al*., 2010). The dispersed bacteria can return to the planktonic form or continue the process of biofilm formation elsewhere. Alternatively, the bacteria can form quiescent reservoirs in the urothelium which are thought to contribute to recurrent infection (Schilling *et al*., 2012).

Many antibiofilm compounds have been discovered and are at various stages of development. Some are naturally occurring compounds while others are synthetically-derived molecules. Natural antibiofilm agents include ajoene from garlic, flavonoids like phloretin and proanthocyanidins from cranberry while synthetic antibiofilm molecules include the pyrrole-imidazole alkaloids (PIA) especially oroidin **1**, sceptrin **2**, and bromoageliferin **3.** However, there are currently no antibiofilm drugs with dosage regimen in clinical use today. Therefore, bacteria forced into forming stronger biofilms will become more difficult to treat and will cause more severe chronic infections. Adults will suffer protracted lung infections as the bacteria hunker down into their protective slime, and children will have repeated ear infections. What may appear to be antibiotic resistance when an infection does not clear up may actually be biofilms at work. (Siva *et al.*, 2014).

In this work, we beam our search light into *Senna siamea* plant. *Senna siamea* which belongs to the sub-family fabaceae (Caesalpinioideae) of family leguminosae has its leaf being ethno medicinally used as laxative, blood cleaning agent, cure for digestive system, urinogenitory disorders, herpes and rhinitis (Aliyu, 2006). It is our aim to evaluate its leaf, stem bark and root extracts for antibacterial activities with a view to using sub-minimal inhibitory concentrations of these extracts against established biofilm-forming *Escherichia coli* isolates. Furthermore, we intend to fractionate the most biofilm-inhibiting of the three extracts above using n-hexane, ethyl acetate and n-butanol successively. The obtained fractions will then be tested against the test *Escherichia coli* isolates. *Senna siamea* plant

MATERIALS AND METHODS

Leaves, stem bark and root parts of *Senna siamea* tree behind Ahmadu Bello University Fire Service were collected while the plant was identified at the herbarium section of the Biological Science Department, Ahmadu Bello University, Zaria, with the voucher number 613. They were shade dried, homogenised and extracted with ethanol through cold extraction for three days. After decantation, the filtrates were concentrated and dried in a water bath at temperature of 40^{o} C. The pellets of standard strain *Escherichia coli* WDCM 00013 (from Sigma Aldrich, Germany) were propagated as provided by the American Type Culture Collection, 2014. The test isolates, *Escherichia coli* 2094, 2088, 587, 367 (from urine samples), 034, 555, 304 (from stool samples) and 520 (from higher vaginal swab) of patients attending Ahmadu Bello University Teaching Hospital, Shika, Kaduna, Nigeria, were donated by the head of the Medical Microbiology department of the hospital. They were purified by regularly subculturing them on tryptic soy agar slant at 37^{o} C for 24 hours. They were then subjected to Microgen^{TM} identification kit and their identity finally confirmed using the Microgen identification system software.

The congo red assay for detection of biofilm-forming capacity as described by Freeman *et al.* (1989). The isolates were then subjected to antibacterial susceptibility testing using agar well diffusion method according to Clinical Laboratory Standards Institute (CLSI) guidelines.

Broth dilution method was adopted to determine the minimum inhibitory concentrations (MIC) of the most active extract based on zones of inhibition of bacterial growth. Minimum bactericidal concentration (MBC) was also determined for the extract using the agar dilution method.

The antibiofilm activity of the most active plant extract was determined by the method described by Filoche *et al.*(2005). Overnight culture of all the test organisms were grown to provide pre-formed biofilms. One hundred microlitre (100µl) of each of these cultures was put into a 96-well microtitre plate. The plate was then incubated for further 4 hours at 37^{0}C to allow cells' attachment. Next, 100 µl of the plant extract at sub-inhibitory concentrations was added to the wells. The plate was then incubated at 37^{0}C for further 24 hours. Following 24 hour incubation, the supernatant was removed and each well was rinsed with sterile saline for three times. The crystal violet assay was then used to assess the biomass of the attached cells using optical density reader at 630 nm.

The extract was then subjected to partitioning. The three solvents used in this solvent-solvent extraction were n-hexane (non-polar), ethyl acetate (moderately polar) and n-butanol (polar).

20 g of the extract was dissolved in 200 ml of water and poured into a mounted separating funnel with the valve tightly stoppered. Next, 300 ml of n-hexane was added to the dissolved extract and the separating funnel shaken until two distinct layers, organic layer (at the top) and the aqueous layer (at the bottom) separates out. The lower aqueous layer was drained out into an ehlenmeyer flask, while the upper n-hexane fraction was kept in a beaker and labeled accordingly. The aqueous fraction was transferred back into the separating funnel and a fresh 300 ml of n-hexane was added to it. The funnel shaken gently until the two distinct layers separate again. The lower aqueous layer drained out and the upper n-hexane layer collected and added to the previously collected one. This process was repeated the third time and the pool of the n-hexane fraction collected was heated to dryness in a water bath at temperature of 40º C.

The aqueous phase of the mixture was transferred back into the separating funnel and 300 ml of ethyl acetate was added to it, the mixture shaken until two distinct upper layer (ethyl acetate) and the lower layer (aqueous fraction) separates out. The lower layer was drained into an ehlenmeyer flask and the upper layer collected in a beaker and labeled accordingly. The aqueous layer was transferred into the separating funnel and a fresh 300 ml of ethyl acetate added to it, the mixture shaken and the two distinct layers collected accordingly. The collected ethyl acetate layer was added to the previously collected one. This process was repeated the third time and the collected pool of ethyl acetate fraction was evaporated to dryness in a water bath at temperature of 40º C.

Next, the aqueous phase of the mixture was poured back into the separating funnel and 300 ml of n-butanol was added and the mixture shaken. A little more n-butanol was added gradually until saturation point between n-butanol and water was reached. This was necessary to bring about the two distinct layers of n-butanol (upper) and aqueous phase (lower). Both layers were collected and labeled accordingly. The aqueous layer was poured back into the separating funnel and another 300 ml of n-butanol added and the separating funnel shaken until the two layers separated distinctly and they were collected into their respective containers. The n-butanol fraction was added to the previously collected one. This process was repeated the third time and

the pool of the n-butanol fraction was evaporated to dryness in a water bath at temperature of 40º C. The aqueous fraction was also evaporated to dryness in a water bath at temperature of 50º C.

The obtained fractions were then subjected to antibiofilm assay. Overnight culture of all the test organisms were grown to provide pre-formed biofilms and 100 µl of each of these cultures was put into a 96-well microtitre plate. The plate was then incubated for further 4 hours at 37^0 C to allow cell attachment after which100 µl of each of the fractions at sub-inhibitory concentrations was added to the wells. The plate was then incubated at 37^0 C for further 24 hours. After 24 hour incubation, the supernatant was removed and each well was rinsed with sterile saline for three times. The crystal violet assay was then used to assess the biomass of the attached cells using optical density reader at 630nm.

RESULTS

Antibacterial Susceptibility Testing results

Though all the three extracts of *Senna siamea* plant did not show a good antibacterial activity, the stem bark extract was chosen for the rest part of this study since it was the only extract with antibacterial activity in at least two concentrations or more in all the test isolates used in this study.

Minimum inhibitory concentration (MIC) of the leaf extract was determined to be 250 mg. The minimum bacteriocidal concentration (MBC) of the extract was found to be 500 mg/ml . Consequently, a sub-MIC concentrations of 200, 100, 50, 25, 12.5 and 6.25 mg/ml were adopted for the extract in the biofilm inhibition assay.

The result for the antibiofilm assay is as follows

Antibiofilm activity of Senna siamea stem bark on bacterial isolates

	Percentage biofilm inhibition (%)					
Concentration (mg/ml) / Isolates	**200**	**100**	**50**	**25**	**12.5**	**6.25**
2094	2.7	14	48	30	40	47
2088	37	51	70	76	77	70
587	34	35	53	42	58	43
034	0	62	87	79	86	89
367	34	59	77	79	80	75

Antibiofilm activities of the fractions

Tables 4.3, 4.4, 4.5 and 4.6 below show the antibiofilm activities of the various fractions on the bacteria isolates at sub-inhibitory concentrations used.

Antibiofilm activity of n-hexane fraction of Senna siamea stem bark on bacterial isolates

	Percentage biofilm inhibition (%)					
Concentration (mg/ml) / Isolates	**200**	**100**	**50**	**25**	**12.5**	**6.25**
2094	46	38	41	32	31	31
2088	85	89	82	86	84	76
587	76	75	66	72	83	70
034	60	65	70	56	66	60
367	76	76	70	75	81	82

Antibiofilm activity of ethyl acetate fraction of Senna siamea stem bark on bacterial isolates

	Percentage biofilm inhibition (%)					
Concentration (mg/ml) / Isolates	**200**	**100**	**50**	**25**	**12.5**	**6.25**
2094	40	20	44	31	46	44
2088	91	87	85	90	91	88
587	43	75	76	78	75	71
034	56	75	70	74	80	82
367	79	86	86	88	92	89

Antibiofilm activity of n-butanol fraction of Senna siamea stem bark on bacterial isolates

	Percentage biofilm inhibition (%)					
Concentration (mg/ml) / Isolates	**200**	**100**	**50**	**25**	**12.5**	**6.25**
2094	72	87	93	92	86	91
2088	91	93	99	96	98	98
587	84	93	86	92	98	97
034	87	88	74	93	97	94
367	88	95	91	91	95	96

Antibiofilm activity of aqueous fraction of Senna siamea stem bark on bacterial isolates

	Percentage biofilm inhibition (%)					
Concentration (mg/ml) / Isolates	**200**	**100**	**50**	**25**	**12.5**	**6.25**
2094	38	18	38	43	38	57
2088	75	83	93	90	90	94
587	60	45	72	72	74	62
034	57	66	65	83	85	73
367	57	75	74	83	85	86

DISCUSSION

Senna siamea plant was reported to possess antimicrobial activities by Mohammed *et al*. (2013). However, in this study, *Senna siamea* plant parts have not shown tangible antibacterial activities. Lack of significant antibacterial activity by the extracts of this plant may be explained by the fact the local usage of the plant for the treatment of typhoid is usually prolonged, perhaps to achieve the needed effective dosage level. This is in tandem with the study carried out by Doughari *et al*.(2008) in which the aqueous leaf extract of *Senna siamea* only sparingly inhibited the growth of *Salmonella typhi* even at the highest concentration used in the study. The absence of zones of inhibition at some of the concentrations used in this study is also similar to the result obtained by Doughari et al. (2008) where the aqueous leaf extract of *Senna siamea* did not show any activity against *Salmonella typhi* isolate at three consecutive concentrations used. This observation may be explained by the fact that some of the bioactive compounds responsible for antibacterial effect may have been interacting with environmental pollutant in the soil through phytoremediation. According to Thassitou et al. (2001), bioremediation which is a naturally occurring process

through which microorganisms transform environmental contaminants to innocuous end products may be responsible for the low antibacterial activities of medicinal plant. The percentage (%) biofilm inhibition show that *Senna siamea* stem bark extract is effective in destroying the biofilms of the isolates. This is evident in the high percentages of biofilm inhibitions observed in table 1. It is noteworthy to mention that in this work, the antibiofilm activities by the various extracts of *Senna siamea* were best at lower concentrations. This dose-independent response of the isolates to the extracts is similar to that observed by Gislene *et al* (2000) where it was reported that plant extracts like *Syzygyum joabolanum* inhibited the growth of resistant bacteria at lower concentration. Supporting this fact is the work of Marco *et al*. (2018) where it was reported that different essential oils extracted from mediterranean plants were able to destabilize biofilm at very low concentration without impairing bacterial viability.

Furthermore, Rui (2003) reported that natural phytochemicals at the low levels present in fruit and vegetables offer health benefits, but these compounds may not be effective or safe when consumed at higher doses, even in a pure dietary supplement form.

It has been discovered that solvent-solvent extraction increases the phytochemical activities of plant extract as elucidated by Samir *et al* (2017). This is evident in the higher percentages (%) of biofilm inhibition on the isolates in tables 2, 3, 4 and 5. It might be possible that the effect of phytochemicals from plant *Senna siamea* could possibly be influenced by dielectric constant, chemical structure of organic solvents, and as well as other chemical properties of plant phytochemicals. For example, the relationships between tannins contents and extraction solvents can be related to the polymerization degree for the tannins extracted by different solvents (Naima et al., 2015). The interactive abilities of solvent and tannins compounds are probably related to chemical reactions suggesting the rationale for the overall best antibiofilm activities exhibited by the n-butanol fraction of the stem bark extract. In addition, butanol is a good example of polar protic solvent which is key to interaction between solvents and secondary metabolites. Aqueous fraction of *Senna siamea* stem bark also showed a level of antibiofilm activity similar to butanol fraction. This according to Brian et al. (2012) may be due to resonance and inductive effects in which compounds with electron-withdrawing groups and extended conjugation ionize better leading to enhanced extractive power of the solvent. However, according to the phytochemical screening conducted at the beginning of this work, *Senna siamea* does not contain essential oil

and its absence in this plant could be the reason why n-hexane, polar compound used for its extraction had the least antibiofilm activity whereas, a moderately polar (polar aprotic) solvent gave an extract with a better antibiofilm activity.

CONCLUSION

In this study, *Senna siamea* stem bark ethanol extract showed antibiofilm activities. Much better antibiofilm activities were exhibited by the fractions of this extract against the same test bacterial isolates.

REFERENCES

Aliyu, B.S. (2006) West African ethnomedicinal plants, Triumph publishing company, kano, Nigeria.

Aminov, R.I. (2011). Horizontal gene exchange in environmental microbiota. *Frontiers in Microbiology*. 2:158. doi: 10.3389/fmicb.2011

Brian, A., Huffman, L., Michael, L., Poltash, M. and Christian A. (2012). Effect of Polar Protic and Polar Aprotic Solvents on Negative-Ion Electrospray Ionization and Chromatographic Separation of Small Acidic Molecules, *American Chemical Society*, 84(22) pp 9942-9950.

American Type Culture Collection. (2014). *Escherichia coli* (ATCC® 25922™)

Doughari J. H and Okafor N. B. (2008). Antibacterial activity of Senna siamae leaf extracts on Salmonella typhi. *African Journal of Microbiology Research*. Vol.(2) pp.042-046.

Filoche SK, Soma K, and Sissons CH. (2005). Antimicrobial effects of essential oils in combination with chlorhexidine digluconate. *Oral Microbiology and Immunology*, 20, 221–225

Flemming H.C., Wingender J. (2010). The biofilm matrix. National Review 0f Microbiology 8:623–633. doi: 10.1038/nrmicro2415

Freeman, D.J., Falkiner, F.R. and Keane, C.T. (1989). New method for detecting slime production by coagulase negative staphylococci. *Journal of Clinical Pathology*, 42 (1989), pp. 872-874.

Gislene, G.F.N., Juliana, L., Paulo, C.F. and Giuliana, L.S., 2000. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian Journal of Microbiology*, 31, 247-256.

Kaplan J.B. (2010). Biofilm dispersal: Mechanisms, clinical implications, and potential therapeutic uses. *Journal of Dental Resucitation* .89:205–218. doi: 10.1177/0022034509359403

Kostakioti M., Hadjifrangiskou M. and Hultgren S.J (2013). Bacterial biofilms: Development, dispersal, and therapeutic strategies in the dawn of the post antibiotic era. *Cold Spring Herbs ; Perspectives in Medicines* 3:a010306. doi: 10.1101/cshperspect.a010306

Madigan, M. T., and Martinko, J. M. (2006). Brock biology of microorganisms, 11th. edition. Chapter19. Upper Saddle River, NJ: Pearson Prentice Hall. ISBN: 0-13-144329-1.

Marco A., Alexandros P., Rosanna P. and Mijat. (2018). Antimicrobial and Antibiofilm Activity and Machine Learning Classification Analysis of Essential Oils from Different Mediterranean Plants against Pseudomonas aeruginosa**.** *Molecules* , *23*(2), 482; https://doi.org/10.3390/molecules23020482

Mohammed A, Liman M.L and Atiku M.K (2013). Chemical composition of the methanolic leaf and stem bark extracts of *Senna siamea* Lam. *Journal of Pharmacognosy and Phytotherapy.* 5(5):98–100.

Naima R., Oumam M., Hannache H., Sesbou A., Charrier B., Pizzi A., Charrier F., Bouhtoury F. (2015). Comparison of the impact of different extraction methods on polyphenols yields and tannins extracted from Moroccan Acacia mollissima barks. Industrial Crops Production. 70:245–252.

Rui Hai Lui (2003) Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals *American Journal Of Clinical Nutrition.* Volume 78, Issue 3, Pages 517S–520S,

Samir, F., Amal, D., Hafedh, H., Kais, M., Neji, G. And Adel, K., 2017. Solvent extraction effects on phytochemical constituent profiles, antioxidant and antimicrobial activities and functional group analysis of *Ecballium elaterium* seeds and peel fruits. *Food and science technology*, vol 37 (3).

Schilling J.D., Lorenz R.G and Hultgren S.J (2002). Effect of trimethoprim-sulfamethoxazole on recurrent bacteriuria and bacterial persistence in mice infected with uropathogenic *Escherichia coli*. Infections in Immunology 70:7042–7049. doi: 10.1128/IAI.70.12.7042-7049.

Siva, W, Xiaojin Li, Manjula M, Gunawardana, A, Kathleen M, Debbie G, Christopher S, Charles W, Marc M. Baum S and Paul W (2014). Beta- Lactam Antibiotics Stimulate Biofilm Formation in Non-Typeable Haemophilus influenzae by Up-Regulating Carbohydrate Metabolism. *PLoS ONE*; 9 (7): e99204 DOI: 10.1371/journal.pone.0099204.

Thassitou**, M** and Arvanitoyannis**, S** (2001). Bioremediation: A novel approach to food waste management. *Trends in Food Science & Technology* 12(5-6):185-196

APPENDICES

Escherichia coli 2094

Escherichia coli 587

Escherichia coli 367