Anti-Bacterial Properties of Leaf Extracts of *Moringa olifera* and *Alchornea cordifolia* Against Biofilm-Forming Strains of *Pseudomonas aeruginosa*

Enya E.* Ekundayo E. O. and Madukwe E. U.
Department of Microbiology, College of Natural Sciences, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria
* Corresponding author: emmy_enya12@yahoo.com

Abstract: Medicinal plants represent potential sources of new antibacterial agents against drug resistant bacteria. The aim of this study was to investigate the antibacterial activities of aqueous and ethanol extracts of the leaves of *Moringa oleifera* and *Alchornea cordifolia* against strains of *Pseudomonas aeruginosa* expressing various virulence and biofilm-forming characteristics. Five hundred grams (500 g) of each of the powdered plant materials was soaked in 1,500 ml of ethanol/water respectively for 24 h at room temperature. The extracts were filtered using non-adsorbent muslin cloth into a clean beaker. The filtrates were dried by evaporating off the solvent at 50 ^{o}C in a hot air oven. The inhibitory activities of the extracts were tested against the strains using the agar well diffusion and microbroth dilution assays. The crude ethanolic and aqueous extracts of the leaves of *Moringa olifera* and *Alchornea cordifolia* produced inhibition zones ranging from 10.0 mm to 20.0 mm at a concentration of 200 mg/ml. The extracts of *Alchornea cordifolia* had higher inhibitory effects on all the test isolates. The antibacterial activities of the extracts varied according to the genetic determinants carried by the various strains with the zone sizes decreasing to between 7.5 mm to 13.0 mm for some strains. The wild-type strain (PA14-GFP) carrying the green fluorescent protein was susceptible to the aqueous and ethanol extracts of both plants while some of the strains carrying mutations for biofilm formation were less susceptible to the plant extracts. It is remarkable that the ethanol extract of *A. cordifolia* had appreciable inhibitory activity against all isolates including strains like PA01-L-wt-PqSR and PA01-L-wt which carry mutations for biofilm formation. The minimum inhibitory concentration against these strains ranged from 6.25 mg/ml to 25 mg/ml while the minimum bactericidal concentration was between 12.5 mg/ml for the ethanol extracts and 25 mg/ml for the aqueous extracts. Findings of the study provides justification for further research on the potentials of these medicinal plants as sources for antibacterial and antibiofilm compounds.
Key word: Antibacterial, Biofilm, Alchornea, Moringa, Antibiotics

INTRODUCTION

Plants have been the main source of pharmaceuticals and healthcare products in many countries (Jamshidi-Kia *et al.*, 2018). This is so because plants possess enormous reservoir of compounds that have numerous reported biological activities including antimicrobial properties (Ruban and Gajalaksmi, 2016). This potential and the growing public health concern resulting from development of multi-drug resistant bacterial strains suggests that investigating plants can yield valuable and innovative pharmaceutical medicines. The medicinal components in plants and plant products are usually in the form of phytochemicals, which are found in all plants (Ruddaraju *et al.,* 2020; Vaou *et al.,* 2021). These secondary metabolites are crucial therapeutic agents in the development of new drugs. Pharmaceuticals can be made by synthesizing, compounding, or transforming these phytochemicals. Plant extracts are the foundation of most pharmacotherapy systems and have been utilized to treat a variety of illnesses (Ayena *et al.*, 2021).

One of the most drug-resistant bacteria observed in healthcare settings is *Pseudomonas aeruginosa*, which is also a major contributor to nosocomial infections (Kamali *et al.,* 2020; Labovska, 2021). According to D'Abbondanza and Shahrokhi (2021), it is one of the pathogens linked to severe burn infections, post-operative surgical site infections, and urinary tract infections caused by catheter use.

Furthermore, due to its high level of antibiotic resistance, which is prominently enabled by its ability to form dense exopolymeric matrices known as biofilms and a variety of other virulence determinants, *P. aeruginosa* can cause a variety of infections in humans that are typically difficult to treat (Waldrop *et al.*, 2014; Gupta *et al.,* 2016). *Pseudomonas*

aeruginosa poses a significant clinical risk due to its resistance to multiple antibiotics (Sonmezer *et al.*, 2016). Therefore, to control these biofilm-mediated diseases, anti-infective medicines that are effective against planktonic and biofilm microbial species must be explored. Multidrug-resistant (MDR) microorganisms pose a serious risk to the welfare and health of people (Lehtinen *et al.*, 2019). Healthcare professionals face challenges when treating infections caused by multi-drug resistant species, such as *P. aeruginosa*, because treating these infections might lead to higher patient morbidity (Kebede *et al.*, 2021).

Alchornea cordifolia has been routinely utilized as a local remedy for cold (Ebenyi *et al.,* 2017). More importantly, according to Siwe *et al.* (2016), it is also utilized to treat illnesses caused by several bacterial and parasitic species. It is also used as a sedative, an antispasmodic, and an antidote to poisons. The leaves and stem bark are the portions that are most commonly used in medicine, however the leaf has greater potency (Ebenyi *et al.,* 2017). Anti-inflammatory properties have been observed in the crude aqueous/methanolic extract of *A. cordifolia* leaves (Djimeli *et al.* 2017).

Discovery of novel pharmaceutical agents is very important for the control of this organism and other pathogenic drug resistant clinical isolates. The emergence of antibiotic-resistant strains among community-acquired diseases and the unfavourable side effects of some medications (Lehtinen *et al.,* 2019) highlight the necessity of screening the numerous overlooked plant materials. *Moringa oleifera* has been used for centuries as a miracle tree and traditional remedy for many diseases. This plant is one of the most valuable multifunctional trees in the world as several component of it may be used to make food, medicine, cosmetics, or even purify water (Mursyid *et al.,* 2019). The leaf of *Moringa oleifera* exhibits antibacterial, antifungal, antihypertensive, antihyperglycemic, antitumor, anticancer, and anti-inflammatory properties from a pharmaceutical standpoint (Deyno, 2014). The search for and creation of novel antimicrobial drugs is thus an important endeavor. Hence this study was undertaken to evaluate the potentials of leaf extracts of *Moringa oleifera* and *Alchornea cordifolia* in controlling growth of biofilm forming strain of *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

Source of Isolate: The *Pseudomonas aeruginosa* strains were laboratory strains obtained from Dr. Blessing Oyedemi of the Department of Biological Sciences, University of Bristol, United Kingdom.

Collection and Extraction of Plant Material: Fresh leaves of *Alchornea cordifolia* and *Moringa oleifera* were collected from Umudike Village, Abia State, Nigeria. The medicinal plants were authenticated by a Plant Taxonomist in the Department of plant science and Biotechnology, Michael Okpara University of Agriculture, Umudike Abia State, Nigeria. The plant materials were air dried for two weeks on cardboards on the floor of a well-ventilated shed used for drying plant materials. The dried parts were pulverized to fine powder using a mechanical grinder. The powdered leaf materials were sieved, weighed and stored in airtight containers at room temperature until they were extracted.

Extraction of plant materials: Five hundred grams (500 g) of each of the powdered plant materials was soaked in 1,500 ml of ethanol/water respectively for 24 h at room temperature. The extracts were filtered using non-adsorbent muslin cloth into a clean beaker. The filtrate was dried by evaporating off the solvent at 50^{o}C in a hot air oven over a period of two days

Screening of the Extracts for Antibacterial Activity: Exactly 0.4 g of each crude extract was reconstituted in 2 ml of dimethyl sulphoxide (DMSO) to obtain extract concentration of 200 mg/ml. This was serially diluted in 2-folds to obtain the following lower extract concentrations: (100, 50, and 25) mg/ml.

Screening of Extracts and Fractions for Antibacterial Activity: The antibacterial activities of the extracts were assessed by the agar well diffusion assay as previously described (Allotey-Babington *et al.*, 2014) with slight modifications. Briefly, a stock solution of 200 mg/ml of each of the plant extracts was made in dimethyl sulfoxide (DMSO). Further dilutions were made to obtain concentrations of 50 mg/ml and 25 mg/ml. The test organisms were reactivated by streaking out on a freshly prepared nutrient agar plate. An aliquot of 100 µl of suspension of each *Pseudomonas aeruginosa* isolate standardized to 0.5 MacFarland standard was aseptically inoculated unto Muller-Hinton agar plate using a cotton swab to create a lawn of the organisms. Wells were created on the agar surface using a flame sterilized cork-borer of 6 mm diameter. An aliquot of 50 µl of each of the plant extracts was loaded into each well. A strain of *Pseudomonas aeruginosa* (ATCC 27853) was used as a control.

The minimum inhibitory concentration (MIC) was determined by microbroth dilution technique. Serial dilutions of the plant extracts were made in test tubes containing sterile Mueller-Hinton broth, to obtain concentrations of 100, 50, 25, 12.5, 6.25, 3.12 and 1.56 mg/ml). The test tubes were inoculated with 50 µl of suspension of the test bacterium standardized to McFarland standard tube No. 0.5. The inoculated tubes were incubated aerobically at 37°C for 18-24 h. After incubation, the tubes were examined for turbidity. The tube with the lowest concentration of extracts which showed no turbidity was recorded as the MIC value for the tested extract. The minimum bactericidal concentration (MBC) was determined by streaking the contents of the two last tubes with no turbidity, separately, on freshly prepared nutrient agar plates. The MBC is the concentration in the tube from which no growth was observed after 18-24 h of incubation (Ekundayo *et al.*, 2020).

RESULTS

The growth of the microorganisms used in this study was inhibited by both the ethanolic and aqueous extracts of the leaves of *Moringa oleifera* and *Alchornea cordifolia*. The zone of inhibition ranged from 10.0 mm to 18.5 mm at the 200 mg/ml concentration for the aqueous extract of *Moringa oleifera*. *Alchornea cordifolia* had the highest inhibitory effect on all the test isolates with zone of inhibition ranging between 10.0 mm to 20.0 mm at the 200 mg/ml concentration. Also, the zone of inhibition decreased to between 7.5 mm to 15.0 mm at the 100 mg/ml concentration (Table 1).

Table 1: Diameter of zone of inhibition (mm) of ethanol extracts of Alchornea cordifolia and Moringa oleifera against the Pseudomonas aeruginosa isolates

Isolate code	Alchornea cordifolia			Moringa oleifera			Control (mm)
	200 mg/ml	100mg/ml	50 mg/ml	200 mg/ml	100 mg/ml	50 mg/ml	
PA14-GFP	18.0	13.0	9.0	0.0	0.0	0.0	33
PA01-L-wt	14.0	11.0	0.0	0.0	0.0	0.0	33
PA01-L-wt-PqSR	16.0	7.5	0.0	0.0	0.0	0.0	35
PA01-N-GFP	16.0	10.0	0.0	0.0	0.0	0.0	41
PA14-wt	20.0	15.0	0.0	12.0	0.0	0.0	45
PA01-Rhl L-Lux	15.0	0.0	0.0	0.0	0.0	0.0	35

Table 2: Diameter of zone of inhibition (mm) of aqueous extracts of *Alchornea cordifolia* and *Moringa oleifera* against the *Pseudomonas aeruginosa* isolates

Isolate code	*Alchornea cordifolia*			*Moringa oleifera*			Control
	200mg/ml	100mg/ml	50mg/ml	200mg/ml	100mg/ml	50mg/ml	(mm)
PA14-GFP	15	0.0	0.0	13.0	10.0	0.0	33
PA01-L-wt	12	0.0	0.0	16.0	10.0	0.0	33
PA01-L-wt-PqSR	15	0.0	0.0	0.0	0.0	0.0	35
PA01-N-GFP	13	0.0	0.0	18.5	12.0	8.0	41
PA14-wt	17	8.0	0	17.0	14.0	7.0	45
PA01-Rhl L-Lux	17	0.0	0.0	0.0	0.0	0.0	35

Table 3: MIC and MBC values (mg/ml) of Ethanol extract of *Alchornea cordifolia* and *Moringa oleifera* against the *Pseudomonas aeruginosa* isolates

Plants	Organisms	100	50	25	12.5	6.25	3.12	MIC (mg/ml)	MBC (mg/ml)
Alchornea cordifolia	PA14-GFP	-	-	-	-	+	+	12.5	25
	PA01-L-wt	-	-	-	+	+	+	25	25
	PA01-L-wt-PqSR	-	-	-	-	+	+	6.25	12.5
	PA14-wt	-	-	-	-	+	+	12.5	25
Moringa oleifera	PA14-GFP	-	-	-	+	+	+	25	25
	PA01-L-wt	-	-	-	+	+	+	25	25
	PA01-L-wt-PqSR	-	-	-	-	+	+	12.5	25
	PA14-wt	-	-	-	-	+	+	12.5	25

Key: +: growth of the organism indicated by turbidity in the broth medium; -= Absence of growth of the test organism shown by no form of turbidity in the medium.

Table 4: The MIC and MBC values (mg/ml) of aqueous extract of *Alchornea cordifolia* and *Moringa oleifera* against the *Pseudomonas aeruginosa* isolates

Plants	Organisms	100	50	25	12.5	6.25	3.12	MIC	MBC
Alchornea cordifolia	PA14-GFP	-	-	-	+	+	+	25	25
	PA01-L-wt	-	-	-	+	+	+	25	25
	PA01-L-wt-PqSR	-	-	-	-	+	+	12.5	25
	PA14-wt	-	-	-	-	+	+	12.5	25
Moringa oleifera	PA14-GFP	-	+	+	+	+	+	50	50
	PA01-L-wt	-	+	+	+	+	+	50	50
	PA01-L-wt-PqSR	-	-	+	+	+	+	25	50
	PA14-wt	-	+	+	+	+	+	50	50

+: growth of the organism indicated by turbidity in the broth medium; -= Absence of growth of the test organism shown by no form of turbidity in the medium.

DISCUSSION

Development of medications with the potential to inhibit biofilm formation by bacteria could serve as a major therapeutic target for the treatment of a variety of bacterial infections. Finding naturally occurring plant-based chemicals that can prevent the production of biofilms is a possible substitute (Slobodnıkova *et al.*, 2016).

The growth of the microorganisms used in this study was inhibited by both the ethanolic and aqueous extracts of the leaves of *Moringa oleifera* and *Alchornea cordifolia*. The activity of the extracts were observed to be concentration dependent. These parts of the plants have medical potential because of their capacity to prevent the growth of certain diseases through leaf extracts.

However, the ethanol extracts of *Moringa oleifera* at the respective concentrations tested had no observable activity against any of the test isolates. The findings of this study is comparable to the report of by Nugraha *et al.* (2020) and Ilanko *et al.* (2019). Results for the aqueous extract varied from those of Shoba *et al.* (2014) and Emmanuel *et al.* (2014) who reported no inhibition or a mean disk diffusion zone of 9.5 mm respectively, while Onsare *et al.* (2013) reported inhibition zones up to 24 mm.

The water extract of *A. cordifolia* had antibacterial activity on all strains of *Pseudomonas aeruginosa* only at the highest concentration. The MIC values of the water extract was 25 mg/ml for both PA14-GFP and PA01-L-wt, while PA01-L-wt-PqSR and PA14-wt had an inhibitory concentration of 12.5 mg/ml each. The findings of this study are consistent with a prior work by Ebenyi *et al.* (2017), which found that *A. cordifolia's* aqueous extract has antibacterial activity against *P. aeruginosa* at a minimum inhibitory concentration (MIC) of 25 mg/ml. The findings of this study are also in agreement with the report of Boniface *et al.* (2016) and

Djimeli *et al.* (2017). The antibacterial efficacy of *A. cordifolia's* aqueous extract against *P. aeruginosa* was also reported by Gatsing *et al.* (2010), with an MBC value of 50 mg/ml and a MIC of 25 mg/ml.

The ethanol extract of *A. cordifolia* had the most remarkable antimicrobial activity on all isolates at the highest concentration tested. The activity was observed to decrease as the concentration decreased. Its minimum inhibitory concentration was 6.25 mg/ml for PA01-L-wt-PqSR and 25 mg/ml for PA01-L-wt. This is in line with the findings of Ngoupayo *et al.* (2015), who reported the antimicrobial activity of the ethanol extract of *A. cordifolia*. The authors reported an MIC value 25 mg/ml of and an MBC value of 50 mg/ml for *P. aeruginosa*. Earlier studies had reported promising antimicrobial properties of this plant in another investigations (Abdullahi and Ali, 2019).

The aqueous extracts of *Moringa oleifera* was found to be more active than the ethanol extract. This observation in line with the submission of Saadabi and Abu (2011) who reported that the aqueous extracts of *Moringa olifera* have inhibition potential against many pathogenic bacteria, such as; *Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli* in a dose dependent manner. These findings corroborate previous studies that the therapeutic agents derived from plants are used as an important alternative or complementary treatment of infectious diseases (Bakal *et al.*, 2017).

The findings of this study provide direction for the creation of novel medications that may be able to meet therapeutic needs. It could serve as a lead to finding other suitable antibiofilm medication that either promotes the dispersal of preformed biofilms or prevents the development of new biofilms *in vivo*.

The susceptibility of the isolates to the study plant extracts implies that chemical compounds in the extracts can be further developed to fight against these drug resistant microorganisms (Uttu *et al.*, 2015).

CONCLUSION

The parent wild type strain PA14-GFP was observed to be susceptible to the aqueous and ethanol extracts of both plants as against some of the mutated strains, further affirming the role of biofilms in mediating drug resistance. However, the activity observed against some of the mutated isolates by these extracts could be regarded as a boost in the search for other medicinal agents against drug resistant organisms.

REFERENCES

Abdullahi, M.S. and Ali, M. (2019). Antibacterial activity of *Moringa oleifera* leaf extracts against bacteria isolated from patients attending General Sani Abacha Specialist Hospital Damaturu. *Journal of Allied Pharmaceutical Sciences*, 61-66.

Allotey-babington, G. L., Nettey, H., Debrah, P., Adi-dako, O., Sasu, C., Antwi, A., Darko, Y., Nartey, N and Asare, J (2014). Screening of Several anti-infectives for in-vitro activity against *Mycobacterium smegmatis*. *Advances in Microbiology*, 4:1197–1203.

Ayena, A.C., Anani, K., Dosseh, K., Agbonon, A. and Gbeassor, M. (2021). Comparative study of antimicrobial, anti-inflammatory, and antioxidant activities of different parts from *Pterocarpus Santalinoides* l'Her. Ex. DC (Fabaceae). *Evidence Based Complementary and Alternative Medicine*, Article ID 8938534, 7.

Bakal, S.N., Bereswill, S. and Heimesaat, M. M. (2017). Finding novel antibiotic substances from medicinal plants—Antimicrobial properties of *Nigella sativa* directed against multidrug-resistant bacteria. *European Journal of Microbiology and Immunology*, 7(1):92–8.

Boniface, P. K., Ferreira, S. B. and Kaiser, C. R. (2016). Recent trends in

phytochemistry, ethnobotany and pharmacological significance of *Alchornea cordifolia*. *Journal of Ethnopharmacology,* 191:216–244.

D'Abbondanza, J.A. and Shahrokhi, S. (2021). Burn infection and burn sepsis. *Surgical Infections (Larchmt),* 22(1):58-64.

Deyno, S.T. (2014). Phytochemistry and pharmacological activities of *Moringa oliefera. Int. Journal of Pharmacognosy,* 1:222–31.

Djimeli, M.N., Fodouop, S.P., Njateng, G.S., Fokunang, C. and Tala, D.S. (2017). Antibacterial activities and toxicological study of the aqueous extract from leaves of *Alchornea cordifolia* (Euphorbiaceae). *Biomed Central Complementary and Alternative Medicine*, 17:349.

Ebenyi, L.N., Nwakaeze, A.E., Moses, I.B., Iroha, I.R., Uzoeto, J.O., Ugochukwu, J.I., Eddison, I.O. and Okamkpa, C.J. (2017). Antibacterial activity of *Alchornea cordifolia* leaves found in Ebonyi State. *International Journal of Advances in Pharmacy, Biology and Chemistry*, 6(1):46-51.

Ekundayo, E.O., Kalu, U.O and Enya, E. (2020). In-vitro Inhibitory Activity of Extracts of Some Medicinal Plants against *Mycobacterium smegmatis. Nigerian Journal of Microbiology,* 34(1):5044 – 5052.

Emmanuel, S.A., Olajide, O.O., Abubakar, S., Idowu, I.D., Orishadipe, A.T. and Thomas, S. A., (2014). Phytochemical and antimicrobial studies of methanol, ethyl acetate, and aqueous extracts of *Moringa oleifera* seeds. *American Journal of Ethnomedicine*, 1:346–354.

Gatsing, D., Nkeuhouapi, C. F., Nji-Nkah, B. F., Kuiate, J. R. and Tchouanguep, F. M. (2010). Antibacterial activity, bioavailabilty and acute toxicity evaluation of leaf extract of *Alchornea cordifolia*. *International Journal of Pharmacology*, 6(3):173.

Gupta, P., Sarkar, S., Das, B., Bhattacharjee, S. and Tribedi, P. (2016). Biofilm, pathogenesis and prevention-a journey to break the wall: A review. *Archives of Microbiology,* 198(1):1–15.

Ilanko, P., McDonell, P. A., Vuuren, S. and Cock, I. E. (2019). Interactive antibacterial profile of *Moringa oleifera* Lam. extracts and conventional antibiotics against bacterial triggers of some autoimmune inflammatory diseases. *South African Journal of Botany,* 124:420–435.

Jamshidi-Kia F, Lorigooini Z, Amini-Khoei H. (2018). Medicinal plants: past history and future perspective. *Journal of Herbmed Pharmacology*, 7(1):1-7.

Kamali, E., Jamali, A., Ardebili, A., Ezadi, F. and Mohebbi, A. (2020). Evaluation of antimicrobial resistance, biofilm forming potential, and the presence of biofilm-related genes among clinical isolates of *Pseudomonas aeruginosa*. *Biomed Central Research Notes,* 13 (1): 1-6.

Kebede, T., Gadisa, E. and Tufa, A. (2021). Antimicrobial activities evaluation and phytochemical screening of some selected medicinal plants: A possible alternative in the treatment of multidrug-resistant microbes. *PLoS ONE,* 16(3): e0249253.

Labovska, S. (2021). *Pseudomonas aeruginosa* as a cause of Nosocomial infections. IntechOpen. doi: 10.5772/intechopen.95908.

Lehtinen, S., Blanquart, F., Lipsitch, M. and Fraser, C. (2019). On the evolutionary ecology of multidrug resistance in bacteria. *Plos Pathogens*, 15:e1007763.

Mursyid, E.M., Annisa, R.N., Zahran, I., Langkong, J. and Kamaruddin, I. (2019). Antimicrobial activity of *Moringa oleifera* extract against the

growth of *Staphylococcus epidermidis*. *Earth and Environmental Science,* 343:012145.

Ngoupayo, J., Nteme-Ebene, M. S., Félicien, M. K. and Mpondo, E. (2015). Phytochemical screening and antibacterial properties from extract of *Alchornea cordifolia*. *Journal of Pharmacognosy and Phytochemistry*, 4(3):176-180.

Nugraha, A. S., Permatasari, A. E. N. and Kadarwenny, C. P. (2020). Phytochemical screening and the antimicrobial and antioxidant activities of medicinal plants of Meru Betiri national park Indonesia. *Journal of Herbs, Spices and Medicinal Plants,* 26:303–314.

Onsare, J. G., Kaur, H. and Arora, D. S. (2013). Antimicrobial activity of *Moringa oleifera* from different locations against some human pathogens. *Academic Journal of Medicinal Plants,* 1:80–91.

Ruban, P. and Gajalakshmi, K. (2016). *In vitro* antibacterial activity of Hibiscus rosa–sinensis flower extract against human pathogens. *Asian Pacific Journal of Tropical Biomedicine*, 2(5):399-403.

Ruddaraju, L. K., Pammi, S. V., Guntuku, G. S., Padavala, V. S. and Kolapalli, V. R. (2020). A review on anti-bacterials to combat resistance: from ancient era of plants and metals to present and future perspectives of green nano technological combinations. *Asian Journal of Pharmaceutical Sciences,* 15:42–59.

Saadabi, A. M. and Abu, Z. (2011). An *in vitro* antimicrobial activity of *Moringa oleifera* L. seeds extract against different group of microor-ganisms. *Asian Journal of Basic and Applied Sciences,* 5:27-30.

Shoba, F. G., Babu, V. A., Parimala, M. and Sathya, J. (2014). *In vitro* evaluation of antimicrobial activity of *Moringa oleifera* and *Momordica charantia* seeds. *International Journal of Pharmaceutical Science Research,* 5:1988–1993.

Siwe, N., Krause, R.M., van Vuuren, S.F., Tantoh, D. and Olivier, D.K. (2016). Antibacterial effects of *Alchornea cordifolia* (Schumach. and Thonn.) extracts and compounds on gastrointestinal, skin, respiratory and urinary tract pathogens. *Journal of Ethnopharmacology,* 179:76-82.

Slobodnıkova, L., Fialova, S., Rendekova, K., Kovac, J. and Mucaji, P. (2016). Antibiofilm activity of plant polyphenols. *Molecules,* 21:1717.

Sonmezer, M. C., Ertem, G., Erdinc, F. S., Kaya Kilic, E., Tulek, N., Adiloglu, A. and Hatipoglu, C. (2016). Evaluation of risk ractors for antibiotic resistance in patients with nosocomial infections caused by *Pseudomonas aeruginosa*. *Canadian Journal of Infectious Diseases and Medical Microbiology*, 1321487.

Uttu, A. J., Sallau, M. S., Ibrahim, H., Dambatta, M. B. and Idris, A. Y. (2015). Phytochemical and antimicrobial screening of stem bark extracts from *Glossonema boveanum* (Decne*). Journal of Pharmacognosy and Phytochemistry,* 4(2): 86-88.

Vaou, N., Stavropoulou, E., Voidarou, C., Tsigalou, C. and Bezirtzoglou, E. (2021). Towards advances in medicinal plant antimicrobial activity: a review study on challenges and future perspectives. *Microorganisms,* 9:2041.

Waldrop, R., McLaren, A., Calara, F. and McLemore, R. (2014). Biofilm growth has a threshold response to glucose *in vitro*. *Clinical Orthopaedics and Related Research,* 472: 3305-3310.