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ANTIBIOFILM ACTIVITY OF LEAF EXTRACTS FROM *JATROPHA CURCAS*, *FICUS PLATYPHYLLA* AND *ANTHOCLEISTA VOGELII* AGAINST *PSEUDOMONAS AERUGINOSA* BIOFILM

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ABSTRACT

One of the reasons for the chronic nature of some infections caused by opportunistic pathogen such as *Pseudomonas aeruginosa* is the ability of it to form biofilms. This study assessed the anti-adhesive and anti-biofilm activities of leaf extracts from *Ficus platyphylla*, *Jatropha curcas* and *Anthocleista vogelii* on biofilms formed by clinical isolates of *Pseudomonas aeruginosa* using standard microbiological techniques. Out of the 59 isolates tested, 35 (59.32%) were biofilm positive. Methanolic extract of *Jatropha curcas* has the highest percentage of adhesion inhibition 29 (82.86%) while ethanolic extract of *F. platyphylla* recorded the lowest percentage of adhesion inhibition 16 (45.75%) at the sub-inhibitory concentrations (0.625-1.88mg/ml) tested. The highest percentage inhibition of pre-formed biofilm was with methanolic extract of *J. curcas* 15 (42.86%) followed by ethanolic extract 12 (34.29%) and the lowest was with the methanolic extract of *F. platyphylla* 10 (28.57%). Methanolic extract of *Jatropha curcas* is the most active against the biofilm producing *Pseudomonas aeruginosa* clinical isolates. The anti-adhesion potentials showed by these plant extracts may provide a complementary medication for biofilm associated infections and damages to industrial surfaces associated with biofilm formation.

KEYWORDS: *Pseudomonas aeruginosa*, Biofilm, Leaf extract, Anti-adhesion, Anti-biofilm.

1.0 INTRODUCTION

Few bacteria live as free floating cells in nutrient rich mediums, and nearly majority of them depend on other microorganisms for energy, carbon and other nutrients and live in micro-ecosystems filled with hundreds of other microorganisms.[1] It is estimated that in the natural world more than 99% of all bacteria exist as biofilms.[2] When bacteria form biofilms, they become more resistant to many harmful environmental factors such as fluctuation of nutrients and oxygen, alteration of pH, and antibiotic effects.[1] One of the reasons for the chronic nature of some infections caused by the opportunistic pathogen *Pseudomonas aeruginosa* is the ability of this bacterium to form biofilms in which the bacteria are protected from host defenses and killing by antibiotics.[3]

A biofilm is a complex community of cells attached to either a biotic or abiotic surface enclosed in an exopolysaccharide matrix.[2,4] Biofilms have been reported to show increased resistance to antimicrobial agents including antibiotics compared to free-floating (planktonic) cells.[5] Several types of biofilms occur in nature as well as in the food and medical industries.[6] In the medical industry, biofilms have been implicated as the cause of serious infections leading to fatalities. Although infections are not exclusively a result of biofilm formation, up to 60% of all human infections are caused by biofilms.[7] *Pseudomonas aeruginosa* is known to form biofilm on both medical and engineered surfaces.[1] These organisms are normal inhabitants of the healthy human skin and mucosal microbial communities, they have emerged as a common cause of numerous nosocomial infections, mostly occurring in immunocompromised hosts or patients with implanted medical devices.[8] In *Pseudomonas aeruginosa* as well as most organisms, biofilm formation is regarded as a major concern as it renders these organisms highly resistant to conventional antibiotics and host defenses.

This can be caused by slow diffusion of these compounds through the extracellular polymeric matrix and slow growth of the bacteria.[9,5] World Health Organization[10] posited the use of herbal medicines as a primary health care source by the vast majority of the world's population, especially in developing countries. This, in turn, reinforces the responsibility of the scientists to devote more attention to the plant kingdom.[10] Moreover, since the high percentage of therapeutically used antimicrobial agents are of natural origin, the interest in investigating plants used in folk medicine with claimed antibacterial activities is a valid quest. In the light of these facts, the plant kingdom and the huge number of constituents in these plants, offers good prospect for the discovery of new, potent extracts and bioactive compounds.

This study was therefore undertaken to evaluate the anti-adhesion and anti-biofilm activities of leaf extracts from selected plants; *Ficus platyphylla*, *Jatropha curcas and Anthocleista vogelii* against biofilm-producing clinical isolates of *Pseudomonas aeruginosa*.

2.0 MATERIALS AND METHODS

Collection of Isolates
A total of 59 clinical isolates from previous study were collected and used in this study.

Identification of the Isolates
The isolates were identified morphologically, culturally and microscopically based on standard microbiological techniques as described by Cheesbrough.[11]

Biochemical Tests
The biochemical test necessary for *P. aeruginosa* were carried out according to the methods described by Cheesbrough.[11]

Collection of Plant Materials
Fresh leaves of *Ficus platyphylla*, *Jatropha curcas* and *Anthocleista vogelii* were collected during the rainy season in the month of August from Gadan Gayam area, Kaduna South L. G. A. of Kaduna State, Nigeria. The leaves were identified and authenticated at the Herbarium Section of the Department of Biological Sciences ABU, Zaria, Nigeria, where voucher numbers 9008 for *Ficus platyphylla*, 1911 for *Jatropha curcas* and 900202 for *Anthocleista vogelii* were assigned and samples deposited in the herbarium. The leaves were air dried at ambient temperature. The dried leaves were reduced to fine powder using laboratory mortar and the powder stored in an air-tight container until needed.

Preparation of the Leaf Extracts (Maceration)
The leaf extracts of *Ficus platyphylla*, *Jatropha curcas* and *Anthocleista vogelii* were prepared using a modified maceration technique. Twenty grams of the powdered samples each was accurately weighed into 500ml conical flasks in triplicates and 400ml of the extractants (water, ethanol and methanol) were added to each of the conical flasks, sealed with aluminum foil, shaken several times and allowed to stand for 48 hours. The extract was filtered using Whatman No. 1 filter paper and the filtrate collected in a clean beaker was concentrated to dryness by evaporation over rotary evaporator and separate the reagents from the extracts. The mass of each powdered extract was obtained and the percentage yield determined.[12]

Determination of Antimicrobial Activity of the Extracts
The antimicrobial activity of the plants' methanol, ethanol and aqueous extracts was assessed against the tested microorganisms using the disc diffusion method as recommended by the Clinical and Laboratory Standard Institute.[13]

Biofilm Production Assay
Biofilm production in the isolates was determined by a modification of the protocol described by Merrit *et al*.[12]

Quantitative Assay of Biofilm
This was carried out according to the protocol described by Merrit *et al.*[12]

Determination of Minimum Inhibitory Concentration (MIC)
Broth micro-dilution method in 96-well plates (Linbro^{Tm} Scientific, Inc. Subsidiary of Flow Laboratories, Inc. Hamden, Conn. 06517, USA)[14] was used to determine the least concentration of the different plant extracts that appeared to inhibit the growth of the microorganisms.[14]

Determination of Minimum Bactericidal Concentration (MBC)
After MIC testing, the 96-microtiter plates set up for the MIC determination were used to determine the minimal bactericidal concentration (MBC) as described by Al-Bakri *et al.*[14]

Determination of Anti-adhesion Activity of the Plant Extracts
The anti-adhesion activity of the plant extracts was determined by the protocol described by Gursoy *et al.*[15]

Determination of Antibiofilm Activity of the Plant Extracts
The antibiofilm activity of the plant extracts was determined by the protocol described by Filoche *et al.*[16]

Statistical Analysis
Microsoft excel^{TM} 2010 and Smith's Statistical Package (SSP) version 2.8 for analysis were used for computational statistics.

3.0 RESULTS

From the fifty nine (59) clinical isolates of *Pseudomonas aeruginosa* subjected to biofilm formation assay, 15 (25.42 %) were strong biofilm formers, 20 (33.89 %) were moderate biofilm formers, 10 (16.95 %) were weak

biofilm formers and 14 (23.73 %) were none biofilm formers (Fig. 1). A total of 35 (59.32 %) of the *P. aeruginosa* isolates were considered biofilm-positive (Fig 2).

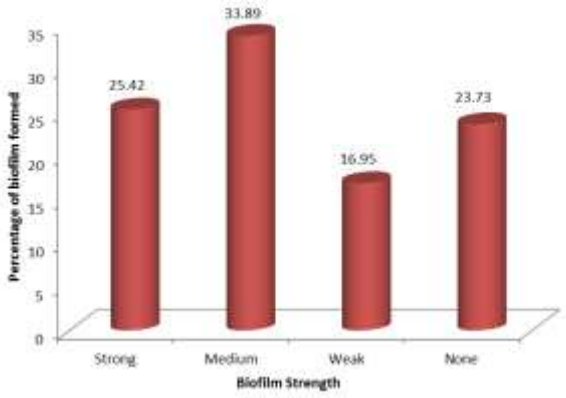

Figure 1: Percentage Biofilm Production by *P. aeruginosa* clinical isolates.

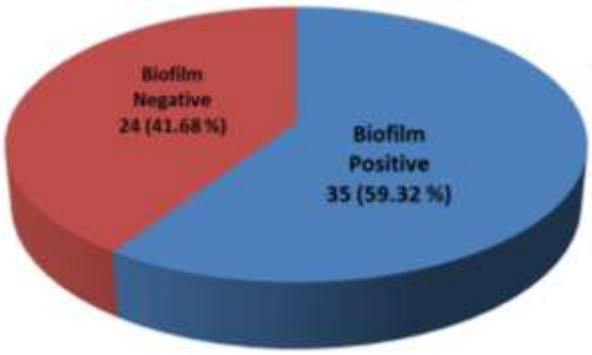

Figure 2: Biofilm Quantification Assay.

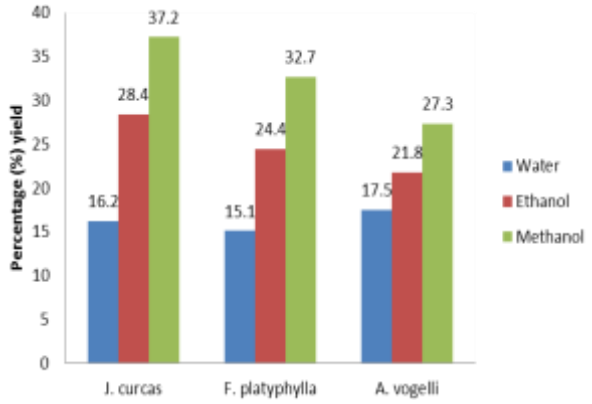

Figure 3: Percentage yields of extracts from sampled plants.

Table 1: Antimicrobial Activities of the Plant Extracts on *P. aeruginosa* clinical isolates.

Plants	Extracts (10mg/ml)	Diameter Zone of Inhibition (mm) S.D
Jatropha curcas	Methanolic	24.00 ± 0.58
	Ethanolic	23.00 ± 0.58
	Water	NA
Ficus platyphylla	Methanolic	23.00 ± 0.58
	Ethanolic	21.30 ± 0.58
	Water	NA
Anthocleista vogelii	Methanolic	NA
	Ethanolic	NA
	Water	NA

NA: indicates no activity at the concentrations (2.5-10 mg/ml) tested; S.D = Standard Deviation.

Table 2: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the Plant extracts on *P. aeruginosa* clinical isolates.

Plants	Extracts	MIC (mg/ml)	Sub-MIC (mg/ml)	MBC (mg/ml)
J. curcas	Methanol	1.25	0.625	2.50
	Ethanol	2.50	1.25	5.0
F. platyphylla	Methanol	1.88	0.94	3.75
	Ethanol	3.75	1.88	7.5

Concentrations tested (0.156 – 20.00 mg/ml)

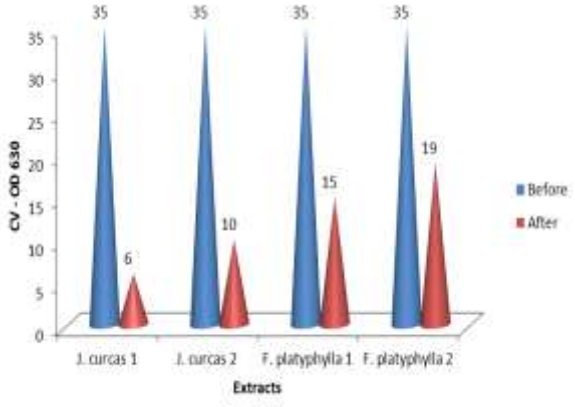

Figure 4a: Biofilm formed before and after exposure to sub-MIC of plant extracts (Anti-adhesion Test).

J. curcas 1 = methanol extract (0.625 mg/ml)
J. curcas 2 = ethanol extract (1.25 mg/ml)
F. platyphylla 1 = methanol extract (0.94 mg/ml)
F. platyphylla 2 = ethanol extract (1.88 mg/ml)

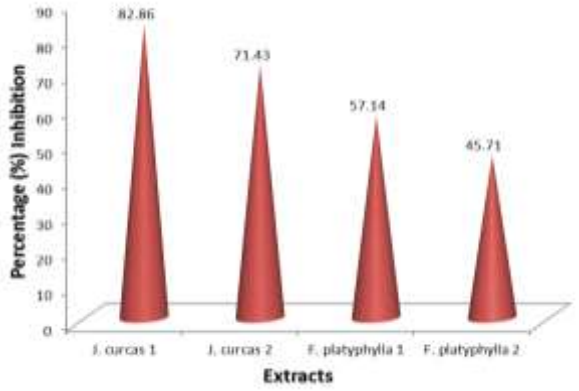

Figure 4b: Percentage Inhibition of *P. aeruginosa* adhesion after exposure to sub-MIC of plant extracts.
J. curcas 1 = methanol extract
J. curcas 2 = ethanol extract
F. platyphylla 1 = methanol extract
F. platyphylla 2 = ethanol extract

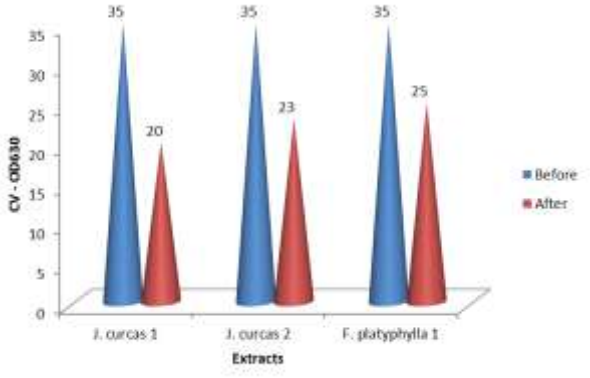

Figure 5a: Biomass of *P. aeruginosa* pre-formed biofilm before and after exposure to sub-MIC of plant extracts.
J. curcas 1 = methanol extract (0.625 mg/ml)
J. curcas 2 = ethanol extract (1.25 mg/ml)
F. platyphylla 1 = methanol extract (0.94 mg/ml)

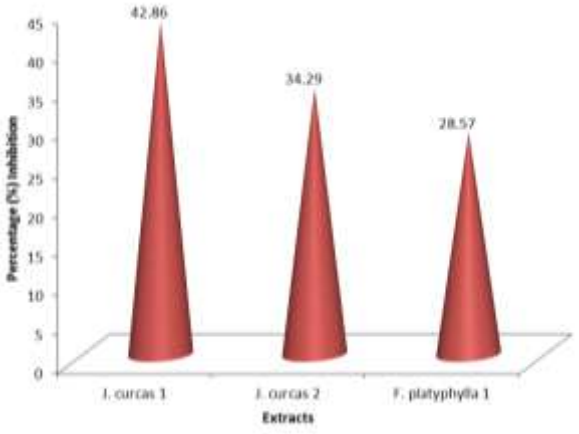

Figure 5b: Percentage Inhibition of the growth of *P. aeruginosa* pre-formed biofilm using plant extracts.
J. curcas 1 = methanol extract
J. curcas 2 = ethanol extract
F. platyphylla 1 = methanol extract

5.0 DISCUSSION

A microbial biofilm is ubiquitous in nature and is characterized by its recalcitrance toward antimicrobial treatment.[7] In clinical, environmental, and industrial settings, microbial growth in form of biofilm poses a serious threat. The urgent need for antibiofilm agents is clear. Efforts toward the discovery of successful antibiofilm agents included reevaluation of the antimicrobial activity of many known antibiotics, biocides, plant extracts, and natural compounds toward sessile populations.[14] Studies evaluating the antibiofilm activity of tested agents include assessing the activities against established biofilms and the antiadhesive properties at subinhibitory concentrations as a prophylactic measure toward biofilm formation.[3,7,17]

In this study, 35 (59.32%) of the *Pseudomonas aeruginosa* clinical isolates were positive biofilm formers, while 24 (41.68%) were confirmed to be negative biofilm formers.

The production of biofilm by clinical isolates of *P. aeruginosa* is in accordance with the works of Willey *et al.*[3] and Ekundayo and Ekekwe[18] which states that, one of the reasons for the chronic nature of some infections caused by the opportunistic pathogen *Pseudomonas aeruginosa* is the ability of this bacterium to form biofilms in which the bacteria are protected from host defenses and killing by antibiotics.[3,18] The higher yield of methanol extract compared with water extract contrast with the result obtained by Remington,[19] which showed that solvents such as methanol selectively extract compounds from plants resulting in lower yields of extracts compared to water, a universal solvent.

The demonstration of antimicrobial activity in methanol and ethanol but not in water extracts of *J. curcas* and *F. platyphylla* against planktonic culture of *P. aeruginosa* clinical isolates is not surprising. Several researchers reported that antimicrobial activity of several plants show antimicrobial activity attributed to their bioactive compounds. [15,1] The absence of activity in aqueous extracts of *J. curcas*, *F. platyphylla* and all the extracts of *A. vogelii* against the planktonic form of *P. aeruginosa* clinical isolates within the concentrations (2.5-10mg/ml) tested is in accordance with Ekundayo and Ekekwe[18] and Kubmara *et al.*[20] reports demonstrated.

The antimicrobial activity observed with methanolic extract compared with ethanolic extract from *J. curcas* may be attributed to the active biochemical components such as steroids, flavonoids, alkaloids, saponins, triterpenoids, tannins and carbohydrate in the leaf extracts of *J. curcas*.[21,22,23] Most water extracts used in this study showed very low or no activity against the tested micro-organism compared to methanolic and ethanolic extracts, this may be due to differences in the type and concentration of the active components across different species of the plants and also the ability of

solvents such as methanol and ethanol to extract a broader spectrum of compounds.

The success of plant extracts in inhibiting cell attachment as shown in this study is a promising tool for reducing microbial colonization on surfaces and epithelial mucosa which subsequently leads to infections. The ability of the plant extracts to inhibit cell attachment is confirmation of previous reports.[24,25,4] Some researchers have also demonstrated the success of coating medical devices with biocides such as silver to reduce microbial adhesion and the subsequent disease pathogenesis.[7,17,24] Al-Bakri *et al.*[14] demonstrated in their work that the extracts of *Salvia triloba* have no activity on biofilms formed by both typed culture and clinical isolate of *P. aeruginosa.* Therefore, the ability of *J. curcas* and *F. platyphylla* extracts to inhibit biofilm formed by clinical isolates of *P. aeruginosa* makes them better antiadhesive agents.

The use of surface disinfectants manufactured from active plant extracts such as those used in this study may be useful in reducing the development of biofilms on surfaces and equipment, thereby reducing food spoilage, rusting, nosocomial infections etc. Isolation and identification of the constituents that possess anti-adhesion properties and those that reduce bacterial development is also essential. This plant extracts when formulated into dosage forms can be used for the treatment of infectious diseases that persist due to biofilm formation.

This inability of the extracts to dissolve preformed biofilm is consistent with reports on resistance of *P. aeruginosa* biofilms to antimicrobial agents.[3,26] In addition to using the EPS as a diffusion barrier, *P. aeruginosa* cells have been reported to synthesize periplasmic glucans that physically interact with the antimicrobial agent thereby preventing them from reaching the site of action.[26] Enhanced biofilm development was observed with these extracts against *P. aeruginosa*.

The weak antibiofilm activity of the plant extracts is evidence that cells in a biofilm are more resistant to antimicrobial agents compared to free-floating cells.[3,26] Several factors have been attributed to the resistance of biofilms to antimicrobial agents. The presence of the EPS (glycocalyx) that surrounds biofilm cells is postulated as the main physical barrier that hinders complete diffusion of antimicrobial agents to inner cells of the biofilm.[3,26] In addition, the negative charge on the EPS restricts penetration of molecules by charge attraction thereby imparting resistance to the biofilm.[7] Other mechanisms are believed to work in synergy, contributing to maintaining biofilm cells intact. Biochemical mechanisms that result in either degradation or inactivation of the antimicrobial agent before it reaches the cells are believed to contribute to the resistance conferred in biofilms. This has been observed in *P. aeruginosa* biofilms where certain enzymes (such as amino glycoside-modifying enzymes) are secreted that inactivate antibiotics during the penetration process.[27,1] Efflux pumps also work in combination with the EPS resulting in the drug being expelled from the cell thus an effective concentration is not reached.[7,26] In this study, the antiadherent activities of *J. curcas* and *F. platyphylla* on clinical isolates of *P. aeruginosa* make them better alternatives for drug development. Nevertheless, these plant extracts were unable to completely eradicate preformed biofilms by the clinical isolates of *P. aeruginosa* under the experimental conditions. Reports on the antiadherent properties of these plants against bacteria have been documented with focus on urinary tract bacteria that include *E. coli*.[7,28,26]

6.0 CONCLUSION

The use of natural products as alternatives or complementary to conventional therapy has gained interest due to the perception that herbal products may be safe and have been used for many years as traditional medicines. Research on the antimicrobial activity of plants has almost exclusively focused on the planktonic form of micro-organisms. Less attention has been given to microbial biofilms as models in research although they have been implicated in most clinical infections and are more resistant to antimicrobial agents than the planktonic form.

FURTHER STUDY

Further research on molecular level (anti-quorum) sensing ability of the *Jatropha curcas* extract should be carried out. Also, further investigation should be carried out to determine if the combination of *J. curcas* and *F. platyphylla* will have better inhibitory effects on biofilm development.

REFERENCES

1. Maryam V, Ahya AA, Parisa M, Azra S. (Effects of Extracts and an Essential Oil from Some Medicinal Plants against Biofilm Formation of *Pseudomonas aeruginosa*). Journal of Medical Microbiology and Infectious Diseases, 2013; 1(1): 36-40.
2. Yerly J, Hu Y, Jones SM, Martinuzzi RJ. (A two-step procedure for automatic and accurate segmentation of volumetric CLSM biofilm images). *Journal of Microbiological Methods*, 2007; 70: 424–433.
3. Willey JM, Sherwood LM, Woolverton CJ. (*Prescott, Harley and Klein's Microbiology).* 7th Edition. McGraw Hill, New York, NY, 10020, 2008; 1088.
4. Chusri S, Chaicoch N, Thongza-ard W, Limsuwan S. (In vitro antibacterial activity of ethanol extracts of nine herbal formulas and its plant components used for skin infections in Southern Thailand). Journal of Medicinal Plant Research, 2012b; 6(44): 4616-5623.
5. Fuente-Nunez C, Mertens J, Smit J, Hancock REW. (The bacterial surface layer provides protection

against antimicrobial peptides). Applied and Environmental Microbiology, 2012; 78(15): 5452–5456.
6. Abdi-Ali A, Mohammadi-Mehr M, Agha AY. (Bactericidal activity of various antibioticsagainst biofilm-producing Pseudomonas aeruginosa). International Journal of Antimicrobial Agents, 2006; 27(3): 196-200.
7. Sandasi M, Leonard CM, Viljoen AM. (The in vitro antibiofilm activity of selected culinary herbs and medicinal plants against Listeria monocytogenes). Letters in Applied Microbiology, 2009; 0266-8254.
8. McCann MT, Gilmore BF, Gorman SP. (*Staphylococcus epidermidis* device-related infections: pathogenesis and clinical management). Journal of Pharmacy and Pharmacology, 2008; 60(12): 1551–1571.
9. Schoenfelder SMK, Lange C, Eckart M, Hennig S, SKozytska S, Ziebuhr W. (Success through diversity—how *Staphylococcus epidermidis* establishes as a nosocomial pathogen). International Journal of Medical Microbiology, 2010; 300(6): 380–386.
10. World Health Organization. (Legal Status of Traditional Medicine and Complementary Alternative Medicine). *A Worldwide Review. WHO, Geneva,* 2011.
11. Cheesborough M. *Medical Laboratory Manual for Tropical Countries, II Micrbiology (ELBS)*, Butterworth, Kent, U.K., 2008; 23-78.
12. Merritt JD, Kadouri E, O'Toole GA. (Growing and Analyzing Static Biofilms). Current Protocols in Microbiology, 2005; 1B.1.1-1B.1.17.
13. Clinical and Laboratory Standards Institute (Methods for Determining Bactericidal Activity of Antimicrobial Agents. Tentative Standard M26-T). National Committee for Clinical Laboratory Standards, Wayne, Philadelphia, 2008.
14. Al-Bakri AG, Othman G, Bustanji Y. (The assessment of the antibacterial and antifungal activities of aspirin, EDTA and aspirin-EDTA combination and their effectiveness as antibiofilm agents). Journal of Applied Microbiology, 2009; 107: 280–6.
15. Gursoy UK, Gursoy M, Gursoy OV, Cakmakci L, Kφnφnen E, Uitto VJ. (Anti-biofilm properties of *Satureja hortensis* L. essential oil against periodontal pathogens). *Anaerobe,* 2009; 15: 164-7.
16. Filoche SK, Soma K, Sissons CH. (Antimicrobial effects of essential oils in combination with chlorhexidine digluconate). Oral Microbiology and Immunology, 2005; 20: 221-5.
17. Chusri S, Sompetch K, Mukdee S, Jansrisewangwong S, Srichai T, Maneenoon K, Limsuwan S, Voravuthikunchai SP. (Inhibition of *Staphylococcus epidermidis* Biofilm Formation by Traditional Thai Herbal Recipes Used for Wound Treatment). Evidence-Based Complementary and Alternative Medicine, 2012a; 8.
18. Ekundayo EO, Ekekwe JN. (Antibacterial activity of leaves extracts of *Jatropha curcas* and *Euphorbia heterophylla).* African Journal of Microbiology Research, 2013; 7(44): 5097-5100.
19. Remington JP. (The science and practice of pharmacy). *20th edition*. Lippincott Williams and Wilkins. USA, 2000.
20. Kubmarawa D, Khan ME, Punah AM, Hassan M. (Phytochemical and Antimicrobial Screening of *Ficus platyphylla* against Human/Animal Pathogens). Pacific Journal of Science and Technology, 2009; 10(1): 382-386.
21. Aiyelaagbe OO, Adeniyi BA, Fatunsin OF, Arimah BD. (*In vitro* antimicrobial activity and phytochemical analysis of *Jatropha curcas* roots). International Journal of Pharmacology, 2007; 3(1): 421-426.
22. Rajesh B, Rattan LI. (Essentials of Medical Microbiology). *4th Edition*. Jaypee Brothers Medical Publishers (P) Ltd. New Delhi 110002, India, 2008; 500.
23. Arekemase MO, Kayode RMO, Ajiboye AE. (Antimicrobial Activity and Phytochemical Analysis of *Jatropha curcas* Plant against Some Selected Microorganisms). International Journal of Biology, 2011; 3(3): 52-59.
24. Cerca N, Martins S, Pier GB, Oliveira R, Azeredo J. (The relationship between inhibition of bacterial adhesion to a solid surface by sub-MICs of antibiotics and subsequent development of a biofilm). Research in Microbiology, 2005; 156: 650–655.
25. Mah TFC, O'Toole GA. (Mechanisms of biofilm resistance to antimicrobial agents). Trends in Microbiology, 2011; 9(1): 34–39.
26. Ceylan O, Ugur A, Sarac N, Sahin MD. (The antimicrobial and antibiofilm activities of *Mentha x piperita* L. essential oil). Journal of Biological Sciences Biotechnology, 2014; 1314-6246.
27. Fazeli MR, Amin G, Attari MMA, Ashtiani H, Jamalifar H, Samadi, N. (Antimicrobial activities of Iranian sumac and avishan-e shirazi (Zataria multiflora) against some food-borne bacteria). Food Control, 2007; 18(6): 646-9.
28. Plyuta V, Zaitseva J, Lobakova E, Zagoskina N, Kuznetsov A, Khmel I. (Effect of plant phenolic compounds on biofilm formation by *Pseudomonas aeruginosa*). Journal of Medical Microbiology infectious Diseases, 2013.