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Original article

Anti-biofilm effect of *Nerium oleander* essential oils against biofilm forming *Pseudomonas aeruginosa* on urinary tract infections

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ABSTRACT

Based on the untreated biofilm formation in urinary tract infections (UTIs), current study was focused on the inhibition of *Pseudomonas aeruginosa* (*P. aeruginosa*) biofilm formation using *Nerium oleander* (*N. oleander*) essential oils. Initially, the LC-MS analysis of antibacterial potential of hydro distillated *N. oleander* essential oils was detected by LC-MS analysis. Initially, the antibacterial nature of essential oils was detected by agar well diffusion method against biofilm forming *P. aeruginosa*. Then, the possible anti-bacterial fractions of the essential oils were purified by preparative HPLC, and confirmed by analytical HPLC peaks. Possible anti-bacterial components of the fractions were merged together and confirmed by agar well diffusion method. Consecutively, the and minimum biofilm inhibition concentration experiment was proved that the concentration of 200 μg/mL of *purified* essential oils was very effective against *P. aeruginosa* biofilm formation. The decrease survival rate of the MTT assay result was also supported the minimum biofilm inhibition concentration result. Finally, all the results were more evident; the HPLC purified *N. oleander* essential oils as a promising anti-biofilm factor against *P. aeruginosa* biofilm formation.

1. Introduction

Biofilm is one of the serious threats worldwide due to the development of recurrent MDRs in UTIs (Wugao et al., 2020). More cells that linked each other in sticky nature among themselves that form self-protective extracellular polysaccharides. Biofilm forming pathogens are very dangerous, because, it is 1000 fold resistant to currently available antibiotics compared to other pathogens (Abinaya and Gayathri, 2019). The mechanism of biofilm formation as very complex and difficult, which formed by more bacteria due to their signaling factors. The controlling of biofilm is not possible by chemical compounds. Recent years, the researchers are concentrated seriously to inhibit the biofilm formation and responsible pathogens by natural product (Na et al., 2020). Among the all infections, biofilm forming pathogens are very serious issue, because the mortality rate was increased frequently. Sometimes, it attached with related infections and lead to develop continuous resistant against external antibiotics and drugs. Among the various pathogens *P. aeruginosa* is one of the most important pathogens. It is a Gram negative rod shaped opportunistic bacteria, which cause recurrent UTIs due to the biofilm formation (Muhammad et al., 2017). Based on the Drug and Developmental Society reports, the *P. aeruginosa* formed biofilm formation is very dangerous thread worldwide, especially developing countries (Carel et al., 2019). Based on the above fact, the emerging threat of biofilm formation was forced the researcher to discover new classes of drugs to fight against the biofilm mechanism.

Essential oils are an odors material having excellent biological activities related chemical derivatives including hydrocarbon, aldehyde, ketones, phenols, oxides and etc. All the chemical derivatives of the compounds are used in various fields such as aromatic, therapeutic, pharmaceutical, biomedical and spiritual activities (Isabela et al., 2016). Till-date 4,000 essential oils have been studied form different plants and acted as excellent preservative agents

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in the field of sedative, food, antimicrobial, biopharmaceutical and some industries (Ygor Jessé et al., 2020). All the essential oils are able to link with phytochemical and aromatic compounds and they are terpenes, monoterpenes, sesquiterpenes, ketonated compounds and nitrogen-sulphur rich compounds (Lin et al., 2019). EOs is most acceptable medicine traditionally in India. It also heightened the economic rate of the Country (Sana et al., 2020; Houdkova et al., 2018). It is a natural flora that is consumed orally and dental treatment in the form of plant or essential oils. Recent years, essential oils are competitor to most of the drugs, and essential oils are succeeded than others. In particular, *N. oleander* is an evergreen shrub used as a folk medicine in South Asia against diabetes, rheumatic pain and various skin infectious diseases (Balkan et al., 2018). Also, it is used very limit in traditional medicine, because of poisonous nature to human (Santos et al., 2019). In addition, it has some other biological properties like anti-oxidant, anti-biofilm, cytotoxic, hepatoprotective anti-inflammatory and neurogenerative agent. It has the ability to produce various biological properties with producing effect of steroids, pregnanes and flavonoids (Kars et al., 2013). Based on the above advantages, the present study was concentrated to inhibition of biofilm forming *P. aeruginosa* using medicinal plant *N. oleander* essential oils.

2. Materials and methods

2.1. Extraction of EOs from Nerium oleander by hydrodistillation method

The *Nerium oleander* leaves were washed properly washed using with tap water initially and followed by double distilled water. The surface contaminates were also effectively removed by using 70% ethanol and grounded well using mixer grinder. After proper removal of contamination, the leaves were dried at room temperature and subjected to perform using Clevenger model apparatus by hydro distillation for 5 h. The solvent of n-hexane was used to recovery of the essential oils from solvent phase. The procedure was followed by previously reported article of Samia et al. (2020). Briefly, the 500 g of the *Nerium oleander* sample was diluted in 1 L (1:2) 2 L of double distilled water containing bottle. The sample was heated at over 90 ℃ continuously 1 h using heating mantle properly. After 1 h, the colloidal nature of the oil surface layer was extracted from the sample mixture of apparatus and filtered by receiver tube. The separating funnel was used to separate the water-EOs complex in two different tubes and the volumes were measured in measuring jar. Finally, the sodium sulphate was used to dry the EOs from water. After complete removal, the extracted EOs was separated safely in new container and stored at 4 ℃.

2.2. Analysis of chemical components of extracted EOs by LC-MS

The presence of EOs, other chemical constituents, phytochemical compounds and bioactive compound derivatives of extracted EOs sample was analyzed by Gas chromatography-mass spectrometer according to the previous reported evidence of Bibi and Mohamad Fawzi (2020). The LC-MS (Allient 6650B/7560C) was attached with capillary column (HP-5MS) using Chrome-Pack CP-Silicon 5 and CK capillary column of 30 m × 250 μm × 0.25 μm. The initial and final oven temperature was arranged with 40 °C and 120 °C at a rate of 4 °C/min with 5 and 10 min. The 250 °C was used for constant temperature and followed by 260 °C temperature was used for injector and detector. The carrier gas and flow rate was set as 30 cm/s linier velocity and 2 mL/min. Finally, the obtained EO_S, aromatic and phytochemical compounds mass spectra were interpreted with retention time, retention percentages, occupied area and occupied percentages. Also, the results were cross checked with previously reported data and confirmed by Wiley library, Bharathidasan University, Thiruchirappalli, Tamilnadu, India.

2.3. Inactivation of P. Aeruginosa by Nerium oleander essential oils

The agar well diffusion assay was used to check the anti-bacterial efficiency of *Nerium oleander* essential oils followed by previous method of Mariadhas et al., 2019. Briefly, first day culture was transferred to fresh nutrient broth, and allowed to grow 12 h at normal temperature. After 12 h of well turbidity containing culture was spread on the nutrient agar plate and then minimum, maximum, distilled water and of the three wells were cut properly using gel borer. Whereas, the positive control of ceftazidime was added on the agar surface as disc model. The plate was kept in room temperature for one day. After one day, the zones of the four wells were noted.

2.4. Purification of active EOs by preparative HPLC method

The detection of specific anti-microbial compounds of the *Nerium oleander* essential oils was purified using preparative HPLC (Shimadzu, Japan) using the ratio of 50:20:20:40 of acetonitrile : methanol : ammonium acetate : water respectively (Ramachandran et al., 2020). After purification, the purified sample was analyzed by analytical HPLC to detect the compound fraction peaks. Based on the preparative HPLC, the various peaks of the fractions were performed against biofilm forming *P. aeruginosa* by agar well diffusion method. In addition, the antibiotic of ceftazidime was used for positive control. The active antibacterial metabolites fraction was purified separately and lyophilized for pure EOs identification. The instrument was previously programmed with C30 column consist of100 mm × 5 mm, the initial temperature of 0 ℃ and final extension temperature of 100 ℃ for 2 h was used. The flow rate was set at 2 mL/min with 5 μm linier gradient. At 50 ℃, 50 μL column volume injector was used for sample.

2.5. Validation of preparative HPLC fractions

Based on the anti-bacterial activity of preparative HPLC, the potential fraction was further cross checked the anti-bacterial activity by agar well diffusion method (Taoufiq et al. 2020). The nutrient agar made 6 mm wells and filled with separately purified essential oils along with previously swabbed biofilm forming *P. aeruginosa* culture. Then, monitored till one day at ordinary room temperature. After one day, the zone of inhibition around the wells was calculated and interpreted the preparative HPLC result.

2.6. Minimum biofilm inhibition concentration

One day old biofilm forming *P. aeruginosa* culture was inoculated into the 24-well polystyrene plate (which was used to adhere the biofilm in bottom of the plate) by microbroth dilution experiment. The modified procedure of Rajivagndhi et al. (2020); Anjugam et al. (2018), 50 μg/mL and 100 μg/mL concentration of EOs and sterile nutrient broth was diluted properly in the presence of 10 μg/mL of one day old *P. aeruginosa* culture. The plate was kept in incubator with normal temperature one day. After one day, the turbidity of incubator maintained culture was shown a naked eye and confirmed by using 600 nm O.D value of microtitre plate reader (Shimadzu, Japan). The result was triplicated and converted to percentages using universal calculation,

Inhibition (%) = 100 ($OD_{SNEOs\ treated\ samples}$-$OD_{Without\ SNEOs\ treated\ sample}$) × 100 -----1.

After identification, the lowest concentration of the EOs that inhibited the highest biofilm was selected for biofilm inhibition concentration (Ramachandran et al., 2019).

2.7. Biofilm metabolic activity assay

One day old biofilm forming *P. aeruginosa* culture was taken in 1 mL nutrient broth containing 6-well plate using 2,3-bis(2-methoxy-4-nitro-5 sulfophenyl)–2H-tetrazolium-5-carboxanilide as a solution. Consecutively, 25–200 μg/mL of purified NOEOs was added into the respective wells. After 1 h time interval, the menadione acetone solution was diluted in all the wells before addition of 200 μL of PBS. The plate was rotate gently to mix all the solutions each other and maintained in atmospheric temperature for 1 h. As same as without treatment of NOEOs containing well served as a control. Finally, the turbidity formed viable cells and absence of turbidity containing wells were calculated using microtitre plate reader at 600 nm (Maruthupandy et al. 2020). The triplicated result was changed to inhibition percentage using bellowed formula,

Inhibition (%) = 100 ($OD_{SNEOs\ treated\ samples}$-$OD_{Without\ SNEOs\ treated\ sample}$) × 100 ------ 2.

3. Result

3.1. Analysis of chemical components of extracted EOs by LC-MS

The LC-MS peaks of the NOEOs were showed with 13 essential oils peak along with different phytochemical and aromatic hydrocarbons. Among the all peaks and their respective chemical composition, the essential oils peaks were only indicated in this study. The retention time, occupied area and occupied percentages of NOEOs was correlated with respective 13 peaks, and confirmed by NIST data base of the Bharathidasan University Wiley library. In the result, the terpenes, monoterpenes sesquiterpenes was highly present along the phytochemical and aromatic hydrocarbons compounds (Fig. 1). Based on the highest RT, the EOs of 2-Thujene, α-terpineol, α-pinene, α-terpenyl acetate, borneol, spathulenol and Octanone was highly correlated. Also, respective occupation area and occupation percentages were noted including 22.30, 16.70, 10.06, 21.98, 12.40, 18.70 and 8.20. This result was indicated that the chosen *Nerium oleander* was suitable plant for purification of EOs with biological activity. In addition, anti-bacterial, anti-biofilm, anti-cancer, anti-oxidant, nurogenerative, larivicidal, anti-viral effect of 1-nonadecene, 5-Pyrrolidino-2-pyrrolidine, 1, 4-diaza-2, 5-dioxo-3-isobutyl bicyclo[4.3.0]nonane, Oleanolic acid, pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3, hexadecane, (1-h ydroxypenta-2,4-dien1-yl)oxy)anthracene-9,10-dione, Phthalic acid, isobutyl nonyl ester, Phenol, 2,5-bis (1,1-dimethyl ethyl), Thieno[3,2-e] benzofuran, 2-propppropenal, 3–91-aziridinyl)-3-(di methylamino) were also present in the *Nerium oleander* extract (Rajivgandhi et al., 2020a, 2020b, 2020c, 2019; Bibi and Mohamad Fawzi, 2020; Ramachandran et al., 2020). The plant essential oils are an important source for biological activity and agreed by Matías et al. (2020). The various environmental factors such as pH, temperature, organic nutrients and salinity were different in region to region and its biological activity also different (Ramachandran et al., 2020). Recently, the plant EOs as an

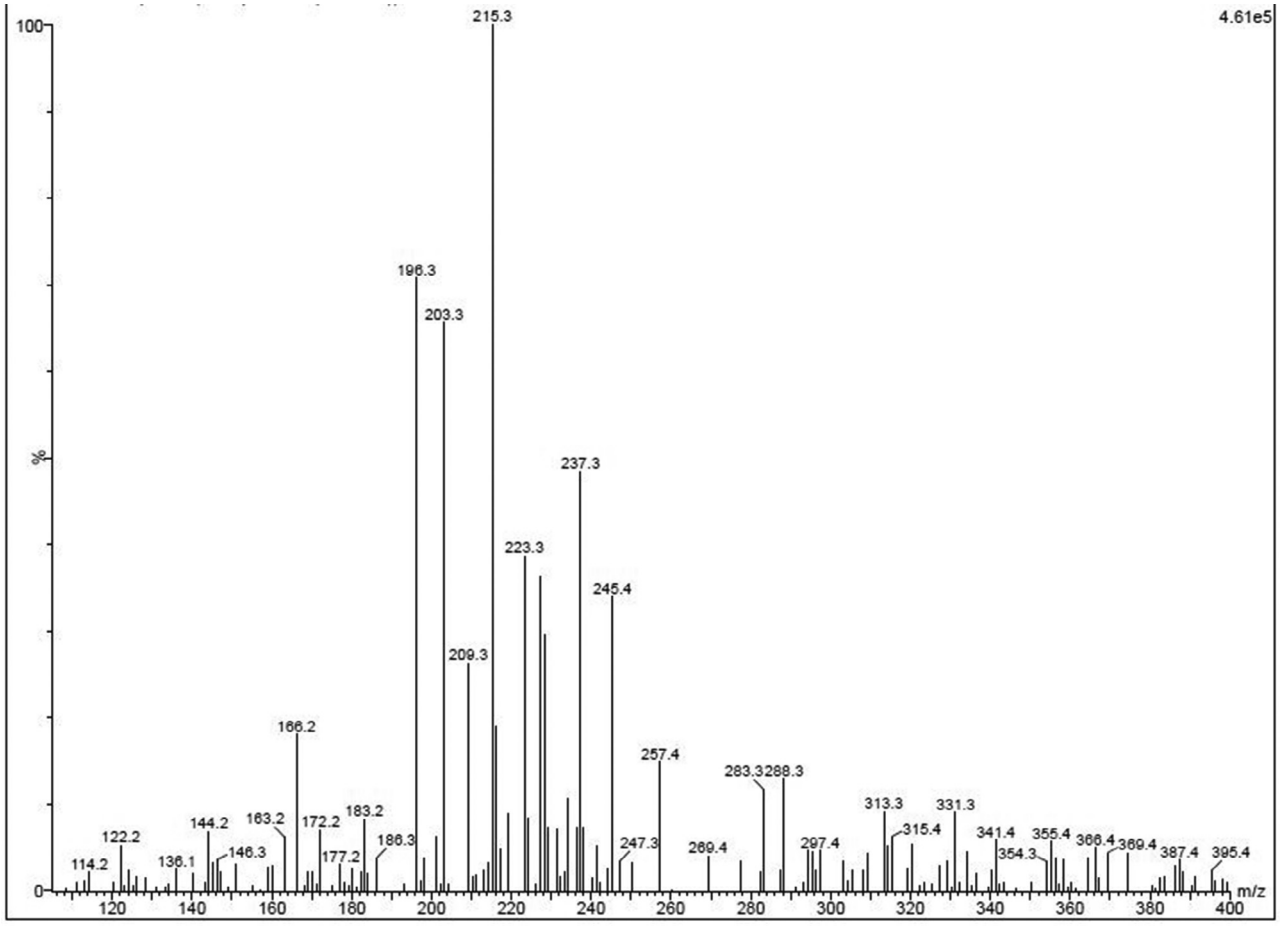


Fig. 1. The chemical components of *Nerium oleander* essential oils using LC-MS analysis.

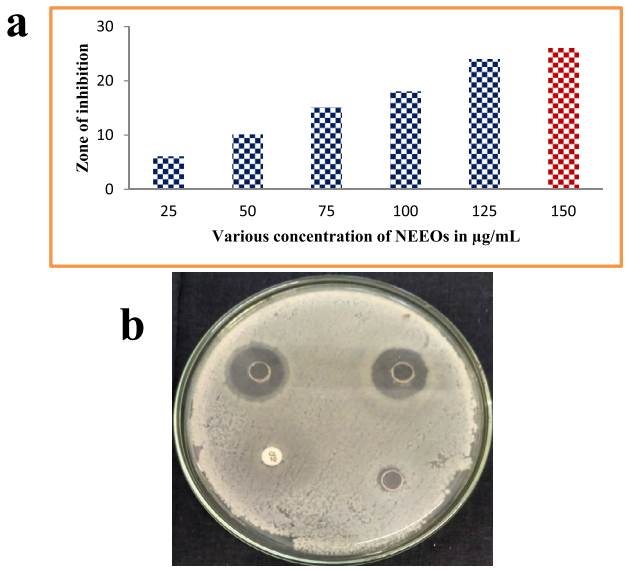


Fig. 2. Inactivation of biofilm forming *P. aeruginosa* by agar well diffusion method using *Nerium oleander* essential oils.

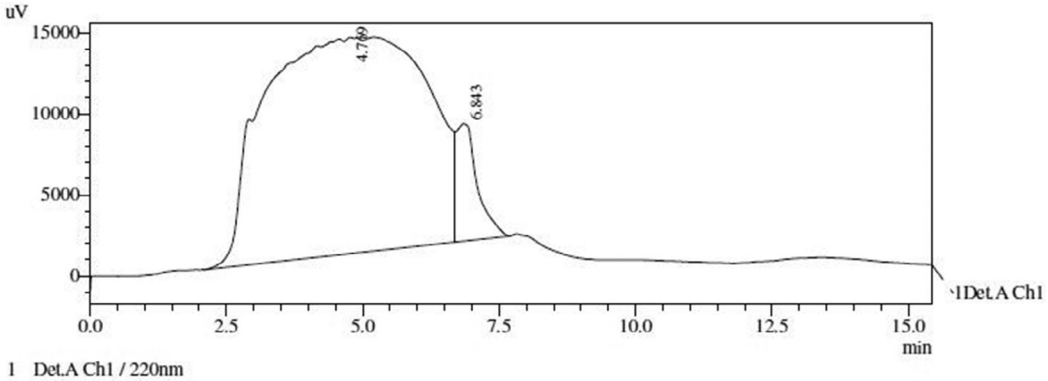
excellent anti-biofilm agent by Kanika et al. (2020) and targeted the virulence genes. The altered genes of in the stresses condition of soil environment also important factors to improve the biological activities against MDRs bacterial infections. Previously, the *Nerium oleander* essential oils were shown excellent activity against multi drug resistant bacteria (Mahjoub et al., 2018). The research reports of Mohammad Reza et al. (2015); Naira and Gamal (2020) were supported to *Nerium oleander* essential oils as promising anti-bacterial agents. The LC-MS was important analytical tool for detection of EOs, phytochemicals and bioactive compounds (Xinjun et al., 2020). All the facts were proved that the *Nerium oleander* was excellent anti-bacterial agent against biofilm forming *P. aeruginosa*.

3.2. Inactivation of P. Aeruginosa by Nerium oleander essential oils

The result of complete bacterial inactivation in the NOEOs treated nutrient agar plate was shown at 150 µg/mL concentration. In this concentration, the bacterium was exhibited 26 mm zone of inhibition and also 10 mm of inhibition at 25 µg/mL. In addition, the positive control of ceftazidime was shown with no zone of inhibition (Fig. 2a, b). The result was indicates, the purified EOs has potential anti-bacterial activity against biofilm forming *P. aeruginosa*. Mechanistically, the EOs was bind on the bacterial surface and attached strongly. After, it permeated in inside of the cell wall and affects the cytoplasmic membrane due to the ATP synthesis. Them the signaling factors are lost their native role and form unmatured cells. Therefore, the bacteria lost exopolysachharide production. The absence of exopolysachharide mediated *P. aeruginosa* lost their biofilm forming effect (Ahmed et al., 2020). The similar statement was reported by Iqbal and Farrukh, 2007. Sometimes, the unfavorable environmental conditions are advantages to plant to synthesis potential essential oils (Kalpna and Sumitra, 2012). Previously, the plant essential oils have the ability to inhibit the biofilm forming pathogens due to the exopolysachharides and hydrophobicity degradation (Mohamed Khaled et al., 2017). When the exopolysachharides was degraded, the bacteria unable to synthesize the signaling molecules and lost connectivity between the bacteria. Therefore, the bacterial pathogenicity was lost and sometimes antigenicity was remain available (Benchaar et al., 2008).

3.3. Purification of active EOs by preparative HPLC method

The potential anti-bacterial effect of the NOEOs was subjected to purified using preparative HPLC for separate the exact anti-bacterial properties form mixed oils. Initially, the anti-bacterial activity of the essential oils was visualized by analytical HPLC with two different major peaks (Fig. 3a). Both the peaks were exhibited with retention time, occupied area, height, area percentages and height percentages. The total number of the retention time was observed more than 11 and their occupied area was 2942644. It was very high and confirmed as an anti-bacterial lead compounds. Preparative HPLC result was agreed by previous result of Ramchandran et al. (2020) using the plant EOs. Based on the result, both the peaks were separately performed against *P. aeruginosa* and showed increased zone of inhibition. The zone of inhibition was shown with 28 mm and 24 mm and 14 mm and 12 mm against *P. aeruginosa* at 150 µg/mL and 25 µg/mL respectively (Fig. 4a). This zone of inhibition was higher compared with unpurified crude essential oils. Based on the inhibition zones, both the peaks were merged together and showed increased zone of inhibition 34 mm at same



PeakTable

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.769	2749967	13334	93.452	64.778
2	6.843	192677	7250	6.548	35.222
Total		2942644	20584	100.000	100.000

Fig. 3. Purification of antibacterial potential containing essential oils compounds by analytical HPLC.

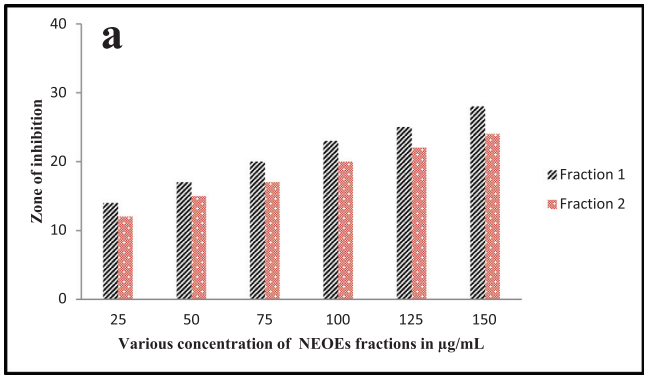


Fig. 4. Anti-bacterial activity of purified essential oils fraction 1, fraction 2 (a) and merged fractions (b) against biofilm forming *P. aeruginosa*.

150 µg/mL and 20 mm zone at 25 µg/mL (Fig. 4b). So, the result was suggested that the purification was successful and it improved the anti-bacterial activity against *P. aeruginosa*. These merged compounds were stored in freezer at 4℃ for for further biological activity. The similar result was reported by Ramachandran et al., 2020, the plant Hi-biscus rosa-sinensis was purified by preparative HPLC for increased anti-bacterial activity. The supportive evidence of Xinjun et al., 2020, also reported that the purified essential oils were improved their anti-bacterial properties against various Gram negative bacteria.

3.4. Minimum biofilm inhibition concentration

The more turbidity of the 96-well plate result was exhibited at the concentration of 200 µg/mL. At this concentration, the turbidity was shown more compared with other wells due to the efficiency of NOEOs. After conversion to percentage, the 94% inhibition rate at 200 µg/mL and half inhibition rate of 51% was shown at 75 µg/mL (Fig. 5). The result was revealed that the NOEOs have anti-biofilm effect at increasing concentration. When the concentration of NOEOs increased, the turbidity and percentage of inhibition also increased due to the intracellular permeation. This 200 µg/mL concentration was very low compared with other concentrations (Benchaar et al., 2008). The biofilm degradation ability was identified by absorption of crystal violet in the treated bacterial wells. In 200 µg/mL concentration treated wells were shown with more crystal violet absorption capacity, and it indicated that the damaged cells were absorbed the crystal violet (Rajivgandhi et al., 2018). Previously, the more concentration of the *N. oleander* was reported against *Nerium oleander* plant extract. Also, the extract of *N. oleander* has excellent anti-bacterial and arrhythmogenic activities, and it also confirmed by MIC with increasing concentration (Botelho et al., 2017; Jamal et al., 2012; Kars et al., 2013). Compared with previous study, the current study result was exhibited excellent anti-biofilm activity against biofilm forming *P. aeruginosa* at very lowest concentration. Finally, the 24-well plate result was confirmed that the NOEOs were potential anti-biofilm agent against biofilm formation at very lowest concentration and it can be used for further *invitro* studies.

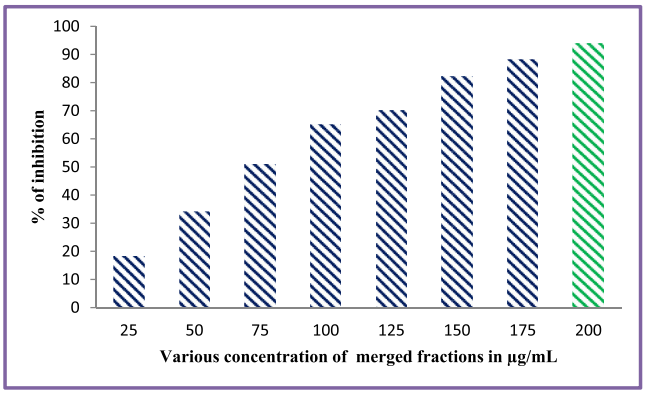


Fig. 5. Detection of minimum biofilm inhibition concentration of *Nerium oleander* essential oils by microtitre plate.

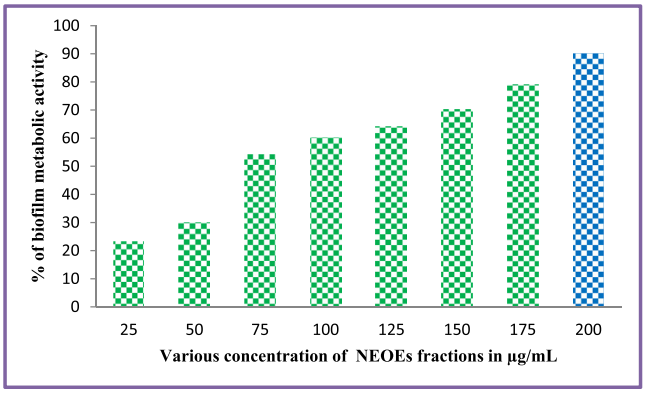


Fig. 6. Identification of biofilm metabolic assay *Nerium oleander* essential oils by microtitre plate using the solution of XTT.

3.5. Biofilm metabolic assay

The decreased survival of 24-well containing wells were shown after treatment with NOEOs at the concentration of 200 µg/mL. Previously, all the metabolic activity result of essential oils treatment was exhibited with higher concentration. Instead, our result was shown with increased death cells at very lowest concentration. The decreased survival and increased death cells of the result was indicated that the essential oils were very effective against biofilm formation. It may influence the inactivation of virulence factors production, arrest of signaling molecules and deactivation of metabolic pathways (Maruthupandy et al., 2020). The converted percentage result of XTT assay was exhibited with 90% and 54% death cells at the concentration of 200 µg/mL and 75 µg/mL concentration (Fig. 6). This result was most supported to BIC result. Our result was also supported with previous result of Milica et al., the plant EOs has more inhibition ability against biofilm forming Gram negative bacteria. The XTT result was suggested that the NOEOs inhibit the biofilm forming *P. aeruginosa* at concentration dependent inhibition.

4. Conclusion

In this study, the anti-bacterial activity of the *Nerium oleander* essential oils was extracted by hydro distillation method. The anti-oxidant activity result was revealed that the extract has more anti-oxidant activity. In addition, the improved anti-bacterial activity of the essential oils was purified using preparative HPLC method. At minimum concentration, the *Nerium oleander* essential oils were inhibited the *P. aeruginosa* biofilm, and it proved the decreased viability result of XTT assay. Finally, all the *invitro* experiments were strongly supported to *Nerium oleander* essential oils, and it is a potential anti-biofilm agent for biofilm forming *P. aeruginosa*.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Wugao, L., Hongjia, L., Xinyu, C., Tianzheng, L., Ning, Z., Bao, Z., Weihua, C., 2020. Tea polyphenols inhibits biofilm formation, attenuates the quorum sensing-controlled virulence and enhances resistance to *Klebsiella pneumoniae* infection in *Caenorhabditis elegans* model. Microb. Pathog. 147, 104266.

Abinaya, M., Gayathri, M., 2019. Inhibition of biofilm formation, quorum sensing activity and molecular docking study of isolated 3, 5, 7-Trihydroxyflavone from *Alstonia scholaris* leaf against *P.aeruginosa*. Bioorg. Chem. 87, 291–301.

Na, L., Stephen, F., Qingping, W., Yu, D., Jumei, Z., Haiyan, Z., 2020. Insights into *Cronobacter sakazakii* Biofilm Formation and Control Strategies in the Food Industry. Engineering 6, 393–405.

Muhammad, K., Danial, H., Muhammad, B., Zahid, A.B., Muhammad, H., Ayaz, A., Danfeng, H., Anwar, H., 2017. Identification of oral cavity biofilm forming bacteria and determination of their growth inhibition by *Acacia arabica*, *Tamarix aphylla* L. and *Melia azedarac*h L. medicinal plants. Archiv. Oral Biol. 81, 175–185.

Carel, B.O., Nomasomi, G., Chris, J.H., Namrita, L., 2019. Inhibition of mycothione disulphide reductase and mycobacterial biofilm by selected South African plants. S. Afr. J. Bot. 120, 291–297.

Isabela, C.J., Gladslene, G.S.F., Arie Fitzgerald, B., Luciana, Cristina Linsde, A.S., 2016. *Myrcia ovata* Cambessedes essential oils: A proposal for a novel natural antimicrobial against foodborne bacteria. Microb. Pathog. 99, 142–147.

Ygor Jessé, R., Daniel, B.M., George, A.Q., Elsie Franklin, G., Anna Carina, A.D., Davyson, L.M., 2020. Chemical composition of the essential oils of circadian rhythm and of different vegetative parts from *Piper mollicomum* Kunth – A medicinal plant from Brazil. Biochem. Systemat. Ecol. 92, 104116.

Lin, L., Mao, X., Sun, Y. Rajivgandhi, Cui, H., Antibacterial properties of nanofibers containing chrysanthemum essential oil and their application as beef packaging, Int. J. Food Microbiol. 292, (2019), 21-30.

Sana, A., Walid, B.S., José, R.V., Mohamed Ali, S., Ridha, T., Jalel, B., Jésus, N., Hedi, B. M., 2020. Anti-oxidant, antibacterial, anti-biofilm, and anti-quorum sensing activities of four essential oils against multidrug-resistant bacterial clinical isolates. Curr. Res. Translat. Med. 68, 59–66.

Houdkova, M., Urbanova, K., Doskocil, I., Rondevaldova, J., Novy, P., Nguon, S., Chrun, R., Kokoska, L., 2018. *In vitro* growth-inhibitory effect of Cambodian essential oils against pneumonia causing bacteria in liquid and vapour phase and their toxicity to lung fibroblasts. S. Afr. J. Bot. 118, 85–97.

Balkan, I.A., Dogan, H.T., Zengin, G., Colak, N., Ayaz, F.A., Goren, A.C., Kirmizıbekmez, H., Yesilad, E., 2018. Enzyme inhibitory and antioxidant activities of Nerium oleander L. flower extracts and activity guided isolation of the active components. Indus. Crops Prod. 112, 24–31.

Santos, R.S., Sanches, F.A.C.R.A., Leitão, R.G., Leitão, C.C.G., Oliveira, D.F., Anjos, M.J., Assis, J.T., 2019. Multielemental analysis in Nerium Oleander L. leaves as a way of assessing the levels of urban air pollution by heavy metals. Appl. Radiat. Isotopes 152, 18–24.

Kars, M.D., Günduz, U., Uney, K., Bas, A.L., 2013. Exploring a natural MDR reversal agent: potential of medicinal food supplement *Nerium oleander* leaf distillate. Asian Pacif. J. Trop. Biomed. 3, 644–649.

Samia, A., Shiv, S., Peter, F., Farah, H., Monique, L., 2020. Potential synergistic antimicrobial efficiency of binary combinations of essential oils against *Bacillus cereus* and *Paenibacillus amylolyticus*-Part A. Microb. Pathog. 141, 104008.

Bibi, S.J., Mohamad Fawzi, M., 2020. Essential oils from 9 exotic and endemic medicinal plants from Mauritius shows *in vitro* antibacterial and antibiotic potentiating activities. S. Afr. J. Bot. 132, 355–362.

Mariadhas, V.A., Ponnuswamy, V., Soundarrajan, I., Naif Abdullah, A.D., Ki Choon, C., 2019. Essential oil of four medicinal plants and protective properties in plum fruits against the spoilage bacteria and fungi. Indust. Crops Prod. 133, 54–62.

Taoufiq, B., Abdelhakim, B., Khaoula, H., Gokhan, Z., Abdelmajid, K., El Hassan, A., Khalil, H., 2020. Chemical composition and antibacterial activity of the essential oil and extracts of *Cistus ladaniferus* subsp. *ladanifer* and *Mentha suaveolens* against phytopathogenic bacteria and their ecofriendly management of phytopathogenic bacteria. Biocatal. Agricult. Biotechnol. 28, 101696.

Rajivgandhi, G.N., Ramachandran, G., Jia-Ling, L., Lingzi, Y., Manoharan, N., Rajesh Kannan, M., Antony Joseph Velanganni, A., Alharbi, N.S., Kadaikunnan, S., Khaled, J.M., Wen-Jun, L., 2020a. Molecular identification and structural detection of anti-cancer compound from marine *Streptomyces akiyoshiensis* GRG 6 (KY457710) against MCF-7 breast cancer cells. J. King Saud Univers. Sci. 32, 3463–3469.

Anjugam, M., Vaseeharan, B., Iswarya, A., Divya, M., Prabhu, N.M., Sankaranarayanan, K., 2018. Biological synthesis of silver nanoparticles using β-1,3 glucan binding protein and their antibacterial, antibiofilm and cytotoxic potential. Microb. Pathog. 115, 31–40.

Ramachandran, G., Rajivgandhi, G., Maruthupandy, M., Manoharan, N., 2019. Extraction and partial purification of secondary metabolites from endophytic actinomycetes of marine green algae *Caulerpa racemosa* against multi drug resistant uropathogens. Biocat. Agricult. Biotechnol. 17, 750–757.

Maruthupandy, M., Rajivgandhi, G., Kadaikunnan, S., Veeramanid, T., Alharbi, N.S., Muneeswaran, T., Khaled, J.M., Li, W.J., Alanzi, K.F., 2020. Anti-biofilm investigation of graphene/chitosan nanocomposites against biofilm producing *P. aeruginosa* and *K. pneumonia*. Carbohyd. Polym. 230, 115646.

Rajivgandhi, G., Saravanan, K., Ramachandran, G., Jia-Ling, L., Lingzi, Y., Quero, F., Alharbi, N.S., Kadaikunnan, S., Khaled, J.M., Manoharan, N., Wen-Jun, L., 2020b. Enhanced anti-cancer activity of chitosan loaded *Morinda citrifolia* essential oil against A549 human lung cancer cells. Int. J. Biolog. Macromol. 164, 4010–4021.

G.N. Rajivgandhi, G. Ramachandran, C. Chenthis Kanisha, L. Jia-Ling, Y. Lingzi Yin, N. Manoharan, N.S. Alharbi, S. Kadaikunnan, J.M. Khaled, L. Wen-Jun, Anti-biofilm compound of 1, 4-diaza-2, 5-dioxo-3-isobutyl bicyclo[4.3.0]nonane from marine Nocardiopsis sp. DMS 2 (MH900226) against biofilm forming K. pneumonia, J. King Saud Univers. Sci. 32, 2020, 3495-3502.

Matías, A.R., María, B.V., Alfonsina, E.A., Héctor, R.J., 2020. Antioxidant and antimicrobial activities of citrus essential oils from Argentina and the United States. Food Biosci. 36, 100651.

Ramachandran, G., Rajivgandhi, G.N., Murugan, S., Alharbi, N.S., Kadaikunnan, S., Khaled, J.M., Almanaa, T.N., Manoharan, N., Wen-Jun, L., 2020. Anti-carbapenamase activity of *Camellia japonica* essential oil against isolated carbapenem resistant *klebsiella pneumoniae* (MN396685). Saud. J. Biolog. Sci. https://doi.org/10.1016/j.sjbs.2020.06.044.

Kanika, S., Sanjay, G., Vijay, K.R., Vikash, B., 2020. Synergistic antioxidant and antimicrobial activities of essential oils of some selected medicinal plants in combination and with synthetic compounds. Indus. Crops Prod. 154, 112569.

Mahjoub, J., Najeh, T., Khiari, R., Tawfik, A.S., 2018. Physicochemical characteristics and dyeing properties of lignin-cellulosic fibers derived from *Nerium oleander*. J. Molecul. Liq. 249, 1138–1144.

Mohammad Reza, F., Mohammad Djaefar, M.F., Kourosh, A., Fariba, M., 2015. Mosquitocidal efficacy of medicinal plant, *Nerium oleander* (Apocynaceae), leaf and flower extracts against malaria vector, *Anopheles stephensi* Liston (Diptera: Culicidae) larvae. Asian Pacif. J. Trop. Dis. 5, 33–37.

Naira, I., Gamal, E.A., 2020. Phytoremediation uptake model of heavy metals (Pb, Cd and Zn) in soil using *Nerium oleander*. Heliyon 6, e04445.

Xinjun, Y., Govindan Nadar, R., Govindan, R., Naiyf, S.A., Shine, K., Jamal, M.K., Taghreed, N.A., Natesan, M., Rajan, Viji, 2020. Preparative HPLC fraction of *Hibiscus rosa-sinensis* essential oil against biofilm forming *Klebsiella pneumonia*. Saudi J. Biolog. Sci. https://doi.org/10.1016/j.sjbs.2020.07.008.

Ahmed, E., Mervat, G.H., Mahmoud, M.A., Soheir, A., Ahmed, M.H., Hagar, A.A., Mohamed, F.F., 2020. Antimicrobial activity of certain natural-based plant oils against the antibiotic-resistant acne bacteria. Saudi J. Biolog. Sci. 27, 448–455.

Iqbal, A., Farrukh, A., 2007. In vitro efficacy of bioactive extracts of 15 medicinal plants against ESβL-producing multidrug-resistant enteric bacteria. Microbiolog. Res. 162, 264–275.

Kalpna, R., Sumitra, C., 2012. *In vitro* interaction of certain antimicrobial agents in combination with plant extracts against some pathogenic bacterial strains. Asian Pacif. J. Trop. Biomed. 2, S876–S880.

Mohamed Khaled, I., Zakaria Ahmed, M., Hanan, H.A.K., Yara Mostafa, A., 2017. Evaluation of antibacterial efficacy of anise wastes against some multidrug resistant bacterial isolates. J. Radiat. Res. Appl. Sci. 10, 34–43.

Benchaar, C., Calsamiglia, S., Chaves, A.V., Fraser, G.R., Colombatto, D., McAllister, T. A., Beauchemin, K.A., 2008. A review of plant-derived essential oils in ruminant nutrition and production. Animal Feed Sci. Technol. 145, 209–228.

Rajivgandhi, G., Senthil, R., Ramachandran, G., Maruthupandy, M., Manoharan, N., 2018. Antibiofilm activity of marine endophytic actinomycetes compound isolated from mangrove plant *Rhizophora mucronata*, Muthupet Mangrove Region, Tamil Nadu, India. J. Terr. Mar. Res. 2, 1–7.

Rajivgandhi, G., Ramachandran, G., Maruthupandy, M., Vaseeharan, B., Manoharan, N., 2019. Molecular identification and structural characterization of marine

endophytic actinomycetes *Nocardiopsis* sp. GRG 2 (KT 235641) and its antibacterial efficacy against isolated ESBL producing bacteria. Microb. Pthog. 126, 138–148.

Botelho, A.F.M., Santos-Miranda, A., Cavalcante Joca, H., Scabelo Mattoso, C.R., Souza de Oliveira, M., Pierezan, F., Santos Cruz, J., Soto-Blanco, B., Martins Melo, M., 2017. Hydroalcoholic extract from Nerium oleander L. (Apocynaceae) elicits arrhythmogenic activity. J. Ethnopharm. 206, 170–177.

Jamal, M.H.M., Rahman, S., Islam, A., Karim, R., Alam, S., Rahman, Z., 2012. Minimum inhibitory concentration analysis of nerium oleander against bacterial pathogens, Asian Pacific. J Trop. Biomed., S1664–S1666