*Malays. Appl. Biol. (2022) 51(4): 45-56*
https://doi.org/10.55230/mabjournal.v51i4.10

# ANTIBACTERIAL AND ANTIBIOFILM ACTIVITIES OF *Swietenia macrophylla* King ETHANOLIC EXTRACT AGAINST FOODBORNE PATHOGENS

CHE AMIRA IZZATI CHE MAN, WAN RAZARINAH WAN ABDUL RAZAK
and MOHD FAKHARUL ZAMAN RAJA YAHYA*

*Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia*
**E-mail: fakharulzaman@uitm.edu.my*

Accepted 3 October 2022, Published online 31 October 2022

## ABSTRACT

*Swietenia macrophylla* is known to possess several medicinal uses, however, its antibacterial and antibiofilm activities against foodborne pathogens remain not well investigated. The present work was performed to examine the phytochemical compounds, antibacterial and antibiofilm activities of *S. macrophylla* ethanolic extract (SMEE) against four foodborne pathogens namely, *Salmonella typhimurium* ATCC 14028, *Escherichia coli* ATCC 25922, *Shigella sonnei* ATCC 33862 and *Pseudomonas aeruginosa* ATCC 10145. The phytochemical analysis of SMEE was performed using gas chromatography-mass spectrometry while the antibacterial activities of SMEE were determined by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays. On the other hand, the antibiofilm and time-killing activities of SMME were evaluated using a crystal violet assay. The result demonstrated that SMEE contained major phytochemical compounds such as olean-12-ene (27.37%), resorcinol (16.45%), 24-noroleana-3,12-diene (13.4%), and germanicol (11.50%). The MIC values of SMEE ranged from 31.25 to 500 µg/mL, while all the MBC values were found to be greater than 1000 µg/mL. At the 12 h exposure to SMEE, all the biofilms were inhibited by 50% except *E. coli*. Biofilm inhibitory concentration (BIC50) values of SMEE ranged between 5.19 and 42.47 µg/mL. In conclusion, *S. macrophylla* is a promising source of natural antibacterial and antibiofilm agents to treat foodborne diseases.

**Key words:** *Swietenia macrophylla*, biofilm, gas chromatography-mass spectrometry, time-killing assay

## INTRODUCTION

Food poisoning and foodborne infections caused by microbial contamination during processing and storage are now becoming major public health problems worldwide (Patra & Baek, 2016). In the United States, a total of 17,094 outbreaks of foodborne disease were documented between 1990 and 2008. A total of 370,266 people were reported to be ill as a result of these incidents (CDC, 2009a). Foodborne disease in Malaysia is also a concern, whereby the incidence rate of food poisoning (45.71 per 100,000 population) in 2018 was the fourth highest among communicable diseases after dengue, hand foot, and mouth disease, and tuberculosis (MOH, 2019). In food processing, synthetic additives and antimicrobial agents are used to alleviate bacterial growth, minimize contamination, and extend food shelf life. Unfortunately, synthetic additives may be harmful, and the usage of preservatives has been linked to respiratory and other health issues. *Shigella flexneri, Escherichia coli,* and *Salmonella typhimurium* are a few of the pathogens that cause food poisoning (Scallan *et al*., 2011) and they belong to the Enterobacteriaceae family. Nearly all foodborne pathogens can attach to the surface and form a biofilm (Yahya *et al*. 2014). It has been well accepted that medicinal plants play a major role in infection control.

*Swietenia macrophylla*, also known as big-leaf mahogany, is a tropical lumber tree that can grow to a height of 40-60 m. It is also locally known in Malaysia as *tunjuk langit* (Mohammed *et al*., 2014). The soils and the environmental conditions of *S. macrophylla* can be very different. Usually, this plant species is present in alluvial soils, volcanic soils, hard clay, lateritis, and soils resulting from the sedimentary, igneous, or metamorphic rock formation. *S. macrophylla* is effective against malaria, anemia, diarrhea, fever, dysentery, hypertension, cancer, cough, chest pain, intestinal parasitism, and anti-ulcer, antibacterial, antioxidant as well as anti-diabetic properties (Goh & Abdul Kadir, 2011). According to Swati and Richa (2011), *S. macrophylla* consists mainly of triterpenoids and limonoids.

To date, there are very few published reports about the biological activities of *S. macrophylla* King in Malaysia. Antibiofilm and antibacterial activities of *S. macrophylla* against foodborne pathogens remain

* To whom correspondence should be addressed

not well investigated. Therefore, this study aims to investigate the antibiofilm and antibacterial potential of *S. macrophylla* leaves against four foodborne pathogens namely, *Pseudomonas aeruginosa* ATCC 10145*, Escherichia coli* ATCC 25922*, Salmonella typhimurium* ATCC 14028 and *Shigella sonnei* ATCC 33862.

## MATERIALS AND METHODS

### Sample collection and plant extraction

Leaves of *S. macrophylla* (MFI 0161/20) collected in Taman Samudra, Batu Caves (3°14'03.1"N, 101°42'05.1"E) were taxonomically identified and certified by the botanist of the Biodiversity Unit, Institute of Bioscience, University Putra Malaysia (UPM). The sample was air-dried for two weeks before being milled into a fine powder with a coffee grinder. The crude *S. macrophylla* ethanolic extract (SMEE) was prepared by mixing 100 g of the powdered sample with 500 mL of 70% (v/v) ethanol. The solution was incubated at room temperature with continuous shaking at 150 rpm for three days in an orbital shaker. After constant shaking at 150 rpm for three days, the sample was filtered using Whatman filter paper (Whatman No. 2, 4.25 cm diameter). To obtain crude extract paste, the filtrate was concentrated using a rotary evaporator at 50 °C under reduced pressure. The crude extract obtained was weighed and stored in sterile universal bottles and refrigerated at -20 °C until further use. Formula to calculate the yield of leaves extraction showed as below (Equation 1) (Adam *et al*., 2019):

Equation 1:

$$\frac{\text{Total weight of crude extract}}{\text{Amount of leaves powder}} \times 100$$

### Preparation of varying concentrations of the extracts

Various concentrations of SMEE were prepared in the range between 31.25 and 1000 µg/mL. To prepare the stock solution, 0.02 g of SMEE was dissolved in a solution containing 1 mL of 2% (v/v) DMSO and 19 mL of distilled water. The initial concentration of SMME (1000 µg/mL) was then serially diluted two-fold in distilled water to produce 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, and 31.25 µg/mL.

### Preparation of test microorganisms

*P. aeruginosa* ATCC 10145*, E. coli* ATCC 25922*, S. typhimurium* ATCC 14028, and *S. sonnei* ATCC 33862 were obtained from Microbiology Laboratory, Faculty of Applied Sciences, UiTM Shah Alam. All bacteria were cultured overnight in nutrient broth at 37 °C. For all assays, the bacterial density was measured using a spectrophotometer and standardized to OD 600 of 0.5.

### GC-MS analysis

GC-MS analysis of SMEE was performed as previously reported (Rukshana *et al*., 2017) using THERMO Gas Chromatography-TRACE ULTRA VER: 5.0. The temperature of the oven was maintained at 220 °C at a rate of 6°C per min while the carrier gas was maintained at a rate of 1 mL per min. The split sampling procedure was used to insert the sample at a ratio of 1:10. By comparing the retention times of the series, the retention indices (RI) value was identified. Moreover, the classification of each element was verified by comparing its retention index with the information in the literature. The mass-Spectrum interpretation was performed using the National Institute of Standard and Technology (NIST) database with more than 62,000 patterns. By using the NIST library database, the spectrum of the unidentified components was compared to the spectrum of known components contained in the NIST library. The molecular weight, name, chemical composition, and molecular formula of the test product components were determined.

### Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) assay for SMEE was performed in 96-well microplates as previously reported by Debalke *et al*. (2008) with few modifications. Fifty µL of nutrient broth was loaded to each well, followed by 100 µL of SMME (1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL and 31.25 µg/mL). Then, 50 µL of bacterial suspension was added to each well, making each well have a total volume of 200 µL. The wells containing broth and bacterial suspension were used as negative growth controls whereas the wells containing ciprofloxacin (64 µg/mL) and bacterial suspension were used as positive controls. Subsequently, the microplate was incubated for 24 h at 37 °C incubators. Then, 30 µL of 0.02% (w/v) resazurin was added to the wells and incubated at 37 °C for at least 2 h. The reduction of the blue dye to the pink color indicated the presence of viable bacteria. The lowest concentration that was able to maintain the blue color of the dye was considered MIC.

### Determination of minimum bactericidal concentration (MBC)

The results from the MIC assay were used to determine the MBC values. A loopful of bacterial suspension from the microplate wells at the lowest SMEE concentration showing no bacterial growth was streaked on Muller-Hinton agar plates to determine the viability of the bacterial cells. The plates were

incubated at 37 °C for 24 h. The minimal bactericidal concentration (MBC) of SMEE was defined as the lowest concentration at which the bacterial cells were killed.

**Pellicle assay**

The pellicle assay was used to screen all the bacteria for biofilm formation. Pellicle assay was performed as previously reported by Yahya *et al*. (2017). Two mL of bacterial suspension was loaded into a sterile test tube and incubated at 37 °C for 24 h. The nutrient broth was then discarded, whilst the pellicle fraction was washed twice with distilled water before being heat-fixed at 60 °C for 30 min. The biofilm fraction was then stained with three mL of 0.5% (w/v) crystal violet for 10 min, 25% (v/v) methanol (Sigma, USA) for 5 min. Lastly, the biofilm fraction was rinsed with distilled water gently. Biofilm formation is characterized by the formation of pellicles and/or adherent bacterial cells on the internal surface of the test tube at the air-liquid interface.

**Antibiofilm screening assay**

Antibiofilm screening assay was performed using a method as previously described by Yaacob *et al*. (2021) with some modifications. One hundred uL of bacterial suspension (approximately $9 \times 10^8$ CFU/mL) was loaded into microplate wells. Twenty µL of fresh nutrient broth and 80 µL of SMEE were then added. The microplate was incubated at 37 °C for 24 h. The wells containing 120 µL of bacterial suspension and 80 µL of fresh nutrient broth were used as negative controls. The wells containing 120 µL of bacterial suspension and 80 µL of intellectual property (IP)-protected antibiofilm cocktail were used as positive controls.

The nutrient broth containing stationary-phase planktonic cells was discarded from 96-well microplates while the biofilm fractions were rinsed twice using a saline buffer to remove non-adhesive cells. Then, the biofilm fractions were heat-fixed at 60 °C for 15 min and further stained with 200 µL of 1% (w/v) crystal violet solution. The dye was allowed to sit for 15 min at room temperature. The plates were then washed three times with sterile distilled water to remove any remaining stains. Two hundred µL of absolute ethanol was used to dissolve the stained biofilm fractions and the absorbance was measured at 600 nm using a microplate reader (BioTek Synergy). The mean absorbance value was calculated, and the percentage inhibition of the biofilm was estimated by using the formula (Equation 2) below (Das *et al*., 2017):

Equation 2:

$$\text{Percentage (\%)inhibition} = \frac{\text{OD (Negative Control)-OD (Experimental)}}{\text{OD(Negative Control)}} \times 100$$

**Time-killing assay**

The time-killing assay of SMEE was carried out by the standard protocol for the time-kill kinetics procedure of the Antimicrobial Susceptibility Testing Method (CLSI, 2007) with minor modifications. The bacterial suspension (150 µL) and nutrient broth (50 uL) were loaded into the microplate wells. The microplates were then incubated at 37 °C for 24 h. The nutrient broth containing stationary-phase planktonic cells was discarded from the microplates whilst the preformed biofilm fractions were rinsed twice using a saline buffer to remove non-adhesive cells.

The microplate wells loaded with 200 uL of SMEE were used as the treated group. The concentration of SMEE showing the highest percentage of inhibition on each bacterial biofilm was tested in the time-killing assay. The microplate wells loaded with 200 µL of fresh nutrient broth were used as negative controls. Meanwhile, the microplate wells loaded with 200 uL of IP-protected antibiofilm cocktail were used as positive controls. The microplates were then incubated for different periods namely 6 h, 12 h, 18 h, 24 h, and 30 h. At the end of each incubation period, the nutrient broth was then discarded while biofilm fractions were rinsed twice using a saline buffer.

The biofilm fractions were heat-fixed at 60 °C for 15 min and stained with 200 µL of 1% (w/v) crystal violet solution. The dye was allowed to sit for 15 min at room temperature. The microplates were then washed three times with sterile distilled water to remove any remaining stains. Two hundred µL of absolute ethanol was used to dissolve the stained biofilm fractions and the absorbance was measured at 600 nm using a microplate reader (BioTek Synergy). The mean absorbance value was calculated, and the graph was plotted according to the respective periods of incubation.

**Statistical analysis**

Experimental results from the antibiofilm screening and time-killing assays were represented as mean ± standard error of the mean (SEM) of three replicates. Where applicable, differences between treated and control groups were analyzed using an independent T-test. A significant difference was considered at the level of $p<0.05$. For biofilm inhibition concentration ($BIC_{50}$), the calculations were performed by using AAT Bioquest ($IC_{50}$ Calculator Tool).

## RESULTS AND DISCUSSION

**Yield of leaves extract and phytochemical composition**

Total crude extract obtained from *S. macrophylla* was 13.82 g, equivalent to 13.82% yield of leaves extraction.

Table 1 shows the phytochemical compounds of

SMEE belonging to different chemical classes. Olean-12-ene (27.37%), resorcinol (16.45%), 24-noroleana-3,12-diene (13.4%), and germanicol (11.50%) were found to be the major phytochemical compounds of SMEE. Other phytochemical compounds identified in SMEE were 6-fluorocumarin, phenol, 3,4-altrosan, 4H-1-benzopyran-4-one, 2,4-di-tert-butylphenol, and palmitic acid.

Oleanane-type triterpenoids represent the most ubiquitous and important group of triterpenoids in the plant kingdom. Olean-12-ene is known to be directly derived from the oleanane skeleton. In the present study, olean-12-ene (27.7%) was identified as a major phytochemical compound in SMME. This compound has also been isolated from methanolic extract of *Drypetes tessmanniana* and shown to inhibit *E. coli, P. aeruginosa, S. typhimurium,* and *S. faecalis* (Kuete *et al*., 2010). Another study has also reported the antifungal and antibacterial activities of olean-12-ene (Katerere *et al*., 2003), however, there is no information on its effect on the biofilm. Therefore, the present study provides the first evidence for the antibiofilm activity of plant extract containing olean-12-ene.

The present study demonstrated germanicol as a major phytochemical compound in SMEE. Fatima *et al*. (2011) revealed that germanicol (12.8%) represented the major constituent of *Pistacia lentiscus* leaf extract. They also showed that germanicol possessed antibacterial activity against two Gram-negative bacteria, namely *Klebsiella pneumonia* and *P. aeruginosa*. On the other hand, Afrouzan *et al*. (2018) demonstrated that ethanolic extract of *Iranian propolis* contained terpene derivatives such as germanicol as its major component. The combination of or/and synergism between flavonoids, aromatic acids, and terpenes may contribute to the antimicrobial activities of *Iranian propolis* (Afrouzan *et al*., 2018).

In the present study, 3,4-Altrosan (1.44%) was identified in SMEE. This finding is in line with Kushwaha *et al*. (2019) demonstrating that 3, 4-Altrosan (6.92%) was a major component of methanolic extract of *Ziziphus mauritiana* fruit. The extract exhibited bacteriostatic and fungicide properties. Another study by Nirubama *et al*. (2014) revealed the presence of 3, 4-Altrosan (15.29%) as a major compound in *Andrographis echiodes* (L.) Nees. leaves.

Herein, 2,4-Di-tert-buylphenol (0.49%) was identified in SMEE. This compound is an alkylbenzene and a member of the class of phenols. It is also known as phenol,2,4-bis(1,1-dimethylethyl). In 2016, Viszwapriya *et al*. investigated the effect of 2,4-Di-tert-butylphenol from the seaweed surface associated with the bacterium *Bacillus subtilis* against the presence of Group A Streptococcus (GAS). They demonstrated that the seaweed (*Gracilaria gracilis*) surface associated with *Bacillus subtilis* has antibiofilm potential when tested against Group A Streptococcus. This is due to the presence of an active principle in the seaweed, which is phenol,2,4-bis(1,1-dimethylethyl) or 2,4-di-tert-butylphenol referred to as DTBP. Viszwapriya *et al*. (2016) discovered that 2,4-Di-tert-butylphenol (DTBP) targeted the first adhesion step of the biofilm development cascade by influencing cell surface hydrophobicity and EPS synthesis. Apart from biofilm inhibition, there were also other potential modes of action performed by the DTBP. Microscopic examination indicated that DTBP treatment resulted in changes in cell surface architecture, reduction in thickness, lower biofilm biomass, and lower extracellular polymeric substance (EPS) (Viszwapriya *et al*., 2016). This finding is supported by Dehpour *et al*. (2012) as they reported the antibacterial, antifungal, antioxidant, and anticancer activity of DTBP isolated from various bacterial, fungal, and plant sources. The antimicrobial activities of *S. macrophylla* may attribute to the presence of 2,4-Di-tert-butylphenol. Furthermore, Mishra *et al*. (2020) investigated the effect of 2,4-Di-tert-butylphenol isolated from endophytic fungi, *Daldinia eschscholtzii* on the quorum sensing activity in *P. aeruginosa.* They discovered that 2,4-DTBP treated *P. aeruginosa* significantly reduced the production of virulence factors, as well as biofilm and its related components regulated by quorum sensing,

**Table 1.** Phytochemical compounds identified in SMME by GC-MS

| Retention time | Compound | Molecular formula | Peak Area (%) |
|---|---|---|---|
| 26.064 | Olean-12-ene | $C_{30}H_{50}$ | 27.37 |
| 6.348 | Resorcinol | $C_6H_4(OH)_2$ | 16.45 |
| 25.062 | 24-noroleana-3,12-diene | $C_{29}H_{46}$ | 13.4 |
| 27.919 | Germanicol | $C_{30}H_{50}O$ | 11.50 |
| 9.591 | 6-fluorocumarin | $C_9H_7FO_2$ | 4.84 |
| 3.547 | Phenol | $C_6H_5OH$ | 3.21 |
| 4.675 | 3,4-altrosan | $C_6H_{10}O_5$ | 1.44 |
| 10.165 | 4H-1-benzopyran-4-one | $C_9H_6O_2$ | 0.5 |
| 4.883 | 2,4-di-tert-butylphenol | $C_{14}H_{22}O$ | 0.49 |
| 11.987 | Palmitic acid | $CH_3(CH_2)_{14}COOH$ | 0.23 |

in a dose-dependent form. Meanwhile, Padmavathi *et al*. (2015) demonstrated the ability of 2,4-Di-tert-butylphenol in inhibiting and disrupting biofilms of *Candida albicans*. They stated that a key virulence factor that aids in the invasion of *C. albicans* includes the inhibition of the production of hemolysins, phospholipases, and secreted aspartyl proteinase.

The present work identified palmitic acid (0.23%) in SMEE. This result corroborates Elmarzugi *et al*. (2013) demonstrating the presence of some fatty acids and terpenoids, which extracted from the seeds included palmitic acid (12.50%), stearic acid (16.42%), arachidic acid (0.56%), oleic acid (25.30%), linoleic acid (33.87%) and linolenic acid (11.32%). Palmitic acid is a long-chain fatty acid, which is a type of organic compound. Many fatty acids are known to have antibacterial and antifungal properties (Aparna *et al*., 2012). Palmitic acid is also the major constituent in three varieties of *Labisia pumila*. In addition, *L. pumila* aqueous extract has been shown to exhibit a variable degree of antibacterial activity against eight Gram-positive and Gram-negative bacteria. Elmarzugi *et al*. (2013) demonstrated that several chemical elements were examined from the *S. macrophylla* terminal shoots, senescent and mature leaves as the essential oil components in form of fatty acids and terpenoids such as γ-himachalene, germacrene D, germacrene A, cadina-1,4-diene, hexadecanoic acid and ethyl hexadecanoate.

In 2017, Husain *et al*. investigated the efficacy of *Mangifera indica* L. (ML) leaf extracts on QS-regulated virulence factors and biofilm formation in Gram-negative pathogens. It shows that n-palmitic acid (9.96%) is one of the major compounds found in ML extracts. Moreover, scanning electron microscopy images indicated that ML extract significantly decreased bacterial biofilm formation, demonstrating that ML extract treatment lowers biofilm strength. The potential function of several synthetic and natural substances as efflux pump inhibitors of Gram-negative bacteria and QS interference has been revealed (Amaral & Molnar, 2012). In addition, Suliman *et al*. (2013) investigated the oil content, fatty acids compositions, and antibacterial efficacy of *S. macrophylla* seed oil against four multiple-drug-resistant bacteria namely: *S. aureus*, *S. typhimurium*, *P. aeruginosa*, and *E. coli.* They showed that palmitic acid (14.62-15.47%) is one of the major fatty acid compounds found in diethyl ether seed oil extraction.

**MIC and MBC**

Table 2 shows the MIC and MBC values of SMEE against four foodborne pathogens. The MIC value of SMEE against *S. typhimurium* and *S. sonnei* was 500 µg/mL respectively. On the other hand, SMEE showed a lower MIC value against *P. aeruginosa* and *E. coli* namely 31.25 µg/mL. The MBC value recorded for all test microorganisms was 1000 µg/mL. According to Mohammed *et al*. (2014), the growth of *E. coli* and *P. aeruginosa* were inhibited by seeds and leaves extracts of *S. macrophylla* at 25 and 50 mg/mL respectively. Gopalan *et al*. (2019) demonstrated that the MBC value of *Swietenia macrophylla* seed extract against Gram-negative bacteria, *Shigella boydii,* and *Acinetobacter anitratus*, was 25 mg/mL respectively. Meanwhile, the antibacterial activities of petroleum ether and ethanolic extract of *S. macrophylla* leaves are due to the presence of terpenoids (Ayyappadhas *et al*., 2012).

The microdilution technique was used to evaluate the quantitative measurement of antimicrobial susceptibility (Jorgensen & Ferraro 2009). The modification of the microdilution method used in this study involved the use of resazurin dye as a redox indicator, which eliminates the issues associated with sparingly soluble test materials. Active bacterial cells convert non-fluorescent resazurin (blue) to fluorescent resorufin (pink), which may then be converted further to hydroresorufin (O'Brien *et al*. 2000).

**Pellicle**

In an aerobic environment, biofilm tends to develop pellicles at the air-liquid interface. The pellicle provides the culture a cloudier look than the planktonic population. A glucose-rich, cellulose-like polymer is required for the development of a pellicle at the air-liquid contact (Yaacob *et al*., 2021). In the present study, *S. typhimurium*, *S. sonnei, P. aeruginosa,* and *E. coli* biofilm were confirmed to form the pellicle at the air-liquid interface (Figure 1).

**Table 2.** The MIC and MBC values of SMEE against the foodborne pathogens

| Microorganisms | Inhibitory concentrations | | |
|---|---|---|---|
| | Ciprofloxacin (64 µg/mL) | MIC (µg/mL) | MBC (µg/mL) |
| *S. typhimurium* | No growth | 500.00 | >1000.00 |
| *S. sonnei* | No growth | 500.00 | >1000.00 |
| *P. aeruginosa* | No growth | 31.25 | >1000.00 |
| *E. coli* | No growth | 31.25 | >1000.00 |

**Biofilm biomass**

Figure 2 shows the effect of different concentrations of SMEE on biofilm biomass. Treatment with SMEE at 250 µg/mL significantly ($p$<0.05) reduced *S. typhimurium* biofilm biomass (Figure 2a). The percentage of biofilm inhibition was found to range between 3.08% and 13.85%. Treatment with SMEE significantly ($p$<0.05) inhibited *S. sonnei* biofilm biomass at all tested concentrations except at 31.25 µg/mL concentration (Figure 2b). The percentage of biofilm inhibition was found to range between 7.75% and 29.58%. Treatment with SMEE at 125 µg/mL and 250 µg/mL significantly ($p$<0.05) reduced *E. coli* biofilm biomass (Figure 2c). The percentage of biofilm inhibition was found to range between 2.68% and 8.93%. Treatment with SMEE at 31.25 µg/mL, 62.5 µg/mL and 125 µg/mL significantly ($p$<0.05) reduced *P. aeruginosa* biofilm biomass (Figure 2d). The percentage of biofilm inhibition was found to range between 29.88% and 58.88%.

It has been established that biofilm formation begins with the initial attachment of floating microbial cells to the surface, followed by microcolony formation, synthesis of extracellular matrix, and formation of heterogeneous three-dimensional structure. Various factors are known to regulate biofilm formation including nutrients, temperature, hydrophobicity, and surface roughness (Sooriyakumar *et al*. 2022). Crystal violet (CV) staining is the commonly used method to quantify biofilm (Yahya *et al*. 2018; Kamaruzzaman *et al*., 2022). This method stains both living and dead cells, as well as some biofilm matrix components, making it ideal for determining total biofilm biomass (Azeredoa *et al*., 2017). A previous study by Ta *et al*. (2014) showed that Meliaceae species including *S. macrophylla* bark exhibited more significant biofilm inhibition than the positive control allicin (74.4 ± 8.1% growth). They also showed that *S. macrophylla* bark was able to inhibit the biofilm of *Pseudomonas aeruginosa* PA14 with the $IC_{50}$ value of 202 µg/mL. The inhibition of biofilm formed by foodborne pathogens using various plant extracts has also been reported in other studies (Namasivayam & Roy 2013; Zawawi *et al*., 2020; Johari *et al*., 2020). To the best of our knowledge, this is the first report on the antibiofilm activity of *S. macrophylla* (which belongs to the Meliaceae family) ethanolic leaves extract against foodborne pathogens.

**Time-killing kinetics**

Figure 3a shows the killing curve of the *S. typhimurium* biofilm treated with SMEE at 250 µg/mL. The negative control showed an exponential phase within 0 to 6 h and entered a stationary phase after 6 h. The biofilm continued to grow after 12 h of incubation. The time-killing curve of the treated group showed a short lag phase. The biofilm entered an exponential phase after 6 h of incubation time. At the end of the incubation period, a significant death phase was observed after 24 h. The biofilm formation by *S. typhimurium* treated with positive control depicted a consistent decrease in viable cells. The biofilm was continuously reduced after 24 h incubation. The present finding is in line with Mamman *et al*. (2013) showing that *Salmonella* spp was killed by *A. indica* aqueous extract at 175 mg/mL in 1200 s. They also found that *Salmonella* spp was killed by *A. indica* methanolic extract at 175 mg/mL in 600 s.

Figure 3(b) shows the killing curve of the *S. sonnei* biofilm treated with SMEE at 125 µg/mL. The negative control showed an exponential phase at a time interval from 0 to 6 h and then entered the stationary phase after 6 h. The biofilm continued to grow after 18 h of incubation. The killing curve of the treated group showed a short lag phase. However, the biofilm population showed a noticeable reduction after 6 h, and then slightly increased after 24 h of incubation. The biofilm formation by *S. sonnei* treated with positive control depicted a consistent decrease in viable cells. The biofilm was continuously reduced

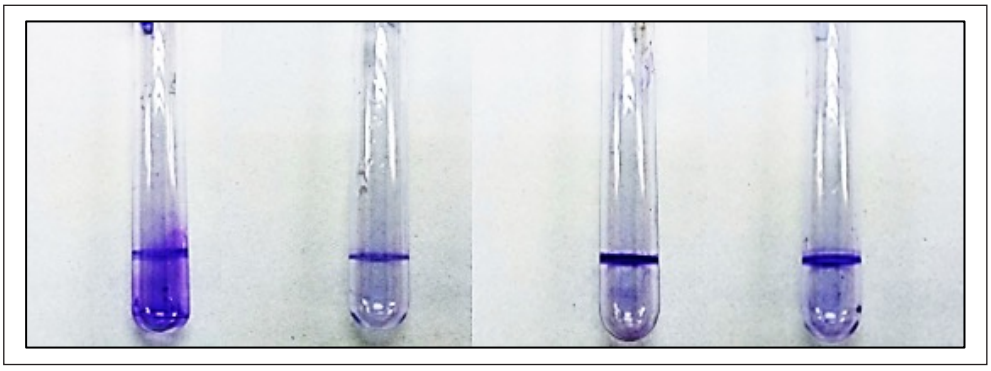

**Fig. 1.** Formation of pellicle biofilm at the air-liquid interface. A) *S. typhimurium*; B) *S. sonnei*; C) *P. aeruginosa* and D*) E. coli*. After 5 min, sufficient staining with 0.5% (w/v) crystal violet and 25% (v/v) methanol were accomplished.

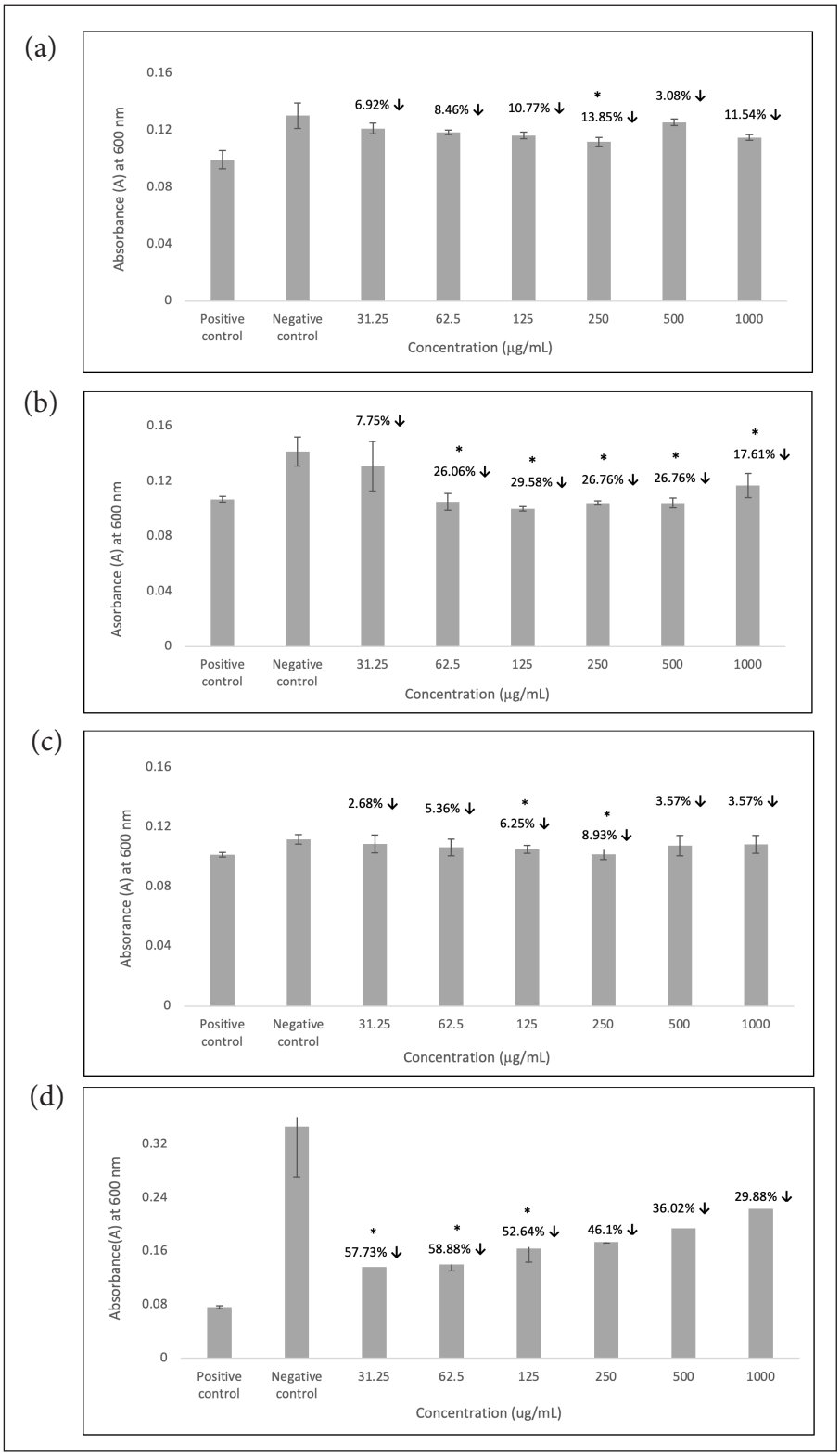


**Fig. 2.** Biofilm biomass exposed to SMEE. (a) *S. typhimurium;* (b) *S. sonnei;* (c) *E. coli;* (d) *P. aeruginosa.* Positive control: IP-protected antibiofilm cocktail; negative control: fresh broth. Values are represented as mean ± SEM. A statistically significant difference ($p<0.05$) is indicated by an asterisk (*). Inhibition of biofilm biomass is defined as a percentage (%) of biofilm inhibition.

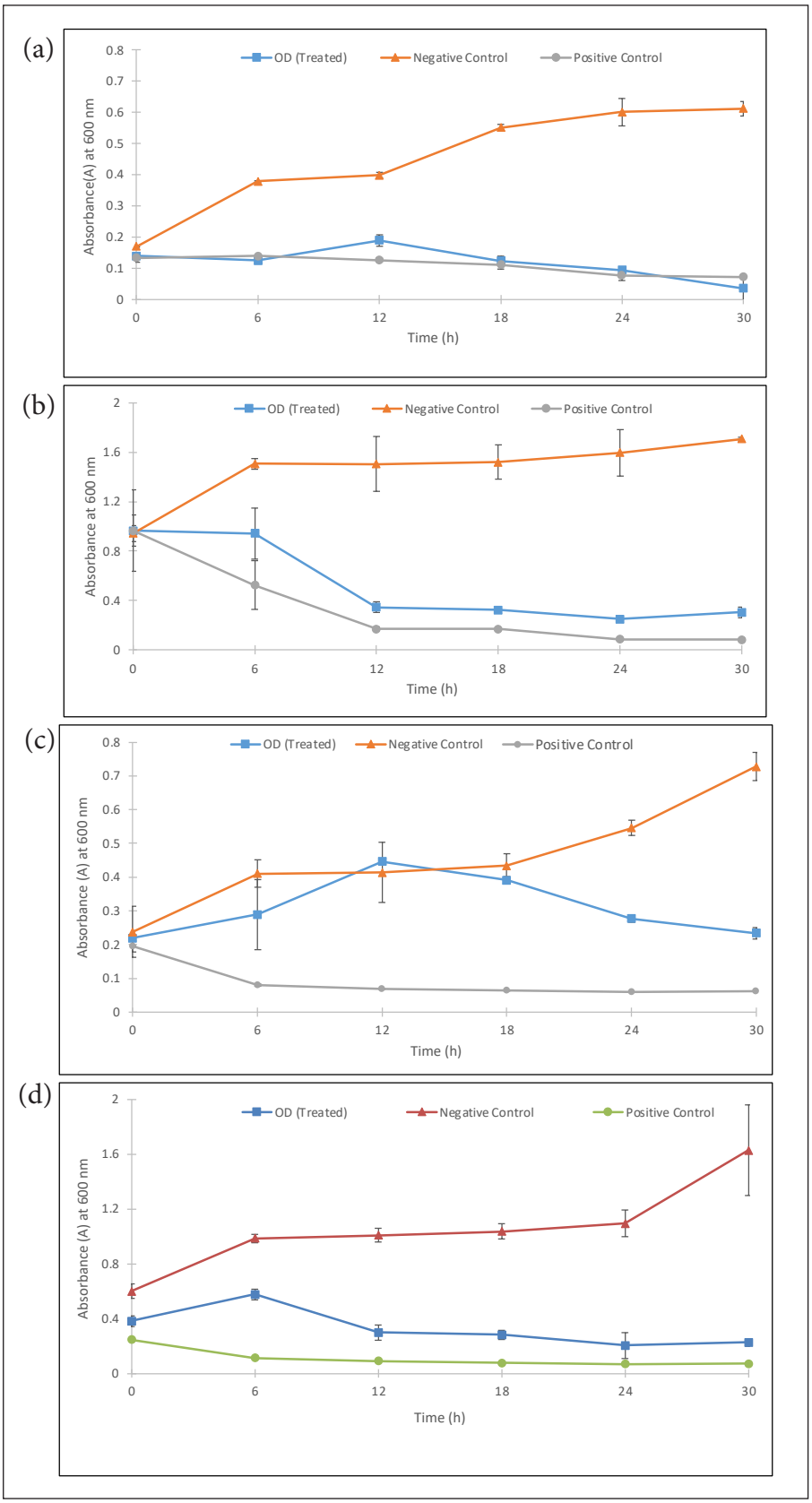


**Fig. 3.** The killing curves of biofilm exposed to SMEE. (a) *S. typhimurium* (250 µg/mL)*;* (b) *S. sonnei* (125 µg/mL)*;* (c) *E. coli* (250 µg/mL)*;* (d) *P. aeruginosa* (62.5 µg/mL).

after 24 h, and a significant death phase was observed.

Figure 3(c) shows the killing curve of *E. coli* biofilm treated with SMEE at 250 µg/mL. The negative control showed an exponential phase at time intervals of 0 to 6 h and then entered the stationary phase after 6 h. The lag phase was not detected at the initial stage of the killing curve of the treated group. The reduction of viable biofilm cells was noticed after 12 h of incubation. Biofilm formation by *E. coli* treated with positive control depicts a consistent decrease in the viable cells. The biofilm was continuously reduced until 30 h incubation. This result corroborates Okemo *et al*. (2001) showing that the highest concentration of *A. indica* extract (8 mg/mL) reduced the *E. coli* population slightly at the initial stage. However, after 8 h the population was drastically reduced and killed after 24 h. A previous study by Mamman *et al*. (2013) found that *E. coli* was eliminated at 87.5 mg/mL in 600 s. The present result is also in line with Ahmed (2008) showing that all concentrations of *A. indica* leave extract initially reduced the *E. coli* population in the first 6 h. A substantial reduction in viable cells was observed at 20 mg/mL, especially after 12 h of incubation.

Figure 3d shows the killing curve of the *P. aeruginosa* biofilm treated with SMEE at a concentration of 62.5 µg/mL. The negative control showed an exponential phase at a time interval of 0 to 6 h and then entered the stationary phase after 6 h. The lag phase was not detected at the initial stage of the killing curve of the treated group. The viable biofilm cell was noticeably reduced after 6 h. The positive control significantly reduced the biofilm population of the initial inoculum. A previous study performed by Harjai *et al*. (2013) showed that *P. aeruginosa* PAO1 biofilm was significantly ($p \leq 0.01$) reduced on day 5 in the presence of *A. indica* leaves extract. They found accumulation of biofilm was maximum on day 5, which later showed a slight decline until day 7. The present result also by Okemo *et al*. (2001) shows that the concentration of *A. indica* extract at 8 mg/mL had some little effects in the first 6 h. However, the *P. aeruginosa* population showed a noticeable reduction to 3.4 x $10^2$ CFU/mL after 8 h of incubation.

The time-killing kinetics antibacterial study has been widely used to evaluate a wide spectrum of antimicrobials, and it is also frequently used as the foundation for *in vitro* studies of pharmacodynamic drug interactions (Agbo *et al*., 2020). Although the bacteriostatic activity of *S. macrophylla* leaves extract against *S. paratyphi* (Ushie *et al*., 2016), *E. coli, P. aeruginosa,* and *S. sonnei* (Dewanjee *et al*., 2007) have been demonstrated, literature information of its efficacy on biofilm killing is completely lacking. In the present study, the time-kill kinetics of SMEE against foodborne pathogens was time-dependent, the increase in incubation time resulted in the reduction of biofilm growth. The strong correlation between time-kill kinetics and membrane damaging potential has previously been reported (Tyagi *et al*. 2015). Thus, the antibacterial and antibiofilm activities of SMME may be mediated by cytoplasmic membrane damage. Further studies examining the morphology, cell leakage, and membrane permeabilization of foodborne pathogens after exposure to SMME are required. The sensitivity trend of the biofilm species towards SMEE is also obvious, as follows: *S. sonnei* (killed within 6 h) > *S. typhimurium* (killed within 6 h) > *P. aeruginosa* (killed within 12 h) > *E. coli* (killed within 18 h). A rapid removal of these biofilms by SMEE may reduce the risk of severe symptoms from foodborne infections (Chimnoi *et al*. 2018).

**Biofilm inhibitory concentration ($BIC_{50}$)**

Table 3 displays the biofilm inhibitory concentration of *S. macrophylla* against foodborne pathogens. Biofilm inhibitory concentrations for *S. typhimurium*, *S. sonnei*, *E. coli,* and *P. aeruginosa* were found to be 2.28 µg/mL, 30.41 µg/mL, 36.20 µg/mL, and 309.90 µg/mL respectively. This indicates that SMME is most effective against *S. typhimurum* as it shows the lowest $BIC_{50}$ against the pathogen.

A previous study by Ta *et al*. (2014) showed that the $IC_{50}$ values against *P. aeruginosa* PA14 biofilm for some active Meliaceae species such as *S. mahogani* L., *C. guianensis* Aubl., *C. tabularis* A. Juss. and *T. martiana* C. DC. were 202 µg/mL, 208 µg/mL, 45 µg/mL, 127 µg/mL respectively. On the other hand, Jaisankar *et al*. (2020) demonstrated that from the crystal violet assay, the essential oil of *Azadirachta indica* exhibited a minimum biofilm eradication concentration ($MBEC_{50}$) of 20 mL, indicating a 50% suppression of biofilm formation by *A. baumannii*.

**Table 3.** $BIC_{50}$ values of SMEE against the foodborne pathogens

| Microorganisms | $BIC_{50}$ value (µg/mL) |
|---|---|
| *S. typhimurium* | 2.28 |
| *S. sonnei* | 30.41 |
| *E. coli* | 36.20 |
| *P. aeruginosa* | 309.90 |

## CONCLUSION

SMEE was found to contain olean-12-ene, resorcinol, 24-noroleana-3,12-diene, and germanicol. It also exhibited antibacterial and antibiofilm activities against all the selected foodborne pathogens. The killing kinetics of SMEE against the biofilms were found to be time-dependent. Furthermore, SMEE was most effective against *S. typhimurium* biofilm. The bioactive compounds identified in SMEE may mediate the antibacterial and antibiofilm activities of SMEE against the selected foodborne pathogens. The findings of the present study demonstrate the potential use of *S. macrophylla* in the management of foodborne diseases.

## ACKNOWLEDGEMENTS

The authors are grateful to the Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM) Shah Alam, Malaysia for providing the test microorganism. This research was funded by the Malaysian Ministry of Higher Education under a research grant, 600-IRMI/FRGS 5/3 (417/2019).

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## REFERENCES

Adam, O.A.O., Abadi, R.S.M. & Ayoub, S.M.H. 2019. The effect of extraction method and solvents on yield and antioxidant activity of certain Sudanese medicinal plant extracts. *The Journal of Phytopharmacology*, **8(5)**: 248-252. https://doi.org/10.31254/phyto.2019.8507

Afrouzan, H., Tahghighi, A., Zakeri, S. & Es-haghi, A. 2018. Chemical composition and antimicrobial activities of *Iranian propolis*. *Iranian Biomedical Journal*, **22(1)**: 50-65. https://doi.org/10.22034/ibj.22.1.50

Agbo, E.C., Achi, O.K., Nwachukwu, E., Obeta, M.U., Obiora, E.O., Maduka, K.M., Oraekei, N.P. & Lote, N.I. 2020. Time kill kinetics study of commonly used disinfectants against biofilm forming *Pseudomonas aeruginosa* in Federal Medical Centre, Umuahia-Nigeria. *American Journal of Biomedical Science & Research*, **7(3)**: 262-268. https://doi.org/10.34297/AJBSR.2020.07.001155

Ahmed, I.M. 2008. Bioactivity of Neem (*Azadirachta indica*) callus extract [WWW Document]. URL http://inis.iaea.org/search/search.aspx?orig_q=RN:40089514 (accessed 06.03.21).

Amaral, L. & Molnar, J. 2012. Inhibitors of efflux pumps of Gram-negative bacteria inhibit quorum sensing. *Open Pharmacology Journal*, **2**: 2-15. https://doi.org/10.1016/j.molmed.2005.06.006

Aparna, V., Dileep, K.V., Mandal, P.K., Karthe, P., Sadasivan, C. & Haridas, M. 2012. Anti-inflammatory property of n-Hexadecanoic acid: Structural evidence and kinetic assessment. *Chemical Biology and Drug Design*, **80**: 434-439. https://doi.org/10.1111/j.1747-0285.2012.01418.x

Aryal, S. 2019. Pyruvate broth tests: Principles, procedures, uses and interpretation [WWW Document]. Microbiology Info. URL https://microbiologyinfo.com/pyruvate-broth-test/ (accessed 05.10.21).

Ayyappadhas, R., Jestin, C., Kenneth, N., Dayana, N. & Dhanalekshmi, U.M. 2012. Preliminary studies on antimicrobial activity of *Swietenia macrophylla* leaf extract. *International Journal of Pharmaceutical Sciences Review and Research*, **16(2)**: 1–4. https://doi.org/10.4314/csj.v7i2.11

Azeredo, J., Azevedo, N.F., Briandet, R., Cerca, N., Coenye, T., Costa, A.R., Desvaux, M., Bonaventura, G.D., Hébraud, M., Jaglic, Z., Kačániová, M., Knøchel, S., Lourenço, A., Mergulhão, F., Meyer, R.L., Nychas, G., Simões, M., Tresse, O. & Sternberg. C. 2017. Critical review on biofilm methods. *Critical Reviews in Microbiology*, **43(3)**: 313–351. https://doi.org/10.1080/1040841X.2016.1208146

CDC. 2009a. Outbreak surveillance data. [WWW Document]. Centers for Disease Control and Prevention. URL http://www.cdc.gov/outbreaknet/surveillance_data.html (accessed 05.25.21).

Chimnoi, N., Reuk-Ngam, N., Chuysinuan, P., Khlaychan, P., Khunnawutmanotham, N., Chokchaichamnankit, D., Thamniyom, W., Klayraung, S., Mahidol, C. & Techasakul, S. (2018). Characterization of essential oil from *Ocimum gratissimum* leaves: antibacterial and mode of action against selected gastroenteritis pathogens. *Microbial Pathogenesis*, **118**: 290–300. https://doi.org/10.1016/j.micpath.2018.03.041

CLSI. 2007. Performance standards for antimicrobial disc susceptibility test; approved standard 9th edition. Clinical and Laboratory Standard Institute (CLSI) document 3: M2-M9.

Das, A., Das, M.C., Sandhu, P., Tribedi, P. De, U.C. Akhter, Y. & Bhattacharjee, S. 2017. Antibiofilm activity of *Parkia javanica* against *Pseudomonas aeruginosa*: a study with fruit extract. *RSC Advances*, **7**: 5497-5513. https://doi.org/doi.org/10.1039/C6RA24603F

Debalke, D., Birhan, M., Kinubeh, A. & Yayeh, M. 2008. Assessments of antibacterial effects of aqueous-ethanolic extracts of *Sida rhombifolia*'s Aerial Part. *Hindawi The Scientific World Journal*, **2018**: Article ID 8429809. https://doi.org/doi.org/10.1155/2018/8429809

Dehpour, A.A., Yousefian, M., Jafary Kelarijani, S.A., Koshmoo, M., Mirzanegad, S., Mahdavi, V. & Javad Bayani, M.J. 2012. Antibacterial activity and composition of essential oils of flower *Allium rotundum*. *Advances Environmental Biology*, **6**: 1020–1025.

Dewanjee, S., Mazumder, R., Kundu, M. & Mazumder, A. 2007. *In vitro* evaluation of antimicrobial activity of crude extract from plants *Diopyros peregrina*, *Coccinia grandis* and *Swietenia macrophylla*. *Tropical Journal of Pharmaceutical Research*, **6(3)**: 773-778. https://doi.org/doi.org/10.4314/tjpr.v6i3.14658

Elmarzugi, N.A., Eid, A.M.M. & El-Enshasy, H.A. 2013. A review on the phytopharmacological effect of *Swietenia macrophylla*. *International Journal of Pharmacy and Pharmaceutical Sciences*, **5**: 47-53.

Fatima, Z.M., Badiaa, L. & Abdelfattah, A. 2011. Antibacterial activity of the essential oils of *Pistacia lentiscus* used in Moroccan Folkloric Medicine. *Natural Product Communications*, **6(10)**: 1505-1506.

Goh, B.H. & Kadir, H.A. 2011. *In vitro* cytotoxic potential of *Swietenia macrophylla* King seeds against human carcinoma cell lines. *Journal Medicinal Plants Research*, **5**: 1395-1404.

Gopalan, H.K., Hanafiah, N.F.M., Ring, L.C., Tan, W.N., Wahidin, S., Hway, T.S. & Yenn, T.W. 2019. Chemical composition and antimicrobial efficiency of *Swietenia macrophylla* seed extract on clinical wound pathogens. *Natural Product Sciences*, **25(1)**: 38-43. https://doi.org/10.20307/nps.2019.25.1.38

Harjai, K., Bala, A., Gupta, R.K. & Sharma, R. 2013. Leaf extract of *Azadirachta indica* (neem): a potential antibiofilm agent for *Pseudomonas aeruginosa*. *Pathogens and Disease*, **69**: 62–65. https://doi.org/10.1111/2049-632X.12050

Husain, F.M, Ahmad, I., Al-thubiani, A.S., Abulreesh, H.H., Alhazza, I.M & Aqil, F. 2017. Leaf extracts of *Mangifera indica* L. inhibit quorum sensing – regulated production of virulence factors and biofilm in test bacteria. *Frontiers in Microbiology,* **8**: 727. https://doi.org/10.3389/fmicb.2017.00727

Jaisankar, A.I., Girija, A.S.S., Gunasekaran, S. & Priyadharsini, J.V. 2020. Molecular characterisation of csgA gene among ESBL strains of *A. baumannii* and targeting with essential oil compounds from *Azadirachta indica*. *Journal of King Saud University-Science*, **32**: 3380-3387. https:// doi.org/10.1016/j.jksus.2020.09.025

Johari, N.A., Amran, S.S.D., Kamaruzzaman, A.N.A., Man, C.A.I.C. & Yahya, M.F.Z.R. 2020. Anti-biofilm potential and mode of action of Malaysian plant species: a review. *Science Letters*, **14**: 34–46. https://doi.org/10.24191/sl.v14i2.9541

Jorgensen, J.H. & Ferraro, M.J. 2009. Antimicrobial susceptibility testing: A review of general principles and contemporary practices. *Clinical Infectious Diseases*, **49**: 1749–1755. https://doi.org/10.1086/647952

Kamaruzzaman, A.N.A., Mulok, T.E.T.Z. & Yahya, M.F.Z.R. 2022. Inhibitory action of topical antifungal creams against *Candida albicans* biofilm. *Journal of Sustainability Science and Management*, **17(2)**: 27-34. https://doi.org/10.46754/jssm.2022.02.003

Katerere, D.R., Gray, A.I., Nash, R.J. & Waigh, R.D. 2003. Antimicrobial activity of pentacyclic triterpenes isolated from African Combretaceae. *Phytochemistry*, **63(1)**: 81-88. https://doi.org/10.1016/S0031-9422(02)00726-4

Kuete, V., Dongfack, M.D., Mbaveng, A.T., Lallemand, M.C., Van-Dufat, H.T., Wansi, J.D., Seguin, E., Tillequin, F. & Wandji J. 2010. Antimicrobial activity of the methanolic extract and compounds from the stem bark of *Drypetes tessmanniana*. *Chinese Journal of Integrative Medicine*, **16(4)**: 337-343. https://doi.org/10.1007/s11655-010-0527-8

Kushwaha, P. Yadav, S.S., Singh, V. & Dwivedi, L.K. 2019. GC-MS analysis of bio-active compounds in methanolic extract of *Ziziphus mauritiana* fruit. *International Journal of Pharmaceutical Sciences and Research*, **10(6)**: 2911-2916. https://doi.org/10.13040/IJPSR.0975-8232.10(6).2911-16

Mamman, P.H. Mshelia, W.P., Susbatrus, S.C. & Sambo, K. W. 2013. Antibacterial effects of crude extract of *A. indica* against *Escherichia coli*, *Salmonella* spp and *Staphylococcus aureus*. *International Journal of Medicine and Medical Sciences*, **5(1)**: 14-18. https://doi.org/10.5897/IJMMS12.017

Mishra, R., Kushveer, J.S., Khan, M.I.K., Pagal, S., Meena, C.K. Murali, A., Dhayalan, A. & Sarma V.V. 2020. 2,4-Di-Tert-Butylphenol isolated from an endophytic fungus, *Daldinia eschscholtzii*, reduces virulence and quorum sensing in *Pseudomonas aeruginosa. Frontiers in Microbiologyy*, **11**: 1668. https://doi.org/doi.org/10.3389/fmicb.2020.01668

Ministry of Health. 2019. Health facts 2019: reference data for year 2018 [WWW Document]. URL www2.moh.gov.my (accessed 23.7.2022).

Mohammed, S.B., Azhari, N.H., Mashitah, Y.M., Abdurahman, N.H. & Mazza, A.S. 2014. Growth inhibitory effect on bacteria of *Swietenia macrophylla* King seeds and leaves crude alkaloid extracts. *International Journal of Pharmaceutical Sciences and Research*, **5(8)**: 3204-3208. https://doi.org/10.13040/IJPSR.0975-8232.5(8).3204-08

Namasivayam, S.K.R. & Roy, E.A. 2013. Antibiofilm effect of medicinal plant extracts against clinical isolate of biofilm of *Escherichia coli*. *International*

*Journal of Pharmacy and Pharmaceutical Sciences*, **5(2)**: 486-489.

Nirubama, K., Kanchana, G. & Rubalakshmi, G. 2014. Bioactive compounds in *Andrographis echiodes* (L.) Nees. leaves by GC-MS analysis. *International Journal of Current Research in Bioscience and Plant Biology*, **1(3)**: 92-97.

O'Brien, J., Wilson, I., Orton, T. & Pognan, F. 2000. Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. *Europian Journal of Biochemistry*, **267(17)**: 5421-6. https://doi.org/10.1046/j.1432-1327.2000.01606.x

Okemo, P.O., Fabry, W. & Chhabra, S.C. 2001. The kill kinetics of *Azadirachta indica* A. JUSS. (meliaceae) extracts on *Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans*. *African Journal of Science and Technology*, **2(2)**: 113-118.

Padmavathi, A.R., Bakkiyaraj, D., Thajuddin, N. & Pandian, S.K. 2015. Effect of 2, 4-di-tert-butylphenol on growth and biofilm formation by an opportunistic fungus *Candida albicans*. *Biofouling*, **31(7)**: 565–574. https://doi.org/10.1080/08927014.2015.1077383

Patra, J. & Baek, K.H. 2016. Antibacterial activity and action mechanism of the essential oil from *Enteromorpha linza* L. against foodborne pathogenic bacteria. *Molecules*, **21(3)**: 388. https://doi.org/doi.org/10.3390/molecules21030388

Rukshana, M.S., Doss, A. & Kumari P.R.T.P. 2017. Phytochemical screening and GC-MS analysis of leaf extract of *Pergularia daemia* (Forssk) Chiov. *Asian Journal of Plant Science and Research*, **7(1)**: 9–15.

Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.A., Roy, S.L., Jones, J.L., & Griffin, P.M. 2011. Foodborne illness acquired in the United States–major pathogens. *Emerging Infectious Diseases*, **17**: 7–15. https://doi.org/10.3201/eid1701.p11101

Sooriyakumar, P., Bolan, N., Kumar, M., Singh, L., Yu, Y., Li, Y., Weralupitiya, C., Vithanage, M., Ramanayaka, S., Sarkar, B., Wang, F., Gleeson, D. B., Zhang, D., Kirkham, M.B., Rinklebe, J. & Siddique, K.H.M. 2022. Biofilm formation and its implications on the properties and fate of microplastics in aquatic environments: A review, *Journal of Hazardous Materials Advances*, **6**: 100077. https://doi.org/doi.org/10.1016/j.hazadv.2022.100077

Suliman, M.B., Nour, A.H., Yusoff, M.M., Nour, A.H., Kuppusamy, P., Yuvaraj, A.R. & Adam, M.S. 2013. Fatty acid composition and antibacterial activity of *Swietenia macrophylla* king seed oil. *African Journal of Plant Sciences*, **7(7)**: 300-303. https://doi.org/10.5897/AJPS2013.1039

Swati, P. & Richa, K. 2011. A review on antidiarrhoeal activity of herbals. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, **2(3)**: 1357-1362.

Ta, C.A., Freundofter, M., Mah, T.F., Rojas, M.O., Garcia, M., Vindas, P.S., Poveda, L., Maschek, J.A., Baker, B.J., Adonizio, A.L., Downum, K., Durst, T. & Arnason, J.T. 2014. Inhibition of bacterial quorum sensing and biofilm formation by extracts of neotropical rainforest plants. *Planta Medica*, **80**: 343–350. https://doi.org/10.1055/s-0033-1360337

Tyagi, P., Singh, M., Kumari, H., Kumari, A. & Mukhopadhyay, K. 2015. Bactericidal activity of Curcumin I is associated with damaging of bacterial membrane. *PLoS ONE*, **10(3)**: e0121313. https://doi.org/10.1371/journal.pone.0121313

Ushie, O.A. & Olumide, V.B. 2016. Phytochemical screening and antimicrobial activities of leaf extracts of *Swietenia macrophylla*. *ChemSearch Journal*, **7(2)**: 64-69. https://doi.org/10.4314/csj.v7i2.11

Viszwapriya, D., Prithika, U., Deebika, S., Balamurugan, K. & Pandian, S.K. 2016. *In vitro* and *in vivo* antibiofilm potential of 2,4-Di-tert-butylphenol from seaweed surface associated bacterium *Bacillus subtilis* against group A streptococcus. *Microbiological Research*, **191**: 19-31. https://doi.org/10.1016/j.micres.2016.05.010

Yaacob, M.F., Murata, A., Nor, N.H.M., Jesse, F.F.A. & Yahya, M.F.Z.R. 2021. Biochemical composition, morphology antimicrobial susceptibility pattern of *Corynebacterium pseudotuberculosis* biofilm. *Journal of King Saud University – Science*, **33(1)**: 101225. https://doi.org/10.1016/j.jksus.2020.10.022

Yahya, M.F.Z.R., Hamid, U.M.A., Norfatimah, M.Y. & Kambol, R. 2014. In silico analysis of essential tricarboxylic acid cycle enzymes from biofilm-forming bacteria. *Trends in Bioinformatics*, **7(1)**: 19-26. https://doi.org/10.3923/tb.2014.19.26

Yahya, M.F.Z.R, Alias, Z. & Karsani, S.A. 2017. Substractive protein profiling of *Salmonella typhimurium* biofilm treated with DMSO. *Protein Journal*, **36(24)**: 286-298. https://doi.org/10.1007/s10930-017-9719-9

Yahya, M.F.Z.R, Alias, Z. & Karsani, S.A. 2018. Antibiofilm activity and mode of action of DMSO alone and its combination with afatinib against Gram-negative pathogens. *Folia Microbiologica*, **63(1)**: 23-30. https://doi.org/10.1007/s12223-017-0532-9

Zawawi, W.M.A.W.M., Ibrahim, M.S.A., Rahmad, N., Hamid, U.M.A. & Yahya, M.F.Z.R. 2020. Proteomic analysis of *Pseudomonas aeruginosa* treated with *Chromolaena odorata* extracts. *Malaysian Journal of Microbiology*, **16(2)**: 124-133. https://doi.org/10.21161/mjm.190512