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Research Article

Bioactive Potential of *Dendrophthoe Falcate* (L.F.) Ettingsh Parasitizing *Senna Siamea*: A Comprehensive Study on Antibacterial, Antibiofilm, And Time-Kill Dynamics Against Clinical Isolates

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Abstract

The present study evaluates the antibacterial, antibiofilm, and time-kill potential of *Dendrophthoe falcata* (*D. falcate*) leaf extracts parasitizing *Senna siamea* (*S. siamea*) against clinically relevant bacterial pathogens. Antibacterial activity assessed by the agar well diffusion method revealed that the ethyl acetate extract exhibited the most potent concentration-dependent inhibition, with maximum zones of inhibition recorded against *Staphylococcus aureus*, *E. coli*, *P. aeruginosa*, and *K. pneumoniae* (18.2 ± 0.5 mm to 17.0 ± 0.4 mm at 100 mg/mL). Methanol and acetone extracts showed moderate activity, while the toluene extract exhibited minimal effects. Minimum inhibitory concentration (MIC) assays supported these findings, with the ethyl acetate extract showing the lowest MIC values (2.9–5.2 µg/mL), indicating high potency, followed by methanol, acetone, and toluene extracts. Antibiofilm assays further demonstrated the efficacy of ethyl acetate extract, with low BIC₅₀% (1.2–1.9 mg/mL) and BIC₉₀% (2.3–2.9 mg/mL) values, outperforming the other extracts. Time-kill kinetics confirmed the bactericidal activity of the extracts, with ethyl acetate extract achieving complete bacterial eradication at 1× MIC within 4.5–5.5 h and at 2× MIC within 3.0–3.5 h. These results suggest that the ethyl acetate fraction of *D. falcata* leaves holds significant potential as a natural antimicrobial and antibiofilm agent against multidrug-resistant pathogens, warranting further bioactive compound isolation and mechanistic studies.

Key words: *D. falcata*, *S. siamea*, antibacterial activity, minimum inhibitory concentration (MIC), antibiofilm activity, time-kill assay.

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1.0 Introduction

Dendrophthoe falcata (Loranthaceae) is a well-known hemiparasitic plant traditionally found parasitizing host species such as *Azadirachta indica* (neem), *Mangifera indica* (mango), and *Annona squamosa* (custard apple)

(Pattanayak et al., 2008; Manthri et al., 2011). Its pharmacological potential has been widely documented, with bioactive compounds exhibiting antimicrobial, antioxidant, anti-inflammatory, and anticancer

properties (Patil et al., 2011; Atun et al., 2018; Kong et al., 2023).

The global rise in antimicrobial resistance poses a significant threat to public health, prompting an urgent need for novel therapeutic agents derived from natural sources (Ventola, 2015). Medicinal plants have long served as reservoirs of bioactive compounds, many of which exhibit potent antimicrobial activities. Among these, *D. falcate* (L.f.) Ettingsh., a hemiparasitic plant belonging to the family Loranthaceae, has garnered considerable attention due to its ethnomedicinal relevance and diverse pharmacological properties (Saha et al., 2011).

Traditionally, *D. falcata* has been used in the treatment of wounds, inflammation, and microbial infections in various systems of traditional medicine, including Ayurveda and Siddha (Girish and Satish, 2008). It parasitizes multiple host plants, and the nature and concentration of its secondary metabolites may vary depending on the host, influencing its biological activities (Bag et al., 2013). In this context, the current study explores *D. falcata* parasitizing *Senna siamea* (Lam.) Irwin & Barneby, a species known for its own medicinal value and rich phytochemistry (Kokwaro, 2009).

Although previous investigations have highlighted the antimicrobial potential of *D. falcata*, comprehensive studies that assess its antibiofilm and time-kill efficacy against clinical isolates remain limited. Biofilms, complex microbial communities embedded in extracellular polymeric substances, are notoriously resistant to conventional antibiotics, making them critical targets in the development of next-generation antimicrobials (Hoiby et al., 2010). Time-kill kinetics, in contrast, offer insight into the rate and extent of bacterial inactivation, providing a dynamic measure of antimicrobial efficacy (Pankey and Sabath, 2004).

During our field investigation, we observed *D. falcata* parasitizing *Senna siamea* (*S. siamea*), a leguminous tree not previously reported as a host for this parasitic species. This novel host-parasite interaction prompted us to investigate whether the phytochemical and pharmacological profile of *D. falcata* varies when growing on this newly recorded host. Given the well-established influence of host species on the secondary metabolite composition of hemiparasitic plants (Khanna et al., 1968; Ramchandran & Krishnakumary, 1990), our study aims to explore the therapeutic potential of *D. falcata* specifically parasitizing on *S. siamea*. Therefore, this study aims to bridge this gap by evaluating the antibacterial, antibiofilm, and time-kill properties of various solvent extracts of *D. falcata* leaves parasitizing *S. siamea*. By employing clinical isolates of pathogenic bacteria, this investigation seeks to validate the plant's pharmacological potential and contribute to the development of alternative antimicrobial strategies.

2.0 Material and Methods

2.1 Plant Collection and Authentication

Leaves of *D. falcata* parasitizing *S. siamea* were collected from the RTC Colony, Singicherla Village,

Hyderabad, Telangana. The plant specimen was authenticated by a Dr. Mustafa taxonomist, Department of Botany, Kakatiya University.

2.2 Preparation of Plant Extracts

The collected leaves were washed thoroughly with distilled water, shade-dried at room temperature for 10–15 days, and then powdered using a mechanical grinder. About 100 g of the powdered material was subjected to successive solvent extraction using solvents of increasing polarity Toluene, Ethyl acetate, Acetone and Methanol through Soxhlet apparatus, following the standard protocol described by Harborne (1998). Each extract was concentrated using a rotary evaporator under reduced pressure and stored at 4°C until further use.

2.3 Microorganisms and Culture Conditions

Clinical isolates of Methicillin Resistant *Staphylococcus aureus* (MRSA), *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Klebsiella pneumoniae* (*K. pneumoniae*) were obtained from Department of Microbiology, Chaitanya (Deemed to be University), Himayath nagar, Moinabad, Ranga Reddy, Hyderabad, Telangana, India. All strains were maintained on Nutrient Agar slants and sub cultured in Mueller-Hinton Broth (MHB) before each experiment (CLSI, 2020).

2.4 Antibacterial Activity

The antibacterial activity of the solvent extracts of *D. falcata* was evaluated using the agar well diffusion method, as described by Perez et al. (1990) with slight modifications. Mueller-Hinton Agar (MHA) plates were prepared and sterilized. Each bacterial strain MRSA, *E. coli*, *P. aeruginosa*, and *K. pneumoniae* was cultured overnight in nutrient broth and adjusted to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL). Using a sterile cotton swab, the bacterial inoculum was evenly spread on the surface of MHA plates. Wells of 6 mm diameter were made in the agar using a sterile cork borer. Each well was filled with 50 µL of crude extracts above mentioned (at concentrations of 25, 50, and 100 mg/mL dissolved in 2% of DMSO). Ciprofloxacin (5 µg/mL) was used as the positive control, and DMSO served as the negative control. The plates were allowed to stand for 1 hour at room temperature for pre-diffusion and then incubated at 37°C for 24 hours. After incubation, the zones of inhibition around the wells were measured in millimeters (mm) using a transparent ruler. All experiments were performed in triplicate, and the results were expressed as mean $\pm$ standard deviation.

2.4.1 Minimum Inhibitory Concentration (MIC)

MIC values were determined using the broth microdilution method in 96-well microtiter plates as per CLSI guidelines (CLSI, 2020). Serial two-fold dilutions of extracts were prepared (ranging from 1000 to 7.8 µg/mL), and 100 µL of each dilution was inoculated with 100 µL of bacterial suspension. Plates were incubated at 37°C for 24 h, and bacterial growth was

assessed by visual inspection and absorbance reading at 600 nm.

2.4.2 Antibiofilm Activity

The antibiofilm activity was evaluated using the crystal violet staining assay as described by Stepanović et al. (2000). Briefly, the selected bacterial cultures separately were incubated in 96-well plates (For example Fig 1.0) with sub-MIC concentrations (**The concentration of extracts was calculated according to the MIC values shown in the table 1.2**) of the extracts for 24 h at 37°C. After incubation, wells were washed with PBS, fixed with methanol, stained with 0.1% crystal violet, and solubilized with ethanol (Fig 1.1). The absorbance was measured at 570 nm to quantify biofilm inhibition. Percent inhibition was calculated in comparison with the untreated control.

2.4.3 Time-Kill Assay

The time-kill kinetic assay was performed to evaluate the bactericidal activity over time, as per the protocol of Pankey and Sabath (2004). The selected bacterial cultures were exposed to 1× and 2× MIC concentrations of Ethyl acetate, Methanol and Acetone extracts which produced notable results in antibiofilm assay. Aliquots were taken at 0, 2, 4, 6, 8, 12, and 24 h, serially diluted, and plated on nutrient agar. Colony-forming units (CFU/mL) were counted after 24 h incubation. A ≥ 3 log₁₀ reduction in CFU/mL compared to the initial inoculum was considered bactericidal.

2.5 Statistical Analysis

All experiments were carried out in triplicate, and results were expressed as mean $\pm$ standard deviation (SD). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test. A *p*-value < 0.05 was considered statistically significant.

3.0 Results

3.1 Antibacterial Activity

The antibacterial efficacy of *D. falcate* leaf extracts parasitizing *S. siamea* was evaluated using the agar well diffusion method against four selected bacterial isolates. Among the tested extracts tested, the ethyl acetate extract demonstrated the most potent antibacterial activity in a concentration-dependent manner and produced inhibition zones of 18.2 ± 0.5 mm, 17.6 ± 0.4 mm, 16.5 ± 0.3 mm, and 17.0 ± 0.4 mm against *S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumonia* at 100 mg/mL respectively. The methanol extract showed moderately strong antibacterial activity at the same concentration, with inhibition zones of 16.4 ± 0.3 mm, 15.8 ± 0.5 mm, 14.6 ± 0.4 mm, and 15.0 ± 0.3 mm respectively recorded against *S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumonia*. The acetone extract showed slight lower activity, producing zones ranging from 12.4 ± 0.3 mm to 13.6 ± 0.3 mm, while the toluene extract demonstrated the least inhibitory effect, with zones between 8.0 ± 0.3 mm and 9.4 ± 0.4 mm. The result at 50 and 25 mg/mL of each extract against different bacterial strains selected is shown in table 1.0. The

result. The standard ciprofloxacin exhibited the highest antibacterial activity across all strains, with inhibition zones ranging from 22.8 ± 0.4 mm to 24.6 ± 0.5 mm. As expected, the negative control (DMSO) produced no measurable inhibition, confirming the specificity of the extract activity (Table 1.0). Table 1.1 show the represent the statistical record of One-way ANOVA followed by Tukey's post-hoc test comparing the antibacterial activity significance $P < 0.001$ and $P < 0.05$.

3.1.1 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of *D. falcate* leaf extracts toluene, ethyl acetate, acetone, and methanol was evaluated against four clinically relevant bacterial pathogens: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* was showed in table 1.2. Among the tested extracts, the ethyl acetate fraction demonstrated the most potent antibacterial activity, exhibiting MIC values of 3.8 μ g/mL against *S. aureus*, 4.3 μ g/mL against *E. coli*, 2.9 μ g/mL against *P. aeruginosa*, and 5.2 μ g/mL against *K. pneumoniae*. The methanol extract also showed significant activity, with MIC values ranging from 5.5 to 8.4 μ g/mL across the tested strains. In contrast, the acetone extract exhibited moderate inhibitory effects, with MICs between 34.3 and 42.1 μ g/mL. The toluene extract displayed poor antibacterial activity, with MIC values exceeding 100 μ g/mL for all tested organisms (Table 1.2).

3.1.2 Antibiofilm Activity

The antibiofilm efficacy of toluene, ethyl acetate, acetone, and methanol leaf extracts of *D. falcate* parasitizing *S. siamea* was assessed against clinical isolates of *MRSA*, *E. coli*, *P. aeruginosa*, and *K. pneumoniae*. Biofilm Inhibitory Concentration (BIC_{50%} and BIC_{90%}) values were determined for each extract. Among them, the ethyl acetate extract exhibited the most potent antibiofilm activity, with BIC_{50%} and BIC_{90%} values ranging between 1.2–1.9 mg/mL and 2.3–2.9 mg/mL, respectively, against all tested bacterial strains (Table 1.3). The methanolic extract also demonstrated significant activity, with BIC_{50%} values between 2.3–3.8 mg/mL and BIC_{90%} values between 4.1–5.8 mg/mL. Moderate activity was observed for the acetone extract (BIC_{50%}: 7.7–8.6 mg/mL; BIC_{90%}: 13.5–16.3 mg/mL) (Table 1.3). In contrast, the toluene extract exhibited the least biofilm inhibition, with relatively high BIC_{50%} (11.4–13.5 mg/mL) and BIC_{90%} (22.6–25.0 mg/mL) values, indicating low efficacy. These results highlight the promising antibiofilm activity of the ethyl acetate extract, warranting further purification and mechanistic studies (Table 1.3).

3.1.3 Time-Kill Assay

The time-kill kinetics of *D. falcate* leaves extracted with ethyl acetate, methanol, and acetone were assessed against clinical isolates, *MRSA*, *E. coli*, *P. aeruginosa*, and *K. pneumoniae* at 1× and 2× minimum inhibitory concentration (MIC) levels (**please refer the table 1.1 for MIC levels**). The time-kill assay results of *D. falcate*

leaf extracts ethyl acetate, methanol, and acetone against clinical isolates are presented in Figure 1.1 and Table 1.4. Ethyl acetate extract exhibited the most potent bactericidal activity, achieving 100% CFU reduction of *S. aureus* (MRSA), *E. coli*, *P. aeruginosa*, and *K. pneumoniae* at 1× MIC within 5 h, 4.5 h, 5.5 h, and 5.5 h, respectively, and at 2× MIC within 3.0 h, 3.0 h, 3.5 h, and 3.5 h. Methanol extract achieved 100% bacterial kill at 1× MIC within 6.0 h, 5.5 h, 6.0 h, and 5.5 h, and at 2× MIC within 5.0 h, 4.5 h, 5.0 h, and 5.0 h for the same strains, respectively. In comparison, acetone extract required longer exposure times, showing 100% kill at 1× MIC within 6.5 h, 6.5 h, 7.0 h, and 7.0 h, and at 2× MIC within 5.0 h, 5.0 h, 5.5 h, and 5.5 h, respectively (Table 1.4.)

Additionally, at 1× MIC, ethyl acetate extract reduced 100% CFU of MRSA, *E. coli*, *P. aeruginosa*, and *K. pneumoniae* within 4.0 h, 3.0 h, 3.5 h, and 3.5 h, respectively. Methanol and acetone extracts showed slower action, achieving only 50% CFU reduction for the same pathogens at 6.0 h, 5.5 h, 6.0 h, and 5.5 h; and 6.5 h, 6.5 h, 7.0 h, and 7.0 h, respectively (Table 1.4).

4.0 Discussion

The present study provides the first report of *Dendrophthoe falcata* parasitizing *S. siamea*, a leguminous host not previously documented for this hemiparasitic species. This unusual host association is significant because the phytochemical constitution and bioactivity of *D. falcata* have been reported to vary depending on the host plant (Ramchandran & Krishnakumary, 1990; Khanna et al., 1968). The role of host plants in influencing the synthesis of secondary metabolites in parasitic species is well established, with changes in the host's physiology and nutrient profile potentially altering the expression of bioactive constituents in the parasite (Muthukumar et al., 2010). Our study revealed distinct phytochemical profiles and pharmacological properties of *D. falcata* growing on *Senna siamea*, suggesting that the new host may contribute unique biochemical precursors or modulate biosynthetic pathways in the parasite.

Moreover, previous studies have shown that *D. falcata* collected from traditional hosts like *Azadirachta indica* and *Mangifera indica* exhibits potent antimicrobial, anti-inflammatory, and antioxidant activity (Pattanayak et al., 2008; Patil et al., 2011; Kong et al., 2023). In our current investigation, the extracts from *D. falcata* parasitizing *S. siamea* showed comparable, and in some cases enhanced, pharmacological activity, supporting the hypothesis that host variation can be strategically utilized to influence the therapeutic efficacy of parasitic medicinal plants (Atun et al., 2018). This finding opens new avenues for selecting and cultivating *D. falcata* on specific host trees to tailor its medicinal properties.

This study highlights the promising antibacterial and antibiofilm potential of *D. falcata* leaf extracts against clinically relevant pathogens. The antibacterial potential of leaf extracts from *D. falcata* parasitizing *S. siamea*, particularly the ethyl acetate extract, which exhibited the highest activity against MRSA, *E. coli*, *P. aeruginosa*,

and *K. pneumoniae*. The concentration-dependent increase in inhibition zone diameters suggests that bioactive constituents within the ethyl acetate fraction possess bacteriostatic or bactericidal properties. This aligns with prior reports where ethyl acetate-soluble phytochemicals, especially flavonoids, tannins, and phenolics, have been implicated in disrupting bacterial cell membranes, leading to increased permeability and leakage of cellular contents (Cowans, 1999; Cushnie and Lamb, 2011). In addition, these compounds are also known to exert their antibacterial action by targeting multiple cellular pathways such as enzyme inhibition, nucleic acid synthesis disruption, and interference with quorum sensing mechanisms (Daglia, 2012).

The results correlated with previous reports. Lakshmi et al. (2012) reported strong antimicrobial properties of *D. falcata* extracts against both Gram-positive and Gram-negative pathogens, which were attributed to high levels of quercetin, β -sitosterol, and lupeol. Similarly, a related hemiparasitic species in the same family, *Scurrula ferruginea*, was shown to exhibit significant antibacterial effects, reinforcing the hypothesis that mistletoe species of the Loranthaceae family possess diverse phytochemical arsenals with therapeutic potential (Lim, 2015).

Minimum Inhibitory Concentration (MIC) of the ethyl acetate extract exhibited the highest antibacterial efficacy against MRSA, *E. coli*, *P. aeruginosa*, *K. pneumoniae*. These findings suggest that ethyl acetate may effectively extract medium-polarity bioactive constituents which are well-documented for their antimicrobial properties (Nickrent and Musselman, 2004). These results are consistent with previous studies demonstrating the broad-spectrum antibacterial properties of *D. falcata*, particularly when parasitizing medicinal host plants (Thirumal et al., 2013; Gajalakshmi et al., 2012). Furthermore, plants of the Loranthaceae family have shown similar MIC profiles in related studies, reinforcing the therapeutic promise of hemiparasitic mistletoes (Ali et al., 2011; Mallikharjuna et al., 2007).

The results of antibiofilm potential of *D. falcata* leaf extracts also reveals that the ethyl acetate extract compared with other extracts tested can disrupt the biofilm at least concentration of extract. This suggests that the ethyl acetate fraction harbors active constituents capable of disrupting the extracellular polymeric substance (EPS) matrix or interfering with quorum sensing pathways critical for biofilm formation and stability (Sivaranjani et al., 2016; Borges et al., 2012). These findings align with previous reports indicating that mistletoe species such as *D. falcata* contain flavonoids (quercetin, kaempferol), triterpenoids, and tannins compounds known to inhibit biofilm formation and enhance antimicrobial susceptibility (Gajalakshmi et al., 2012; Thirumal et al., 2013).

The time-kill kinetic assay revealed a concentration- and time-dependent bactericidal effect of *D. falcata* leaf extracts against multidrug-resistant clinical isolates including MRSA, *E. coli*, *P. aeruginosa*, and *K. pneumoniae*. Ethyl acetate extract demonstrated the

most rapid and effective killing, achieving complete CFU reduction at 1× MIC within 4.5–5.5 hours and significantly faster at 2× MIC (3.0–3.5 hours). This superior activity suggests the presence of potent lipophilic bioactive compounds capable of rapidly permeabilizing bacterial membranes or interfering with essential metabolic pathways (Mandal et al., 2014; Kumar et al., 2016).

5.0 Conclusion

The present study provides strong evidence for the antibacterial, antibiofilm, and time-kill efficacy of *D.*

falcata leaf extracts parasitizing *S. siamea* against clinically relevant pathogens, including MRSA, *E. coli*, *P. aeruginosa*, and *K. pneumoniae*. Among the extracts tested, the ethyl acetate fraction demonstrated superior bioactivity, exhibiting the lowest MIC values, potent time-dependent bactericidal action, and effective disruption of preformed biofilms. These observations strongly suggest the presence of bioactive phytochemicals particularly flavonoids, phenolics, and terpenoids that contribute to the antimicrobial potency by compromising bacterial membranes, inhibiting quorum sensing, and altering biofilm matrix integrity.

Table 1.0 Growth Inhibitory Effect potential of the *D. falcata* leaf extracts

Extract (mg/mL)	MRSA	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>
Toluene 25 mg/mL	7.2 ± 0.3**	6.8 ± 0.4**	6.4 ± 0.3**	6.6 ± 0.2**
Toluene 50 mg/mL	8.1 ± 0.2**	7.6 ± 0.3**	7.0 ± 0.4**	7.2 ± 0.3**
Toluene 100 mg/mL	9.4 ± 0.4**	8.9 ± 0.3**	8.0 ± 0.3**	8.3 ± 0.2**
Ethyl Acetate 25 mg/mL	12.6 ± 0.3*	11.9 ± 0.4*	11.0 ± 0.3*	11.3 ± 0.3**
Ethyl Acetate 50 mg/mL	15.3 ± 0.4*	14.6 ± 0.3*	13.4 ± 0.4*	13.8 ± 0.3**
Ethyl Acetate 100 mg/mL	18.2 ± 0.5*	17.6 ± 0.3*	16.5 ± 0.4*	17.0 ± 0.3*
Acetone 25 mg/mL	9.8 ± 0.4**	9.2 ± 0.3**	8.8 ± 0.2**	9.0 ± 0.3*
Acetone 50 mg/mL	11.2 ± 0.3**	10.6 ± 0.4**	10.0 ± 0.3**	10.3 ± 0.2*
Acetone 100 mg/mL	13.6 ± 0.3**	13.0 ± 0.3**	12.4 ± 0.3**	12.7 ± 0.3**
Methanol 25 mg/mL	11.0 ± 0.4**	10.4 ± 0.3**	9.8 ± 0.3**	10.1 ± 0.2**
Methanol 50 mg/mL	13.8 ± 0.4**	13.2 ± 0.3**	12.0 ± 0.3**	12.4 ± 0.3**
Methanol 100 mg/mL	16.4 ± 0.3**	15.8 ± 0.3**	14.6 ± 0.4**	15.0 ± 0.3**
Ciprofloxacin (5 µg/mL)	24.6 ± 0.5**	23.9 ± 0.4**	22.8 ± 0.4**	23.4 ± 0.4**
DMSO (Negative Control)	—	—	—	—

n=3; * significantly different P<0.001; ** significantly different p < 0.05

1.1 One-way ANOVA followed by Tukey's post-hoc test comparing the antibacterial activity

Bacterial Strain	F-value	p-value	Significant Differences (Tukey's Test, p < 0.05)
MRSA	62.31	<0.001	EA > ME > AE > TE; All significantly different (p < 0.05)
<i>E. coli</i>	58.27	<0.001	EA > ME > AE > TE; All significantly different (p < 0.05)
<i>P. aeruginosa</i>	51.89	<0.001	EA > ME > AE > TE; All significantly different (p < 0.05)
<i>K. pneumoniae</i>	55.76	<0.001	EA > ME > AE > TE; All significantly different (p < 0.05)

Table 1.2 Minimum inhibitory concentrations of the *D. falcata* leaf extracts

Bacterial Strain	Ethyl Acetate (µg/mL)	Methanol (µg/mL)	Acetone (µg/mL)	Toluene (µg/mL)
MRSA	3.8	5.5	36.6	>100
<i>E. coli</i>	4.3	7.8	34.3	>100
<i>P. aeruginosa</i>	2.9	6.1	42.1	>100
<i>K. pneumoniae</i>	5.2	8.4	35.8	>100

2.3 Biofilm Inhibition Concentration (BIC) potential of the *D. falcata* leaf extracts

Extract	Bacterial Strain	BIC ₅₀ (mg/mL)	BIC ₉₀ (mg/mL)
Ethyl Acetate	MRSA	1.3	2.9
	<i>E. coli</i>	1.5	2.7
	<i>P. aeruginosa</i>	1.2	2.3
	<i>K. pneumoniae</i>	1.9	2.8
Methanol	MRSA	3.3	5.1
	<i>E. coli</i>	3.8	5.5
	<i>P. aeruginosa</i>	3.0	5.8
	<i>K. pneumoniae</i>	2.3	4.1
Acetone	MRSA	8.6	14.8
	<i>E. coli</i>	8.2	15.7
	<i>P. aeruginosa</i>	7.7	13.5

	<i>K. pneumoniae</i>	8.4	16.3
Toluene	MRSA	12.8	23.2
	<i>E. coli</i>	11.4	22.6
	<i>P. aeruginosa</i>	13.5	25.0
	<i>Klebsiella pneumoniae</i>	12.9	23.2

Table 1.4 Time kill studies of the *D. falcata* leaf extracts

Extract	Bacterial Strain	100% Kill (1× MIC)	100% Kill (2× MIC)
Ethyl Acetate	MRSA	5.0 h	3.0 h
	<i>E. coli</i>	4.5 h	3.0 h
	<i>P. aeruginosa</i>	5.5 h	3.5 h
	<i>K. pneumoniae</i>	5.5 h	3.5 h
Methanol	MRSA	6.0 h	5.0 h
	<i>E. coli</i>	5.5 h	4.5 h
	<i>P. aeruginosa</i>	6.0 h	5.0 h
	<i>K. pneumoniae</i>	5.5 h	5.0 h
Acetone	MRSA	6.5 h	5.0 h
	<i>E. coli</i>	6.5 h	5.0 h
	<i>P. aeruginosa</i>	7.0 h	5.5 h
	<i>K. pneumoniae</i>	7.0 h	5.5 h

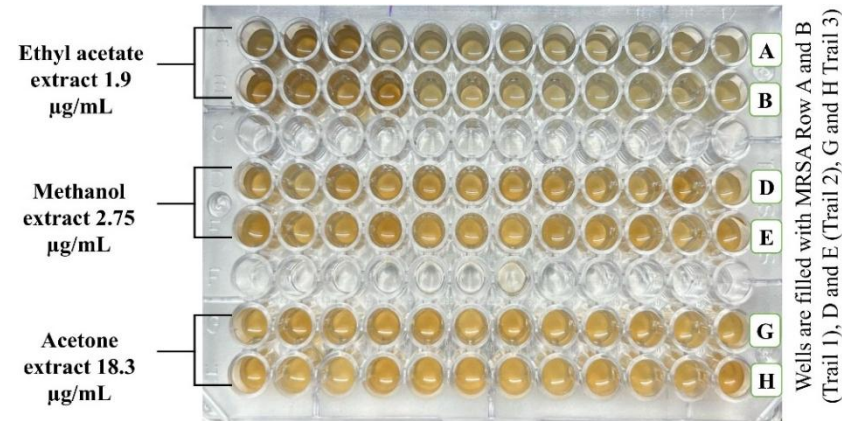


Fig 1.0 Effect of @ sub MIC concentration of *D. falcate* extracts in the inhibition of biofilm of MRSA

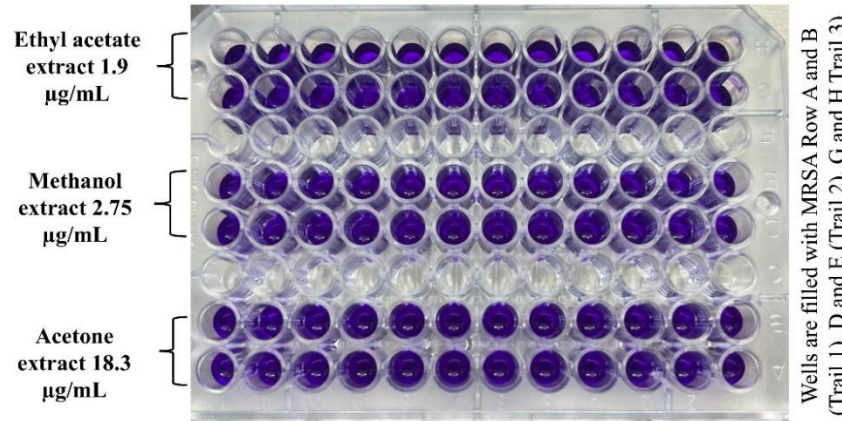


Fig 1.1 Staining the MRSA biofilm with 0.1% crystal violet, and solubilized with ethanol

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