RESEARCH ARTICLE

Antimicrobial Activity of Plant-derived Pongamol and its Derivatives

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Abstract

Many plant-derived phytomolecules have antimicrobial properties, useful against drug-resistant pathogens. In this study, some new derivatives of pongamol were synthesized by treating pongamol with different reagents to produce pyrazole, oxazole, pyrimidine, and diazepine systems and evaluating their antibacterial activity against different strains of bacteria. Pongamol, a phytochemical from *Tephrosia purpurea* seeds and *Pongamia pinnata* oil, exhibits antibacterial properties and can serve as a model for synthesizing novel antimicrobial compounds with the potential for fewer side effects. The synthesized compounds were screened for their in vitro antimicrobial potential against different strains of bacteria, *Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli,* using minimal inhibitory concentration (MIC) and zone of inhibition methods, with clarithromycin as the standard drug. Compound A5 showed greater activity than clarithromycin against *E. coli,* while A2 and A3 have better activity than standard drugs in the case of *S. aureus* in a zone of inhibition. (A1-A5) have greater activity than standard drugs. In MIC, all compounds (A1-A5) are more active than the clarithromycin in *P. aeruginosa.*

Highlights:

- Derivatization of plant-isolated molecules is done to evaluate the effectiveness, selectivity, improved stability and resistance towards different strains and to understand how structural changes affect biological activity.
- Pongamol was isolated from the seeds of *Tephrosia purpurea* plant.
- Five derivatives (A1-A5) of plant-origin pongamol (AO) were synthesized.
- The synthesized derivatives underwent antimicrobial activity against three bacteria: *E. coli. S. aureus* and *P. aeruginosa.*
- The derivatives (A1-A5) were compared to the parent molecule "Pongamol" (A0) and the standard drug clarithromycin.
- Results showed that the Derivatives (A1-A5) are more active than pongamol (AO) and have better results than clarithromycin.

Keywords: Pongamol, *Tephrosia purpurea*, *Pongamia pinnata* Clarithromycin, MIC.

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Introduction

The chemical complexity and structural diversity of natural products offer a vast library of potential bioactive compounds (Bruce, 2022). Thousands of bioactive phytomolecules have served as a valuable source of pharmacologically active compounds for drug discovery (Dias *et al*., 2012). This approach has led to the development of many medically proven drugs, including antibiotics, anticancer agents, and immune suppressants. The emergence and spread of antimicrobial resistance pose significant challenges to global public health, limiting the therapeutic index of drugs (Atanasov *et al*., 2021). The mechanisms through which microorganisms develop resistance to antimicrobial agents are multifaceted and can involve a variety of genetic and biochemical changes, which include alterations in cell membrane permeability, increased expression of efflux pumps that expel antibiotics from the cell, enzymatic modification or degradation of antibiotics, mutations in target sites, the development of alternative metabolic pathways, and the formation of protective biofilms (Barbosa *et al*., 2021). One of the major factors contributing to the problem of resistance is the use of about 50% of existing antimicrobials for purposes other than therapeutic use. The extensive use of antimicrobials in non-therapeutic applications, such as in agriculture for livestock production and as food additives, has contributed significantly to the emergence and spread of antimicrobial resistance (Manyi-Loh *et al*., 2018). Microorganisms indeed employ various strategies to adapt and survive in the presence of antimicrobial drugs, primarily through genetic mutations and horizontal gene transfer (HGT). The combination of genetic mutations and HGT imposes a significant challenge in combating antimicrobial resistance (Manandhar *et al.,* 2019). Biomolecules from microorganisms and plants are crucial for drug discovery (Jahan *et al.,* 2021). The World Health Organization's definition

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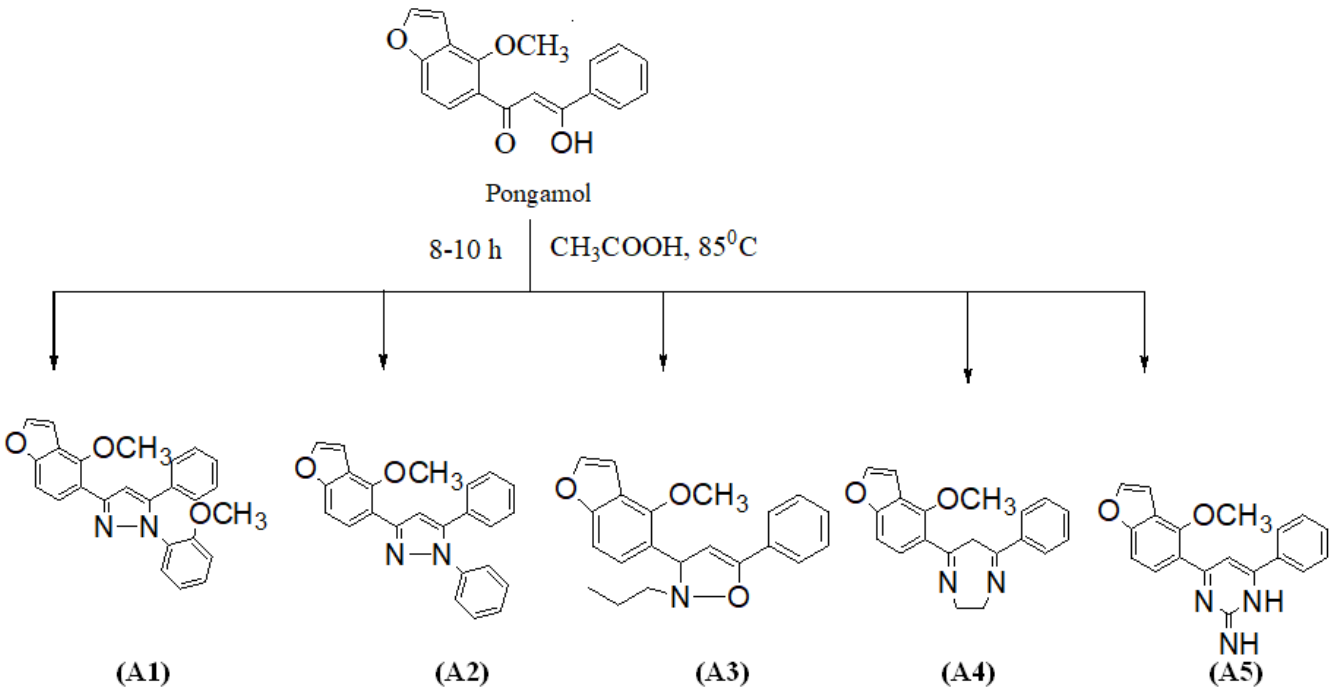
of antimicrobial resistance, which states that "Antimicrobial Resistance occurs when viruses, bacteria, fungi, and parasites no longer respond to antimicrobial medicines," succinctly captures the essence of this global health (Prestinaci *et al*., 2015).

Phytomolecules from plants indeed offer significant potential as antimicrobial agents (Cowan, 1999). Various classes of phytomolecules have been investigated for their antibacterial properties, including flavonoids, alkaloids, terpenoids, phenolics and others (Newman and Cragg, 2016). These compounds can exert their antimicrobial effects through a variety of mechanisms, making them valuable candidates for combating drug-resistant pathogens (Khare *et al*., 2021). The ability of phytomolecules to target specific bacterial pathways or cellular processes underscores their potential as alternative or adjunctive therapies for combating antimicrobial resistance. Additionally, the synergistic effects of phytomolecules with standard antibiotics highlight the possibility of enhancing the efficacy of existing antimicrobial agents while potentially reducing the risk of resistance development (Dias *et al*., 2012).

Research into the antimicrobial properties of phytomolecules continues to expand, with ongoing efforts to identify novel compounds, elucidate their mechanisms of action, and explore their therapeutic potential. By harnessing the diverse array of bioactive compounds present in plants, researchers aim to develop new strategies for combating drug-resistant infections and preserving the effectiveness of antimicrobial therapies (Jhanji *et al*., 2021). The effectiveness of antimicrobial medicines can indeed differ due to the structural differences between their cell wall. Positive bacteria have a thick peptidoglycan layer in their cell wall, readily accessible to many antimicrobial compounds. This thick layer provides structural integrity and shape. In contrast, negative bacteria possess a more complex cell wall structure, including an outer membrane composed of lipopolysaccharides (LPS). This membrane serves as a barrier, providing resistance to many antibiotics and other antimicrobial agents (Qadri *et al*., 2022). This variation in cell wall composition significantly influences the efficacy of antimicrobial treatments. Understanding these structural distinctions is crucial for developing and selecting appropriate antibiotics to target specific bacterial infections effectively (Epand *et al.,* 2016). Exploiting these differences, researchers are working towards overcoming the challenges of bacterial resistance and improving the treatment for infectious diseases. Various plant-sourced chemical moieties are being used for the treatment of infectious diseases.

Pongamol is a flavone derivative present in the seeds and roots of *Pongamia pinnata* (karanjin) (Singh *et al*., 2021) and *Tephrosia purpurea* (Kumari *et al*., 2023), both of which belong to the family Fabaceae. This compound is characterized by the presence of a methoxy group along with enolic or phenolic hydroxyl moieties (Rangaswami and Sesdhari, 1942). The bacteria affected by pongamol include Baki *et al.,* 2007). Additionally, when coupled with other antimicrobial agents or chemicals, it shows synergistic effects that result in greater antimicrobial activity against a wide range of diseases. The superior antimicrobial activity, synergistic effects, and chemical properties of pongamol make it a suitable candidate to be used as a tool for creating the derivatized compound that may have improved antimicrobial efficacy. Combining pongamol with different reagents to produce different derivatives to create antimicrobial agents is an intriguing approach in medicinal chemistry.



Scheme 1: Synthesis of Pongamol derivatives

In view of the above observation, we have synthesized five pongamol derivatives (A1-A5) and evaluated their antimicrobial properties in different strains of pathogenic bacteria *S. aureus, P. aureus and E. coli*. The compounds A1, A2 and A3 have shown significant antimicrobial activity in *in-vitro* settings that may have reduced drug resistance in various pathogens, which needs further validation.

Material and Methods

General Procedure

Pongamol (1-mmol) was dissolved in glacial acetic acid (50 ml, providing homogenous reaction condition), sodium acetate and different hydrazide derivatives (1.2 mmol) o-methoxyphenylhydrazine hydrochloride, phenylhydrazine hydrochloride, N-butyl amine, ethylene diamine and guanidine hydrochloride were mixed to the solution and refluxed for 8-10 h, at 80° C. The progress of the reaction was monitored by a pre-coated TLC plate using ethyl acetate and n-hexane as a solvent system (2:8). Then the solvent was removed by distillation and the residue was partitioned with ethyl acetate and water(50X50). The organic portion was collected and evaporated. The crude product was further purified by column chromatography by using silica gel #60-120 and n-hexane and ethyl acetate (95:05) as solvent system. The synthesized compounds (A1-A5) were characterized by infrared spectroscopy (IR), mass spectroscopy, and NMR spectroscopy (Rao *et al*., 2012). (Scheme: 1)

Bacterial cell culture

The well diffusion assay was used to assess the antimicrobial activity of the pongamol and its derivatives against three bacterial strains: *P. aeruginosa* (MTCC 2453), *S. aureus* (MTCC 96), and *E. coli* (MTCC 739).

Determination of Zone of Inhibition

The antimicrobial activity of pongamol and its derivatives was evaluated using the agar well diffusion method on Mueller Hinton Agar (MHA) plates. Test organisms were cultured in nutrient broth and incubated overnight at 37°C to achieve a turbidity equivalent to 0.5 McFarland standards, corresponding to a final inoculum of 1.5×10^8 CFU/mL. The MHA plates were uniformly lawn-cultured with the standardized microbial suspension. A 50 µg/mL solution of pongamol and its derivatives was prepared in dimethyl sulfoxide (DMSO). Sterile cork borers (2.5 mm) were used to create three wells in the inoculated agar, and each well was filled with 100 µg/mL of pongamol or its derivatives. Positive controls (Clarithromycin at 100 µg/mL) were included for bacterial strains. The plates were allowed to stand at room temperature for 30 minutes to enable diffusion, followed by incubation at 37°C for 18 to 24 hours. Post-incubation, the plates were examined for clear zones around the wells, indicative of antimicrobial activity. The zone of inhibition (ZOI) was measured in millimeters.

Determination of Minimum inhibitory concentration (MIC)

The antibacterial efficacy of new derivatives was assessed by determining their minimum inhibitory concentration (MIC) against different strains of bacteria. Initially, lyophilized bacterial strains in the lag phase were reconstituted in sterile water. The strains were then activated using nutritional broth as activation media (as advised by the vendor's protocol) at 37°C until the broth contained 10^8 to 10^9 colony-forming units (CFU) of bacteria. The inoculum was prepared by diluting the suspension to a 0.5 McFarland standard using a sterile 0.9% w/v saline solution. Subsequently, 100 mL of the bacterial suspension (corresponding to the 0.5 McFarland standard) was inoculated into sterile nutrient broth in culture tubes, followed by the addition of test samples at various concentrations along with positive controls (clarithromycin). The vials were then cultured at 37°C for 24 hours and opacity was measured at 600 nm to assess bacterial growth. Sterile PBS served was used as a control (Sharma *et al*., 2016).

Determination of minimum biofilm inhibitory concentration (MBIC).

Biofilms were initiated by inoculating 0.5 McFarland standard bacterial suspensions into nutrient broth and incubating for 96 hours at 37°C, with media refreshed every 24 hours to remove non-adherent bacteria. After incubation, crystal violet staining confirmed biofilm formation on tube surfaces. Biofilms were rinsed with PBS and sonicated to detach bacteria, then fixed. Following this, biofilms were treated with different conc. of clarithromycin and the synthesized derivatives for 24 hours. Post-treatment samples were taken, sonicated, and incubated in fresh broth to evaluate the antibiofilm effectiveness of each compound. (Cruz *et al.,* 2018).

Result and Discussion

Characterization

3-(4-Methoxy-benzofuran-5-yl)-1-(2-methoxy-phenyl)-5-phenyl-1H-pyrazole(A1)

Isolated from the column as a colorless crystalline solid, yield 45%; mp: 126–129°C; UV Abs (MeOH, λmax): 356; IR bands: 1240.33, 1027.98 1217.47, 1058 (2–OCH_3), 1646.90 (C=N), 1363.34 (C-N), 1461.47 (C=H) cm-1; 1H-NMR (800 MHz, $CDCl_3$): 3.711{OCH_3 (3H)}, 7.259 {4H (1H)}, 7.350 {d, *J* = 8.0 Hz, 2'H (1H)}, 6.903 {d, *J* = 8.01 Hz, 3'H (1H)}, 7.724 {d, *J* = 8.00 Hz, 7.724 {dd, *J* = 8 Hz, 6'H (1H}, 6.83 {d, *J* = 8 Hz, 7'H (1H)}, 7.300-7.023 (2" H-6-H), 6.977–7.023 (2"' H-6" H). 13C-NMR (800 MHz, $CDCl_3$ δ 147.81, 147.02, 129.52, 129.20, 121.24, 113.49. LC-MS calculated for $C_{24}H_{18}N_2O_2$ [M+H] 366.41found 367.41

3-(4-Methoxy-benzofuran-5-yl)-1,5-diphenyl-1H-pyrazole (A2)

Isolated from the column as white crystalline solid, yield 40%; m.p: 99–101°C; UV Abs (MeOH, λmax): 239, 281; IR bands; 1238.40, 1075.61 (–OCH_3), 1302.81 (C-O) 1643.84 (C=N), 1152.97(C-N), 1441.81(C=H), 3017.21 (C-H) cm^{-1}. ^{1}H-NMR (800 MHz, $CDCl_3$): *δ*3.8159 (3H, s, 2'''-OCH_3), 3.4713 (3H, s, 4'-OCH_3), 6.8809 (1H, s, 4-H), 6.9068 (1H, d, *J* = 8 *Hz* , 3'-H), 7.6078 (1H, d, 2'-H), 7.7927 (2H, d, *J* = 2 *Hz* 6'-H, 7'-H), 7.5621–7.5393 (m, 1" -5" (5H); ^{13}CNMR (200 MHz, $CDCl_3$): *δ* 158.53, 156.96, 151.93, 144.27, 133.64, 129.02, 128.59, 127.78, 126.02, 121.93, 119.50, 117.32, 108.18, 104.37, 59.95; LC–MS: calculated for $C_{25}H_{20}N_2O_3$ [M+H] 396.44, found 397.44.

3-(4-Methoxy-benzofuran-5-yl)-5-phenyl-2-propyl-2,3-dihydro-isoxazole (A3)

Isolates from the column as light brown crystalline solid, yield 30%; m.p: 138–140 °C; UV Abs (MeOH, λmax): 263, IR bands (KBr); 1066.83 (C-O), 1252.96, 1034.06 (OCH_3), 1357.83 (C-N), 1644.28 (Ar C=C), 3112.83 (Ali. C-H) cm^{-1}; ^{1}HNMR (800 MHz, $CDCl_3$): δ 7.51 (2-H, dd, *J* = 1.8Hz, 1H), 6.87 (3′-H, dd, *J* = 1.7 Hz, 1H), 6.86 (7′-d, *J* = 8.1 Hz,1H), 1.77 (7-H, 2H), 1.28 (8-H, 2H), 2.04 (4′-OCH_3, 3H), 3.18 (2-H, 2H), 3.68 (s, 3-H, 1H), 4.95 (1-H, d, *J* = 5.4 Hz, 1H); ^{13}C NMR (200 MHz, $CDCl_3$) δ 145.97, 131.86, 126.27, 117.29, 110.36, 108.14, 104.34; LC–MS calculated for $C_{21}H_{21}NO_3$ [M+H] 335.40 found 336.40.

4-(4-Methoxy-benzofuran-5-yl)-6-phenyl-1H-pyrimidin-2-ylideneamine (A4)

Isolated from the column as light yellow crystalline solid, yield 38%; m.p: 115–118°C; UV Abs (MeOH, λmax): 356; IR bands (KBr); 1215.84, 1033.78 (OCH_3), 1255.48 (C-O), 1640.99 (C=N), 1065.31 (C-NH), 3067.05 (Ar. N-H), 3067.05 (NH) cm^{-1}; ^{1}H-NMR (800 MHz, $CDCl_3$): δ 7.23 (6′-H, 1H), 7.55 (2′-H, 1H), 6.89 (3′-H, 1H), 2.05 (OCH_3, 3H); ^{13}C NMR (200 MHz, $CDCl_3$) δ 178.48, 162.86, 158.52, 151.00, 145.99, 131.92, 129.30, 127.36, 126.36, 110.39, 108.20, 104.37, 77.35; LC–MS calculated for $C_{19}H_{15}N_3O_2$ [M+H] 318.37 found 319.40.

5-(4-Methoxy-benzofuran-5-yl)-7-phenyl-3,6-dihydro-2H-[1,4] diazepine (A5)

Isolated from the column as white light crystalline solid, yield 38%; m.p: 136–139°C; UV Abs (MeOH, λmax): 296; IR bands (KBr): 1066.83(C-O), 1252.96, 1034.04 (–OCH_3), 1644.28 (C=N), 748.01, 3112.83(C-H) 1604.45 (C=C) cm-1; 1 H-NMR (800 MHz, $CDCl_3$): δ 7.63 (2′-H, dd, *J*=1.8*Hz*, 1H), 6.79 (3′-H, dd, *J* = 1.8, 1H), 3.82 (4′-OCH_3, 3H), 7.23 (6′-H, d, *J* = 7.9*Hz*, 1H), 7.26 (7′-H, d, *J* =7.9*Hz*, 1H), 3.555 (4-H, d, *J* =7.8, 1H), 3.59 (2‴-H, 3‴-H, 5‴-H, dd, *J*=8.0*Hz*, 4H), 3.47 (2-H, 1H), 3.82 (3-H, 1H); 13C-NMR (200 MHz, $CDCl_3$) δ 162.88, 158.54, 146.00, 131.94, 129.31, 126.38, 117.34, 108.23, 104.38; LC–MS calculated for $C_{25}H_{20}N_2O_3$ [M+H] 317.34 found 318.37.

Biology

Zone of Inhibition (Well diffusion method)
Minimum inhibitory concentration (Turbidity method)

Zone of Inhibition

The pongamol (AO) and its derivatives (A1-A5) showed variable inhibitory effects on the growth of the bacterial strains. (Table 1) (Fig: 1). In *S. aureus*, compounds A2 (10.50) and A3 (12.50 mm) are more active than clarithromycin (9.50 mm) and pongamol (10.25 mm), while compound A5 (12.25 mm) is approximately equivalent to clarithromycin (12.75 mm) and more active than pongamol in *E. coli.* Whereas in the case of *P. aeruginosa,* no compound are active than clarithromycin (10.00 mm).

Table 1: Zone of inhibition against bacterial pathogens showing zone of inhibition (mm)

	Zone of Inhibition (mm) ± SD (n = 3)			
	Dilutions (µg/mL)	*E. coli*	*S. aureus*	*P. aeruginosa*
AO	100	8.00 ± 1.7	10.25 ± 1.1	7.25 ± 0.8
A1	100	8.50 ± 1.4	8.50 ± 1.2	6.00 ± 0.5
A2	100	7.25 ± 2.6	10.50 ± 1.9	3.50 ± 0.0
A3	100	7.25 ± 0.8	12.50 ± 1.6	8.5 ± 2.5
A4	100	7.75 ± 1.1	7.50 ± 1.9	6.75 ± 0.8
A5	100	12.25 ± 4.0	9.50 ± 1.6	5.50 ± 0.7
Clarithromycin	100	12.75 ± 4.3	9.50 ± 3.2	10.00 ± 0.5
Control		-	-	-

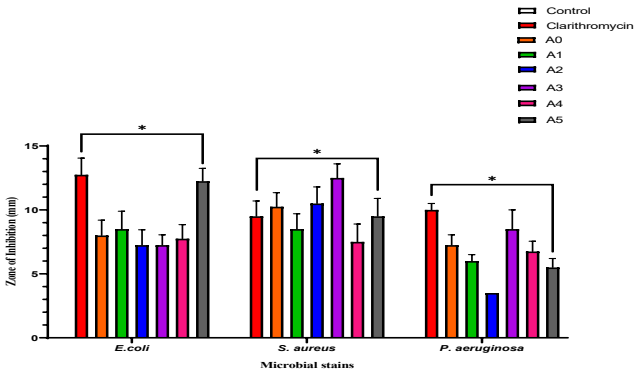


Fig. 1: Zone of Inhibition of different derivatives of pongamol against *different strains of bacteria.* One-way analysis of variance (ANOVA) was utilized for statistical analysis. Data is shown as means ± SD; *$p < 0.05$ compared to control. (*significant)

Minimum Inhibitory Concentration (MIC)

The pongamol (AO) and its derivatives (A1-A5) exhibited stronger antibacterial activity against pathogenic strains. The A1 and A3 (5 µg/mL) are more active than the parent molecule pongamol (8 µg/mL) and the clarithromycin (6 µg/mL) in *E. coli.* and in *S. aureus* A3 (8 µg/mL) is equivalent to clarithromycin (12µg/mL) whereas all compounds (A1-A5) are more active than clarithromycin in the case of *P. aeruginosa*. (Table: 2) (Fig:2)

Minimum Biofilm Inhibitory Concentration (MBIC)

The compound A3 (112 µg/mL) is more active than the standard drug clarithromycin (140 µg/mL). Other compounds are inactive (Table 2).

The MIC and MBIC values in µg/mL, indicate the effectiveness of various pongamol derivatives against various strains *E. coli*, *S. aureus*, and *P. aeruginosa*, with clarithromycin used as the standard drug.

Statistical analysis

All experiments were conducted in triplicates and acquired data are presented as Mean ± SD. Statistical analysis was performed

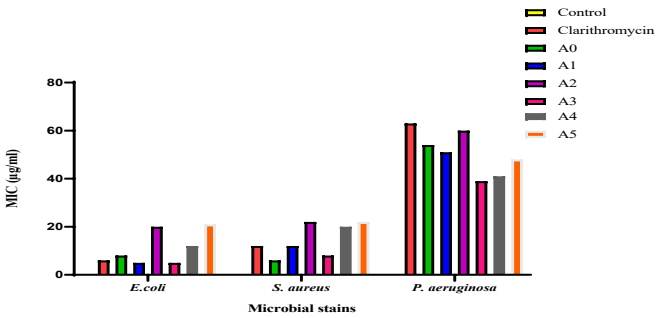


Fig. 2: Minimum inhibitory concentration (MIC) of different derivatives of pongamol against different strains of bacteria

Table 2: Antimicrobial activity (MIC and MBIC = µg/mL) of pongamol and newly synthesized compounds (A1-A5) against different strains of bacteria

S. No.	*Compounds*	*E. coli MIC in µg/mL*	*S. aureus MIC in µg/mL*	*P. aeruginosa MIC in µg/mL*	*MBIC (µg/mL)*
1	A0	8	6	54	-
2	A1	5	12	51	-
3	A2	20	22	60	-
4	A3	5	8	39	112
5	A4	12	20	41	-
6	A5	21	22	48	-
7	Clarithromycin	6	12	63	140
8	Control	-	-	-	-

using Prism 6 (Graph Pad software). One-way analysis of variance (ANOVA) was utilized for statistical analysis. Data is shown as means ± SD; $*p < 0.05$ compared to control. (*significant)

Conclusion

The study highlights that the derivatives (A1–A5) of pongamol exhibit superior antimicrobial activity against *E. coli, S. aureus* and *P. aeruginosa* compared to the parent molecule pongamol (A0) and the standard drug clarithromycin. Among the derivatives, compound A5 demonstrates notable activity against the gram-negative bacterium *E. coli*. Its pyrimidine structure, featuring nitrogen atoms and double bonds, likely facilitates hydrogen bonding and π-π stacking interactions with microbial targets, enhancing its antimicrobial potency.

Additionally, compounds A1 and A2 show significant activity, attributed to the presence of a pyrazole nucleus substituted with electron-withdrawing groups (e.g., phenyl or o-methoxy phenyl) at the nitrogen atom. Similarly, compound A3, containing an oxazole nucleus with an electron-donating group (alkyl) at the nitrogen atom, exhibits good antimicrobial activity. Similarly, the compound A3 is more active than clarithromycin in inhibiting the formation of a biofilm by microorganisms or disrupting an already established biofilm.

These findings underscore the impact of chemical modifications in enhancing the antimicrobial properties of pongamol derivatives. This advancement not only provides insights into the structure-activity relationships of these molecules but also paves the way for further exploration of pongamol derivatives as potent antimicrobial agents.

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Authors Contribution

The first author designed the scheme and synthesized the derivatives. The second author performs the antimicrobial activity. The third author performed the analysis. The fourth author interprets the data.

Conflict of Interest

Authors declare no conflict of interest

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