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Research Article

ANTIBACTERIAL ACTIVITY OF THREE PLANT EXTRACTS AGAINST MULTIDRUG RESISTANCE *PSEUDOMONAS AERUGINOSA*

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ABSTRACT

Objective: The increasing of antibiotic resistance of *Pseudomonas aeruginosa* causes serious worldwide infection problems. Hence, the objective of the study was to investigate the antibacterial activity of some plants against multidrug resistance *P. aeruginosa* isolated from burn-wound infections.

Methods: About 30 burn-wound swab samples taken from hospitalized patients in Al-Hillah Teaching Hospital, Babylon Province. *P. aeruginosa* isolates were isolated and identified depending on cultural, microbial, and biochemical characteristics. Then, the drug susceptibility was detected using different available antibiotics (8) to select multidrug resistance *P. aeruginosa* strains for using as test organisms. Three types of plants, including onion bulbs (*Allium cepa*), leaves of mint (*Mentha asiatica*), and outer peel of pomegranate (*Punica granatum*), were extracted by three types of solvent. The plant extracts were tested as antimicrobial substances.

Results: About 9 samples were found positive by causing bacterial infection who presented invasive burn-wound infection from both sex and average age of 9 to 45 years. *P. aeruginosa* was found to be the most common isolates, 10 samples out of 30 samples. The most of multidrug resistance *P. aeruginosa* was used as test organisms to investigate the antimicrobial activity of three types of plant extracts. The plant extract of *P. granatum* showed the highest antibacterial activity, followed by *A. cepa*, and finally, *M. asiatica*.

Conclusion: From the study, all three studied plants had antibacterial activity against multidrug-resistant *P. aeruginosa* isolated from burn wound. It is a recommendation that natural products can use as therapeutic agents will probably not elicit resistance in bacteria. More research must continue to isolate and purify the active components and applied in experimental animal models.

Keywords: Multidrug resistance *Pseudomonas aeruginosa, Allium cepa, Mentha asiatica, Punica granatum.*

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INTRODUCTION

Burn-wound infection due to *Pseudomonas aeruginosa* poses a significant challenge in terms of graft loss, systemic sepsis, prolonged hospital stay, and even increased mortality. Armour *et al.* [1] reported on 48 adult patients with gentamicin-resistant *P. aeruginosa* colonization compared with case-matched controls; this cohort required a two-fold increase in grafting procedures as well as an average of 15 days' longer hospital stay. Geyik *et al.* [2] reported the only specific series to detail the effect of *P. aeruginosa* in children, and they found that 65% frequency of wound colonization, with systemic sepsis in 7.2%, when associated with bacteremia, was associated with an 80% mortality. Herbal medicines have been important sources of products for developing countries in treating common infectious diseases and the problems of resistance and side effects of the currently available antimicrobial agents [3]. The World Health Organization estimates that 80% of the people living in developing countries almost fully depend on traditional herbal medicines in Iraq known as Arabic medicine. This means that approximately 3300 million people use medicinal plants on a regular basis. Medicinal plants used in traditional medicine should be studied for safety [4]. Hence, we use three types of plants which approved their ability as antimicrobial activity against pathogenic bacteria isolated from burn infection, especially against *P. aeruginosa*, onion bulbs (*Allium cepa*), leaves of mint (*Mentha asiatica*), and outer peel of pomegranate (*Punica granatum*).

Each one of the chosen plant extract has special active chemical compound against the specific chemical compound on/or inside pathogenic cell, and onion extract is very effective against *P. aeruginosa* due to flavonoids and polyphenols which has been reported to have a broad spectrum of antibacterial activity [5]. While mint extract includes monoterpenes, mainly menthol, menthone, and their derivatives [6]. Pomegranate peels reported that phenolic compounds are punicalagin isomers, ellagic acid derivatives, and anthocyanins [7].

The objective of the study was to investigate the antimicrobial activities of three solvent extracts of selected Iraqi plant against multi-resistant *P. aeruginosa* isolated from burns and wounds.

MATERIALS AND METHODS

P. aeruginosa isolation and identification

About 30 burn-wound swaps were taken from both male and female burned patients, and average age 9-40 years, from a burn unit of teaching AL-Hillah Hospital, Babylon Provence, Iraq, during September 2016-January 2017. All specimens were inoculated on 5% blood agar, MacConkey agar, and Chocolate agar plates and incubated overnight at 37°C aerobically. The sample was also put into liquid media (Brain Heart Infusion broth) and was subcultured after overnight incubation onto blood agar and MacConkey agar. Bacterial pathogens were identified by conventional biochemical methods according to standard microbiological techniques [8]. Antimicrobial susceptibility was performed on Mueller-Hinton agar by the standard disk diffusion method [9]. The antibiotics tested for bacterial isolates were: Ciprofloxacin (Cip10), amikacin (AK30), chloramphenicol (C30), tetracycline (T30), oxacillin (Ox1), Cip10, trimethoprim (Tmp5), and doxycycline (Do30). The inhibition zone diameter (mm) of antibacterial activities was calculated.

Preparation of plant extracts

Three plant samples were used in this study; peel of pomegranate, onion bulbs, and mint leaves were purchased from the local markets.

The plants were classified by specialists in the Botanical Garden at the University of Babylon. These plants were air-dried at room temperature, ground to powder with a mechanical grinder.

Aqueous extraction using hot water

20 g of the weighed plant powder was soaked in 100 ml of hot boiled water for 24h.The plant extract was separated from the plant solid residue using filter paper with vacuum, then centrifuges and the supernatant filtered using Whatman filter paper No.1. The filtrate was concentrated by a rotary evaporator (rotavapor R300) at 40°C. The concentrated extract was dried to obtain an extract powder [10]. The powdered was stored in clean containers at 4°C for further analysis.

Alcoholic extraction

Alcoholic extracts were prepared using two solvents such as ethanol and methanol. The plant powder (50 g) was soaked in 250 ml of 99.9% for each ethanol or methanol at room temperature for 24h.The extracts were separated from the other solid plant residues using filter paper with vacuum, and then, it was centrifuged, and the supernatants were filtered using Whatman filter paper No.1. The filtrates were concentrated using a rotary evaporator (rotavapor R300) at 40°C. The concentrated extracts were dried to obtain the extract powders [10,11]. These powdered were stored in clean containers at 4°C for further analysis.

Preparation of the stock solutions and dilutions

A 15 g of each plant extract powder was dissolved in 50 ml sterile hot-distilled water to prepare the stock solution (200 mg/ml). While the ethanol extract was firstly dissolved in 1 ml of 99.9% ethanol due to it was insoluble in water and it was oily nature. Then, diluted in sterile distilled water to give an ethanolic stock solution. All stock solutions were filtrated by Whatman filter paper No. 1 and then by Millipore filter membrane (0.45 µm) for sterilization. Different dilution frequencies (50, 100, and 150 mg/ml) were made with sterile distilled water.

Antibacterial activity determination

The antibacterial activity of the crude extracts against *P. aeruginosa* was determined by the agar-well diffusion method [12,13] using Muller-Hinton agar plates. Bacterial suspension (10^5 CFU/ml) was made, and then, the Muller-Hinton plates were streaked with a bacterial suspension using sterile swab. After that, the wells were made with a diameter of 6 mm by punched aseptically with a sterile cork borer (No. 6). Approximately 50 µl of the crude extract at different concentrations 50, 100, and 150 mg/ml were loaded into the wells, and the negative control was used a sterile distilled water, in triplicate. One an hour pre-diffusion time was allowed, after that, the plates were incubated at 37°C for 18h.The diameter of inhibition zones was measured in millimeter and calculated the mean of triplicate results [14,15].

RESULT AND DISCUSSION

Bacterial isolation and identification

The bacterial isolates (9) were recovered from only 9 burn-wound swabs out of 30 swabs that indicate 30% of examined burn patients had invasive burn-wound infections. These results are consistent with the previous studies [16-18] who showed that the burn-wound infections are one of the most important and potentially serious issues that occur in the acute period following injury. Furthermore, Raja and Singh [9] demonstrated that the infectious complications are considered a major cause of morbidity and mortality and the type and amount of microorganisms onto and into the injured tissues influence wound healing. In the present study, the most commonly isolated organisms from burning patients were *P. aeruginosa* identified by conventional biochemical methods according to standard microbiological techniques [8] and using the specific chromagar for *P. aeruginosa* identification.

Antimicrobial susceptibility testing

Antibacterial susceptible of pseudomonad isolates was detected to determine the multidrug-resistant *P. aeruginosa* isolates. The test was performed by disc diffusion method with different antibiotic discs. The results showed that all *Pseudomonas* isolates appeared multidrug resistant for all used antibiotics, including Ox1, C30, T30, Tmp5, and Do30. However, it had moderate resistance to AK30 and Cip10 as shown in Fig. 1. These results were consistent with the previous studies [17-19]. Increasing resistance to various anti-*Pseudomonas* agents has been reported worldwide, and this poses a serious problem in therapeutic management of the bacterial infections [17,20]. Furthermore, our results explained that most of the isolates were resistant to many antibiotics.

Antibacterial activity of plant extracts against *P. aeruginosa*

Agar well diffusion method was used for antibacterial activity determination of aqueous, ethanolic, and methanolic extracts of plants, including outer peel of pomegranate (*P. granatum*), onion bulbs (*A. cepa*), and mint leaves (*M. asiatica*). Quantitative evaluation of this activity was carried out against *P. aeruginosa* by measuring of inhibition zone surrounded the wells containing the extract.

As shown in Table 1, plant extracts had antibacterial activity against *P. aeruginosa* with clear differentiation among the extracts depending on the concentration of the extract, type of solvent, and type of plant.

The results show high activity to all ethanolic extract of three plant extracts than methanolic and water extracts as shown in Tables 1-3 due to the ability of ethanol to solve solid organic compounds and liberate all chemical components rather than other solvent used in the present study. Peel of *P. granatum* (Table 1) showed the highest anti-pseudomonad activity than other plants in all of the concentrations. The highest concentration of peel of *P. granatum* extract (200 mg/ml) appeared highest inhibition zones for all types of extraction methods (38 mm for ethanolic extract, 36 mm for methanolic extract and 22 mm for the water extract). Peel of *P. granatum* contains substantial amounts of polyphenols such as ellagic tannins, ellagic acid, and gallic acid, which have antimicrobial activity [21]. These results were in agreement with Hayrapetyan *et al.* [22] who reported that the presence of two pure compounds commonly found in the pomegranate-peel extract, namely, ellagic acid and gallic acid. While other study revealed that the phenolic compounds of pomegranate juice are punicalagin isomers, ellagic acid derivatives, and anthocyanins [23]. Ahmad and Beg [24] reported that the phytochemical components found in the alcoholic extract of pomegranate are alkaloid, flavonoid, glycoside, phenol, and tannin. The results of antimicrobial activity of the peel ethanolic extract of *P. granatum* were in agreement with the study of Oskay *et al*. [25] who recorded 16 mm inhibition zone diameter against *P. aeruginosa*.

The methanolic extract showed lower action than the ethanolic extract as antibacterial agents. This may be due to little diffusion properties of the extract in the agar or because fresh plants contain

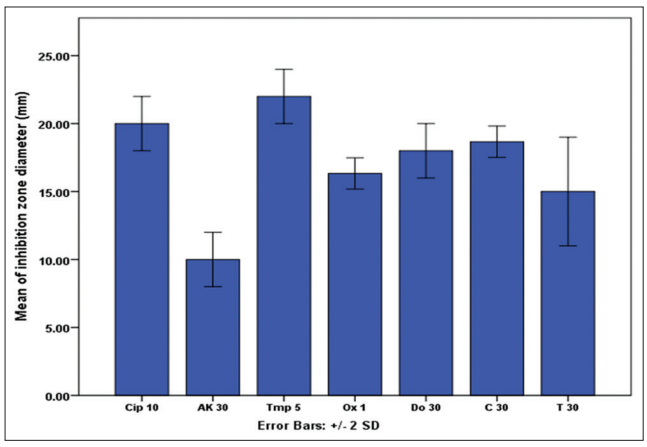

Fig. 1: Multidrug-resistant *Pseudomonas aeruginosa* isolated from burn-wound infections

Table 1: Antibacterial activity of *P. granatum* peel extract against multidrug-resistant *P. aeruginosa* isolated from burn wound

Types of extract	Isolate	Inhibition zone diameter mm±SD				
		200 mg/ml	150 mg/ml	100 mg/ml	50 mg/ml	Control
EtOH	P1	38.000±1.000	35.000±1.000	30.666±0.577	27.666±0.577	0.00
	P2	32.666±1.577	34.666±1.577	33.000±1.000	30.333±1.577	0.00
	P3	35.000±1.000	34.666±1.577	31.000±1.000	25.000±1.000	0.00
	P4	30.666±1.577	20.666±1.157	15.333±0.577	16.000±1.000	0.00
	P5	37.666±1.577	33.333±1.567	28.666±1.577	21.666±1.577	0.00
	P6	31.000±1.000	30.333±1.577	24.000±1.000	20.666±1.577	0.00
	P7	40.666±1.577	35.666±0.577	31.333±1.156	25.000±1.000	0.00
	P8	38.000±1.000	35.333±1.577	29.000±1.000	27.666±1.577	0.00
	P9	40.333±1.577	35.666±1.577	31.666±1.577	26.666±1.156	0.00
MeOH	P1	36.333±1.577	33.000±1.000	29.666±1.527	20.000±1.000	0.00
	P2	34.333±1.527	30.666±1.577	31.000±1.000	24.000±1.000	0.00
	P3	37.333±1.577	32.666±0.577	24.333±1.577	15.666±1.527	0.00
	P4	32.666±0.577	32.666±1.577	30.000±1.000	12.000±1.000	0.00
	P5	36.000±1.000	31.000±1.000	29.333±0.577	15.333±0.333	0.00
	P6	34.666±1.577	33.666±1.577	30.333±1.527	20.666±1.556	0.00
	P7	34.333±0.577	31.666±1.527	25.333±1.577	20.666±1.577	0.00
	P8	35.333±0.333	33.333±1.333	25.666±0.577	20.333±1.333	0.00
	P9	38.333±1.577	35.000±1.000	30.000±1.000	24.666±1.527	0.00
Aquas	P1	22.000±1.000	20.666±0.577	16.000±1.000	11.666±0.577	0.00
	P2	21.333±0.527	18.000±1.000	18.333±0.577	12.666±0.577	0.00
	P3	26.333±0.577	22.000±1.000	19.666±0.577	9.666±1.527	0.00
	P4	21.333±0.577	20.666±0.577	20.000±1.000	18.333±0.577	0.00
	P5	20.000±1.000	18.333±0.577	12.666±0.577	0.00	0.00
	P6	20.000±1.000	15.333±0.577	10.000±1.000	0.00	0.00
	P7	20.666±0.577	14.333±0.577	10.666±0.577	6.666±0.577	0.00
	P8	18.333±0.577	14.000±1.000	11.666±1.527	0.00	0.00
	P9	22.666±0.577	18.666±1.577	14.333±1.000	12.666±0.577	0.00

SD: Standard deviation, *P. granatum: Punica granatum, P. aeruginosa: Pseudomonas aeruginosa*

active substances which may be affected, insoluble, or attributed by the used solvent [5].

Water extract of pomegranate even when it less active than alcoholic solvent still gives obvious 22 mm inhibition zone diameter that means the extract seems to be thermostable because of using hot-water in plant metabolite extraction. These results were consistent with Al-Zoreky who found the inhibition zone diameters were ranged from 13 to 17 mm [7], whereas, the other study [23] found that the inhibition zone diameter was lower (10-40 mm).

The water extract, in the present study, showed lower activity than other extracts that may be due to water extracted less phenolic contents where the phenolic groups are the active component against microbial growth [26]. The effect of the lowest concentration (50 mg/ml) of *P. granatum* of all extractions solvent were still higher than other plant extracts that may be referred to the *P. granatum* extract containing high amounts of active phenolic contents against microbial growt [27].

The antibacterial activity of onion (*A. cepa*) extract (Table 2) can be attributed to the presence of flavonoids and polyphenols which has been reported to have a broad spectrum of antibacterial activity [28]. Furthermore, the polyphenols of plants have been reported to have antibacterial activity [29]. In the present study, the onion ethanolic extract showed inhibition zone about 30 mm at 200 mg/ml concentration. These zones gradually reduced with decreasing of concentration until 50 mg/ml which showed no inhibition zone. While the methanolic extract showed high activity than ethanolic one, with inhibition zone was 29, 25, and 18 mm at concentration 150, 100, and 50 mg/ml, respectively. That means the methanol solvent is the best solvent for phenolic content extraction, which has antibacterial activity as reported in the study of Hendrich [29]. These results were in agreement with the previous studies [30] which reported the inhibition zone was 29, 26, 25, and 24 mm at concentrations 1000, 500, 200, and 100 mg/ml, respectively. Furthermore, it found the antibacterial activity against pseudomonad bacteria of the onion-water extract was 23, 18, 16, and 14 mm at concentrations 1000, 500, 200, and 100 mg/ml, respectively. While in the present study, we showed different results which were lower than ethanolic and methanolic extracts with 20 and 13 mm at 200 and 150 mg/ml concentration, respectively. However, they showed no inhibition zone at concentrations 100 and 50 mg/ml. That is because of water is more polarity than alcoholic solvent, so low level of phenolic and alkaloid compounds was extracted which disruption of the cell membrane [31-35].

M. asiatica leaves extract (Table 3) also shows most antimicrobial activity in the ethanolic extract than methanol and water extract with a range of inhibition zones 36, 30, 25, and 18 mm at concentrations 200, 150, 100, and 50 mg/ml, respectively. These results agreed with the findings of El-Taweil [5] but less activity (3.3 and 7.3 mm inhibition zone at 200 and 100 mg/ml concentration, respectively). The principle active components of peppermint *M. asiatica* are monoterpenes, mainly menthol, menthone, and their derivatives (e.g., isomenthone, neomenthol, acetyl menthol, and pulegone). These essential oils dilate blood vessels and inhibit bacteria. Especially, menthol has a broad-spectrum antibacterial activity [36].

Also, the methanolic peppermint extract was higher antibacterial activity against test organisms than water extract with 30 mm inhibition zone at 200 mg/ml concentration and 19 mm at 50 mg/ml. The results of the present study were higher than other study [5] which used chloroform as extraction solvent instead methanol to solve peppermint that showed 9.2 mm inhibition zone at 150 mg/ml and 14.2 mm at 100 mg/ml of chloroform or methanol increased the suspended higher compounds.

Water extract of *M. asiatica* also has moderately active against *P. aeruginosa* where alcoholic solvents are more effective, and results show an inhibition zone range 21, 19, 13, and 9 mm at concentrations 200, 150, 100, and 50 mg/ml, respectively, which was the lowest effect on *P. aeruginosa* than other plants.

Table 2: Antibacterial activity of *A. cepa* bulbs extract against multidrug-resistant *P. aeruginosa* isolated from burn wound

Type of extract	Isolate	Inhibition zone mm±SD				
		200 mg/ml	150 mg/ml	100 mg/ml	50 mg/ml	Control
EtOH	P1	30.000±1.000	21.333±0.577	16.000±1.000	0.00	0.00
	P2	33.666±1.577	25.666±1.577	20.000±1.000	0.00	0.00
	P3	29.666±0.577	25.666±0.577	14.666±0.577	10.000±1.000	0.00
	P4	33.000±1.000	24.000±1.000	20.666±0.577	10.000±1.000	0.00
	P5	38.666±1.577	34.000±1.000	30.000±1.000	21.666±0.577	0.00
	P6	32.000±1.000	18.666±0.577	16.666±0.577	10.000±1.000	0.00
	P7	30.000±1.000	21.333±0.527	16.666±0.577	0.00	0.00
	P8	30.000±1.000	24.000±1.000	16.000±0.577	0.00	0.00
	P9	31.000±1.000	21.333±0.527	14.666±0.577	0.00	0.00
MeOH	P1	32.000±1.000	29.000±1.000	25.000±1.000	18.333±0.527	0.00
	P2	32.666±1.577	30.666±1.577	22.000±1.000	15.000±1.000	0.00
	P3	33.000±1.000	29.000±1.000	22.000±1.000	14.000±1.000	0.00
	P4	32.333±1.527	30.000±1.000	22.333±1.577	0.00	0.00
	P5	35.666±1.577	30.666±1.577	25.000±1.000	15.000±1.000	0.00
	P6	32.666±1.577	29.000±1.000	25.333±1.527	16.000±1.000	0.00
	P7	32.000±1.000	25.000±1.000	25.000±1.000	18.666±0.577	0.00
	P8	30.000±1.000	27.000±1.000	21.333±0.527	18.666±0.577	0.00
	P9	32.000±1.000	29.000±1.000	25.001±1.000	18.666±0.527	0.00
DW	P1	20.000±1.000	13.666±0.577	0.00	0.00	0.00
	P2	19.000±1.000	15.000±1.000	0.00	0.00	0.00
	P3	24.000±1.000	16.666±0.577	6.666±0.577	2.666±0.527	0.00
	P4	24.333±1.527	16.000±1.000	10.666±1.577	0.00	0.00
	P5	34.000±1.000	29.333±1.527	19.666±1.577	10.333±0.527	0.00
	P6	26.000±1.000	20.333±0.527	9.666±0.577	0.00	0.00
	P7	20.333±0.527	13.666±0.577	6.66±0.577	0.00	0.00
	P8	18.666±0.577	14.666±0.577	0.00	0.00	0.00
	P9	20.000±1.000	13.666±0.577	0.00	0.00	0.00

SD: Standard deviation, *P. aeruginosa: Pseudomonas aeruginosa, A. cepa: Allium cepa*

Table 3: Antibacterial activity of *M. asiatica* extract against multidrug-resistant *P. aeruginosa* isolated from burn wound

Types of extract	Isolate	Inhibition zone mm±SD				
		200 mg/ml	150 mg/ml	100 mg/ml	50 mg/ml	Control
EtOH	P1	36.333±1.577	30.333±1.577	25.000±1.000	18.666±0.577	0.00
	P2	37.000±1.000	33.666±1.577	26.000±1.000	15.000±1.000	0.00
	P3	30.000±1.000	18.666±1.577	19.000±1.000	10.666±1.577	0.00
	P4	39.000±1.000	35.333±1.527	30.666±1.577	29.000±1.000	0.00
	P5	30.666±1.577	30.666±1.577	25.000±1.000	13.333±0.577	0.00
	P6	36.666±1.577	35.666±1.577	31.666±0.577	26.333±0.577	0.00
	P7	36.666±1.577	30.333±1.577	25.333±1.577	17.000±1.000	0.00
	P8	36.000±1.000	30.333±0.577	25.000±1.000	18.333±0.577	0.00
	P9	35.333±1.527	30.000±1.000	25.666±0.577	18.000±1.000	0.00
MeOH	P1	30.000±1.000	27.000±1.000	20.666±1.577	19.666±1.577	0.00
	P2	30.666±1.577	25.000±1.000	19.333±0.527	15.666±0.577	0.00
	P3	35.666±1.577	30.000±1.000	25.000±1.000	15.666±1.527	0.00
	P4	37.000±1.000	31.000±1.000	28.666±0.527	25.000±1.000	0.00
	P5	28.666±0.577	27.000±1.000	20.00±1.000	17.333±0.577	0.00
	P6	31.333±1.577	25.000±1.000	23.333±0.577	19.333±0.577	0.00
	P7	30.000±1.000	27.333±0.577	19.666±0.577	19.000±1.000	0.00
	P8	30.666±1.577	27.333±0.577	20.000±1.000	19.000±1.000	0.00
	P9	30.000±1.000	27.000±1.000	18.666±0.577	19.333±0.527	0.00
DW	P1	21.666±0.577	19.000±1.000	13.666±0.577	9.000±1.000	0.00
	P2	25.000±1.000	18.666±0.577	16.333±0.527	0.00	0.00
	P3	22.333±0.577	20.666±0.577	15.333±0.527	0.00	0.00
	P4	27.000±1.000	20.666±1.577	10.666±0.577	9.666±0.577	0.00
	P5	19.666±0.577	18.666±0.577	14.333±0.577	0.00	0.00
	P6	20.000±1.000	15.666±0.577	9.666±0.577	5.666±0.577	0.00
	P7	21.333±0.577	19.666±0.577	11.666±0.527	9.333±0.577	0.00
	P8	23.000±1.000	19.666±0.577	13.666±0.577	9.666±0.577	0.00
	P9	24.000±1.000	18.666±0.577	13.666±0.577	9.000±1.000	0.00

M. asiatica: Mentha asiatica, SD: Standard deviation, *P. aeruginosa: Pseudomonas aeruginosa*

The result of the study revealed that all solvents, actively effect against the *P. aeruginosa* that are a common cause of infections. *M. asiatica* shows significant activity as because of their leaves contain many potent compounds such as menthol, menthone, menthyl acetate, menthofuran, and limnone [37].

CONCLUSION

From the study, all three studied plants had antibacterial activity against multidrug-resistant *P. aeruginosa* isolated from burn wound. *P. granatum* showed the highest antibacterial activity, followed by

A. cepa, and finally, *M. asiatica*. It is a recommendation that natural products can use as therapeutic agents will probably not elicit resistance in bacteria. More research must continue to isolate and purify the active components and applied in experimental animal models.

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