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In-vitro Antibacterial Activities of Selected Traditional Plants

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The present research work was focused on the antibacterial activity of medicinal plants (*Aegle marmelos*, *Citrus aurantifolia*, *Piper sarmentosum*, *Sesbania grandiflora*, *Carthamus tinctorius*, *Piper longum*, *Morus alba*, Green tea and Oolong tea). Extracts were examined using water, methanol and ethanol as solvents and tested against six human pathogens (*Staphylococcus aureus* DMST4212, *Bacillus cereus* DMST5040, *Staphylococcus epidermidis* DMST518, *Escherichia coli* ATCC25922, Methicillin-resistant *Staphylococcus aureus* (MRSA) DMST20625 and *Pseudomonas aeruginosa* DMST4739) using the agar well diffusion method. The five day methanol extracts of green tea showed significant activity against MRSA and *S. aureus* of around 28.3 mm. The five day methanol extracts of *A. marmelos* exhibited the highest antibacterial activity against *S. epidermidis* (29.7 mm) and lowest in *E. coli* (no inhibition zone). The drop plate technique found that three day ethanol and three day methanol extracts of *P. longum*; water, three day and five day methanol and three day and five day ethanol extracts of green tea and oolong tea; three day and five day methanol and three day and five day ethanol extracts of *C. aurantifolia;* and three day ethanol extract of *S. grandiflora* had no growth for all six human pathogens. The results demonstrated that this plant has strong antibacterial potential against all tested bacteria.

Keywords: Herb; Medicinal plant; Antibacterial; Extract.

Worldwide, significant economic value can be derived from medicinal plants. One of the important sources of control products for bacteria and fungi are natural plant products, and around 30% of the drugs used today come from natural products. Over time, plants as food or as products, such as extracts and powders, have had varying degrees of success when being used to treat and stop diseases. There are many benefits to medicines that comes from plants, such as being cheaper, non-toxic, no side effects and easily available.[1] Due to the level of antibiotic resistance that has been identified in medicinally important bacteria, it is very important to provide a constant stream of new and effective agents.[2]

Higher plants contain antimicrobial substances that can be a source of initial ideas for novel drug compounds that can improve human health. For thousands of years humans have used nature as a source of medicinal agents and a significant amount of drugs used currently have come from natural sources, which were initially used in traditional medicine. At present, about 80% of the world's population still relies on traditional medicine as their main source of health care. The World Health Organization has indicated that

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medicinal plants could be the best source from which to obtain a range of drugs. This means that these plants should be studied to ensure that their properties, safety and efficacy are well known.[3]

Fernandez et al.[4] reported that antimicrobial effects against MRSA were found in an extract of *P. sarmentosum*. While an ethanol extract of green tea showed antimicrobial properties against five pathogens (*Streptococcus mutans*, *S. sobrinus*, *Listeria monocytogenes*, *Shigella flexneri* and *Salmonella enterica*).[5] In addition, a further five pathogens (*S. epidermidis*, *Micrococcus luteus*, *Brevibacterium linens*, *Ps. fluorescens* and *B. subtilis*) were also affected by another green tea extract.[6]

There is an increasing range of bacteria, parasites, viruses and fungi that have resistance to commonly used treatments, which means that their treatment could be affected. Therefore, it is important to develop a range of compounds that can be turned into new medicines with antimicrobial properties.[7] This study will determine the antimicrobial properties of a range of medicinal plants that are available in Thailand (*Aegle marmelos*, *Citrus aurantifolia*, *Piper sarmentosum*, *Sesbania grandiflora*, *Carthamus tinctorius*, *Piper longum*, *Morus alba*, green tea and oolong tea).

MATERIALS AND METHODS

Plant materials and extraction

There were nine plants in this study with different parts sampled from different plants: the leaves of *P. sarmentosum, M. alba*, green tea and oolong tea; the fruits of *A. marmelos*, *C. aurantifolia* and *P. longum*; and the flowers of *S. grandiflora* and *C. tinctorius*. The purchases of the plants were made in May 2016 from markets in the Northeast of Thailand. The reference plant specimens were preserved at the Museum of Reference Plants at Mahasarakham University. Each sample was dried, powdered and 200 g was added to 1 L of methanol and ethanol for three days and five days, respectively, and then boiled for 10 min at 100 °C, after which it was filtered with Whatman No. 1 filter paper. Then the crude extract was obtained via concentration at 60 °C under reduced pressure in a rotary evaporator and kept at -20 °C until needed.[8]

Antibacterial activity assays

The following strains were obtained from the Public Health Ministry, Bangkok and used to determine the antibacterial activity: *S. aureus* DMST4212, *B. cereus* DMST5040, *S. epidermidis* DMST518, *E. coli* ATCC25922, Methicillin-resistant *S. aureus* (MRSA) DMST20625 and *Ps. aeruginosa* DMST4739. After being cultured on nutrient agar, bacterial suspensions were made in 0.85% NaCl and set so their turbidities were the same as McFarland standard No. 0.5 (approximately 1.5×10^8 CFU/ml of bacteria), after which they were swabbed with sterile cotton wool onto Mueller Hinton agar. Agar wells were made with a No. 4 cork borer, and these were filled with 100 µl of the crude extract (negative control - 10% DMSO, water and positive control - gentamycin). Experiments were performed in triplicate.[9,10,11]

The macrodilution method was used to identify the minimal inhibitory concentration (MIC). The following Mueller Hinton broth:crude extract ratios were made 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, 1:1024, and 1:2048. From these, 0.9 ml was taken and added to 0.9 ml of the bacterial suspension (same turbidity as McFarland standard No. 0.5 and diluted 1:2000 in broth). After 24 h of incubation, the MIC was determined. The clear solutions from the MIC were then spread on nutrient agar plates to determine the minimal bactericidal concentration (MBC). The MBC was the dilution that after incubation for 24 h did not result in any growth.[10]

Drop plate technique

The Mueller Hinton agar (liquid ller Hinton agar culture agar 25 ml) was sterilized by autoclaving at a temperature of 45 ^{o} C, mixed with 2.5 ml medicinal plant extract concentrations of 500 µg/ml, then pour the culture media into a petri plate. Then, 10 µl of the bacterial suspension (same turbidity as McFarland standard No. 0.5, approximately 1.5×10^8 CFU/ml of bacteria) was dropped onto the designated quadrant of the petri plate. After the drops on the agar had dried, the petri plates were inverted and incubated at 37 ^{o} C for 18 h. Experiments were performed in triplicate.[12,13]

Statistical analysis

The significance of differences was determined by SPSS version 14.0 by analysis of variance (nonparametric test) with Krustal-wallis

H and differences with P values of < 0.05 was considered to be statistically significant.

RESULTS

Agar well diffusion method, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The results obtained in the present study revealed that the nine tested medicinal plant extracts possessed potential antibacterial activity against *S. aureus* DMST4212, *B. cereus* DMST5040, *S. epidermidis* DMST518, *E. coli* ATCC25922, MRSA DMST20625 and *Ps. aeruginosa* DMST4739 (Table 1). When tested by the agar well diffusion method, the five day methanol extracts of green tea exhibit the highest antibacterial activity against *S. aureus* of around 28.3 mm and the three day methanol extracts of green tea exhibited the secondary antibacterial activity against *S. aureus* of around 27.7 mm, but the five and three day methanol extracts of green tea did not show statistically significant differences and exhibited the highest antibacterial activity against *S. aureus* (Table 1). The five day methanol extracts of *A. marmelos* exhibit the highest antibacterial activity against *S. epidermidis* (29.7 mm) and lowest against *E. coli* (no inhibition zone) (Table 1).

The five day methanol extracts of green tea exhibited the highest antibacterial activity against MRSA of around 28.3 mm, the secondary, the five day methanol extracts of oolong tea, three day methanol extracts of green tea and water extract of green tea exhibited antibacterial activity against *S. aureus* of around 27.0, 26.7 and 25.7 mm respectively, but the five and three day methanol extracts, water extract of green tea and five day methanol extracts of oolong tea showed no statistically significant differences and exhibited the highest antibacterial activity against MRSA. The five day ethanol extracts of green tea and five day methanol extracts of oolong tea showed the significantly highest antibacterial activity against *B. cereus* of around 25.3 mm (Table 1). The three day and five day methanol extracts of *C. aurantifolia* exhibited the highest antibacterial activity against *Ps. aeruginosa* (18.3mm) and 14.7-15.7 mm with similar zones of inhibition as observed in *E. coli*. The five day green tea ethanol and five day oolong tea methanol extracts showed similar zones of inhibition against all the tested bacteria except *B. cereus*, which showed the highest activity (25.3 mm) (Fig. 1). The medicinal plant water extracts (*A. marmelos*, *P. sarmentosum*, *C. tinctorius*, *P. longum* and *M. alba*) did not have inhibition zones.

Table 2 shows the MIC and MBC of nine medicinal plant extracts against the six tested bacteria. The MIC of the green tea five day methanol extract was 1:64 (1.56 µg/ml) and the MBC was 1:2 (50 µg/ml), and it did not inhibit *S. epidermidis*. The oolong tea three day methanol extract MIC was 1:32 (3.125 µg/ml) and MBC was 1:2 (50 µg/ml), and it did not inhibit *S. aureus*.

The oolong tea water extract and five day methanol extract showed the least MIC values of 0.78 µg/ml against MRSA and *S. epidermidis,* respectively. The MICs of the water extract of oolong tea and green tea extracts were 1.56 µg/ml against *S. aureus*; three and five day green tea methanol extracts were 3.125 µg/ml against *B. cereus*; the *P. longum* three and five day and the *C. aurantifolia* five day methanol extracts were 12.5 µg/ml against *Ps. aeruginosa*; and the green tea three day ethanol extract and *P. sarmentosum* three day methanol extract were 12.5 µg/ml against *E. coli* (Table 2).

Drop plate technique

The results obtained in the present study relieved that the nine tested medicinal plant extracts possessed potential antibacterial activity against *S. aureus* DMST4212, *B. cereus* DMST5040, *S. epidermidis* DMST518, *E. coli* ATCC25922, MRSA DMST20625 and *Ps. aeruginosa* DMST4739 (Table3). When tested by the drop plate technique, the three day ethanol extract and three day methanol extract of *P. longum*; water extract, three day and five day ethanol extracts and three day and five day methanol extracts of green tea and oolong tea; three day and five day ethanol extracts and three day and five day methanol extracts of *C. aurantifolia;* and three day ethanol extract of *S. grandiflora* did not affect the growth of the bacteria (Table 3 and Fig. 2). The three day and five day ethanol extracts of *A. marmelos* and *M. alba* did not inhibit the growth of the six bacteria species (Table 3 and Fig. 2). The five day ethanol extract of *P. longum* and five day methanol extract of *P. sarmentosum* can inhibit

Table 1. Antibacterial activity of some medicinal plant extracts (concentrations 500 µg/ml) against bacterial species tested by agar well diffusion method

Microorganisms	Extracts	Inhibition zone diameter (mm) on Petri plates of medicinal plant								
		P. longum	*A. marmelos*	*M. alba*	Green tea	Oolong tea	*C. tinctorius*	*C. auran tifolia*	*P. sarmen tosum*	*S. gran diflora*
S. aureus	Water	-	-	-	21.67±1.53^{c}	18.3±0.58^{de}	-	-	-	19.7±1.15^{cd}
	3 day ethanol	-	16.3±0.58^{ef}	-	22.7±2.31^{bc}	23.7±0.58^{bc}	-	16.7±1.95^{ef}	17.3±1.15^{ef}	16±1^{f}
	5 day ethanol	-	16±1^{f}	-	24.3±1.15^{b}	18.3±0.58^{de}	13.3±0.58^{g}	17±1.73^{ef}	13±1^{g}	15±1^{fg}
	3 day methanol	-	14.7±0.58^{fg}	-	27.7±0.58^{a}	25.7±1.15^{ab}	13.3±0.58^{g}	19±1de	16.7±0.58^{ef}	15±1^{fg}
	5 day methanol	14.33±0.58^{g}	23±1^{bc}	-	28.3±0.58^{a}	26±1^{a}	14.7±0.58^{fg}	19±1de	16±1^{f}	14±1^{g}
	Positive control	19.7±2.57^{cd}	17.9±1.85^{de}	20.3±1^{cd}	22.5±5.41^{bc}	20.5±0.40^{cd}	19.9±1.25^{cd}	20.8±0.81^{cd}	21.8±1.08^{bc}	19.7±1.82^{cd}
S. epidermidis	Water	-	-	-	22.7±1.5^{bcd}	18±1de	-	-	-	-
	3 day ethanol	-	20.7±0.6^{bcde}	-	27±1^{ab}	23±2^{bcd}	-	20±1^{bcde}	17.7±0.6^{de}	17.7±2.3^{de}
	5 day ethanol	-	18.7±0.6^{cde}	-	27.7±1.2^{ab}	18.7±1.5^{cde}	14.3±0.6^{e}	22±2^{bcde}	13±1^{ef}	16.3±1.2^{de}
	3 day methanol	-	18.7±1.2^{cde}	-	26.3±0.6^{ab}	25.3±0.6^{abc}	11.7±1.2^{f}	23.7±0.6^{bc}	19.3±1.2^{cde}	17±1de
	5 day methanol	-	29.7±1.2^{a}	-	26.7±1.5^{ab}	25.3±1.5^{abc}	14.3±1.2^{e}	23.7±1.2^{bc}	19.7±0.6^{cde}	16.7±0.6^{de}
	Positive control	21.6±2.6^{bcde}	21.4±3.4^{bcde}	22.3±1.7^{bcd}	22±2^{bcde}	23.3±1.8^{bc}	22.5±0.7^{bcd}	24.2±1.9^{bc}	23.3±3.5^{bc}	23.1±1.8^{bc}
MRSA	Water	-	-	-	25.7±0.6^{a}	22±1^{bc}	-	-	-	-
	3 day ethanol	-	-	-	24.3±1.5^{b}	22.7±0.6^{b}	-	19±1de	18±2^{e}	16.3±0.6^{f}
	5 day ethanol	-	-	-	24.7±1.5^{b}	18.3±0.6^{e}	15±1^{f}	21.7±1.5^{c}	15±2^{f}	15.3±0.6^{f}
	3 day methanol	-	16±1^{f}	-	26.7±0.6^{a}	25±1^{b}	11.7±0.6^{f}	20.3±0.6^{de}	21±1.73^{cd}	-
	5 day methanol	15.3±0.6^{f}	27±1^{a}	-	28.3±0.6^{a}	27±1^{a}	13.7±0.6^{f}	20.3±0.6^{de}	19.7±2.3^{de}	-
	Positive control	-	-	-	-	-	-	-	-	-
B. cereus	Water	-	-	-	21.7±1.5^{bcde}	20.7±0.6^{bcde}	-	14.3±1.2^{ef}	-	-
	3 day ethanol	17.3±0.6^{def}	16±1^{ef}	14.3±1.5^{ef}	22.3±1.5^{abcde}	24.3±1.2^{abcd}	13±1^{f}	17.7±0.6^{de}	19.3±1.2^{bcde}	17.3±0.6^{def}
	5 day ethanol	17.7±0.6^{de}	16.7±0.6^{ef}	13.3±0.6^{f}	25.3±0.6^{ab}	20.3±1.5^{bcde}	17.7±0.6^{de}	19.7±0.6^{bcde}	17.3±0.6^{def}	17.7±0.6^{de}
	3 day methanol	14.3±0.6^{ef}	16.7±0.6^{ef}	-	24.3±0.6^{abcd}	23.7±0.6^{abcd}	-	21±1^{bcde}	18.7±0.6^{cde}	17.7±0.6^{de}
	5 day methanol	19±1^{cde}	19.7±0.6^{bcde}	-	22.7±0.6^{abcde}	25.3±0.6^{ab}	-	20.3±0.6^{bcde}	18.3±1.5^{cde}	17.7±0.6^{de}
	Positive control	20.7±0.7^{bcde}	20.1±1.6^{bcde}	20.3±0.7^{bcde}	19.7±0.9^{bcde}	19.9±1.3^{bcde}	19.7±1.8^{bcde}	20.2±0.7^{bcde}	19.6±2.5^{bcde}	19.8±0.2^{bcde}
E. coli	Water	-	-	-	-	-	-	-	-	-
	3 day ethanol	-	-	-	12.7±0.6^{e}	-	-	-	14.3±0.6^{c}	-
	5 day ethanol	-	-	-	-	-	13.7±1.2^{d}	-	-	-
	3 day methanol	-	-	-	-	-	-	14.7±0.6^{c}	12.7±0.6^{e}	-
	5 day methanol	-	-	-	-	-	-	15.7±0.6^{b}	13±1.5^{e}	-
	Positive control	18.9±1.2^{a}	19±0.9^{a}	17.8±0.9^{a}	18.2±0.2^{a}	19.5±2.4^{a}	18±0.9^{a}	18.6±0.8^{a}	16.9±1.1^{a}	18.2±1.6^{a}

Ps. aeruginosa	Water	-	-	-	-	-	-	-	-	-	-
	3 day ethanol	-	-	-	-	-	-	15.7 ± 1.2^{ab}	13.3 ± 0.6^{e}	14.7 ± 0.6^{bcd}	
	5 day ethanol	-	-	-	-	-	12.7 ± 0.6^{e}	15.3 ± 1.5^{ab}	-	14.7 ± 0.6^{bcd}	
	3 day methanol	13.7 ± 0.6^{de}	-	-	-	11.7 ± 0.6^{e}	-	18.3 ± 0.6^{a}	15 ± 2^{bc}	-	
	5 day methanol	17.3 ± 0.6^{a}	15.3 ± 1.2^{ab}	-	-	10 ± 1^{f}	-	18.3 ± 0.6^{a}	12.3 ± 0.6^{e}	-	
	Positive control	14.9 ± 0.8^{bc}	13.6 ± 1.5^{e}	12.9 ± 2.8^{e}	16.2 ± 1.3^{ab}	13.8 ± 1^{de}	16.9 ± 1.7^{ab}	15.8 ± 1.5^{ab}	15.1 ± 0.7^{bc}	14.4 ± 1.2^{bcde}	

Remark: Letters "a, b, c, d, e, f, g" show that values in same column are not statistically significantly different at 0.05 level.
Values are mean inhibition zone (mm) ± S.D of three replicates.
- = No zone

the growth of gram positive bacteria, but did not inhibit the growth of gram negative bacteria (Table 3). The water extracts of *P. longum*, *A. marmelos*, *C. tinctorius*, *P. sarmentosum* and *M. alba* as well as three day and five day methanol extracts of *M. alba* did not result in any inhibition zones in any of the bacteria (Table 3).

DISCUSSION

In this study, there were nine plants with different parts sampled from different plants: the leaves of *P. sarmentosum, M. alba*, green tea and oolong tea; the fruits of *A. marmelos*, *C. aurantifolia* and *P. longum*: and the flowers of *S. grandiflora* and *C. tinctorius*. The medicinal plant extracts have the ability to inhibit the growth of microorganisms. In this study the medicinal plant extracts have the ability to inhibit the growth of gram positive bacteria and gram negative bacteria. The efficiency varies depending on the type of medicinal plant and species of bacteria. The medicinal plant extracts can inhibit the gram positive bacteria better than the gram negative bacteria. This is consistent with Parekh et al.[14] where the plant extracts were more active against gram positive bacteria than against gram negative bacteria. This is because the gram positive bacterial cell wall consists of the main substance, peptidoglycan. While the gram negative bacterial cell wall structure is more complex, mainly composed of 80% outer membrane and 20% peptidoglycan.

The present study relieved that the nine tested medicinal plant extracts possessed potential antibacterial activity against *S. aureus* DMST4212, *B. cereus* DMST5040, *S. epidermidis* DMST518, *E. coli* ATCC25922, MRSA DMST20625 and *Ps. aeruginosa* DMST4739. When tested by the agar well diffusion method, the five day methanol extracts of green tea showed significant activity against MRSA and *S. aureus* of around 28.3 mm (Table1) and MIC values of 3.12 and 25 µg/ml, respectively, and BMC values of more than 50 µg/ml (Table2). The five day methanol extracts of *A. marmelos* exhibited the highest antibacterial activity against *S. epidermidis* (29.7 mm) with MIC values of 25 µg/ml and BMC values of more than 50 µg/ml and lowest in *E. coli* (no inhibition zone). This contrasts with the research of Prasannabalaji et al.[15] where the methanol extract (0.4 g/ml) of *A.*

Table 2. MIC (µg/ml) and MBC performance of different medicinal plants extracts against pathogenic organisms.

Medicinal plants	Extracts	Bacteria											
		Sa		*Se*		MRSA		*Bc*		*Ec*		*Ps*	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
A. marmelos	Water	-	-	-	-	-	-	-	-	-	-	-	-
	3 day Ethanol	12.5	>50	25	>50	-	-	12.5	>50	-	-	-	-
	5 day Ethanol	12.5	>50	25	>50	-	-	12.5	>50	-	-	-	-
	3 day Methanol	12.5	>50	25	>50	25	>50	12.5	>50	-	-	-	-
	5day Methanol	6.25	>50	25	>50	12.5	>50	6.25	>50	-	-	25	>50
C. aurantifolia	Water	-	-	-	-	-	-	-	-	-	-	-	-
	3 day Ethanol	25	>50	12.5	>50	25	>50	25	>50	-	-	25	>50
	5 day Ethanol	12.5	>50	12.5	>50	12.5	>50	12.5	>50	-	-	25	>50
	3 day Methanol	12.5	>50	25	>50	12.5	>50	12.5	>50	25	>50	25	>50
	5 day Methanol	6.25	>50	12.5	>50	6.25	>50	6.25	>50	25	>50	12.5	>50
C. tinctorius	Water	-	-	-	-	-	-	-	-	-	-	-	-
	3 day Ethanol	-	-	-	-	-	-	25	>50	-	-	-	-
	5 day Ethanol	12.5	>50	12.5	>50	25	>50	12.5	>50	25	>50	25	>50
	3 day Methanol	12.5	>50	12.5	>50	12.5	>50	-	-	-	-	-	-
	5 day Methanol	6.25	>50	6.25	>50	12.5	>50	-	-	-	-	-	-
M. alba	Water	-	-	-	-	-	-	-	-	-	-	-	-
	3 day Ethanol	-	-	-	-	-	-	25	>50	-	-	-	-
	5 day Ethanol	-	-	-	-	-	-	25	>50	-	-	-	-
	3 day Methanol	-	-	-	-	-	-	-	-	-	-	-	-
	5 day Methanol	-	-	-	-	-	-	-	-	-	-	-	-
P. longum	Water	-	-	-	-	-	-	-	-	-	-	-	-
	3 day Ethanol	-	-	-	-	-	-	25	>50	-	-	-	-
	5 day Ethanol	-	-	-	-	-	-	12.5	>50	-	-	-	-
	3 day Methanol	-	-	-	-	-	-	25	>50	-	-	12.5	>50
	5 day Methanol	25	>50	-	-	25	>50	25	>50	-	-	12.5	>50
P. sarmentosum	Water	-	-	-	-	-	-	-	-	-	-	-	-
	3 day Ethanol	12.5	>50	25	>50	25	>50	25	>50	25	>50	25	>50
	5 day Ethanol	12.5	>50	12.5	>50	25	>50	25	>50	-	-	-	-
	3 day Methanol	12.5	>50	25	>50	25	>50	25	>50	12.5	>50	25	>50
	5 day Methanol	6.25	>50	12.5	>50	12.5	>50	25	>50	25	>50	25	>50
S. grandiflora	Water	6.25	>50	-	-	-	-	-	-	-	-	-	-

green tea	3 day Ethanol	6.25	>50	25	>50	12.5	>50	12.5	>50	-	-	25	>50
	5 day Ethanol	6.25	>50	25	>50	12.5	>50	6.25	>50	-	-	25	>50
	3 day Methanol	6.25	>50	12.5	>50	-	-	12.5	>50	-	-	-	-
	5 day Methanol	6.25	>50	25	>50	-	-	12.5	>50	-	-	-	-
	Water	1.56	>50	1.56	>50	1.56	>50	6.25	>50	-	-	-	-
oolong tea	3 day Ethanol	12.5	>50	12.5	>50	6.25	>50	6.25	>50	12.5	>50	-	-
	5 day Ethanol	6.25	>50	6.25	>50	12.5	>50	12.5	>50	-	-	-	-
	3 day Methanol	3.125	>50	1.56	>50	3.125	>50	3.125	>50	-	-	-	-
	5 day Methanol	3.125	>50	1.56	25	3.125	>50	3.125	>50	-	-	-	-
	Water	1.56	>50	3.125	>50	0.78	>50	12.5	>50	-	-	-	-
	3 day Ethanol	12.5	>50	12.5	>50	12.5	>50	12.5	>50	-	-	-	-
	5 day Ethanol	6.25	>50	6.25	>50	6.25	>50	6.25	>50	-	-	-	-
	3 day Methanol	3.125	25	1.56	>50	3.125	>50	6.25	>50	-	-	12.5	>50
	5 day Methanol	3.125	>50	0.78	>50	3.125	>50	6.25	>50	-	-	12.5	>50

- =not tested

marmelos showed significant inhibitory activity of 22.5 mm and MIC value of 0.156 mg/ml against *E. coli*.

In this study, the three day and five day methanol extracts of *C. aurantifolia* exhibited the highest antibacterial activity against *Ps. aeruginosa* (18.3mm) with MIC values of MIC 25 and 12.5 µg/ml, respectively, MBC values of more than 50 µg/ml and 14.7-15.7 mm zones of inhibition similar to those observed in *E. coli*. The study demonstrates that the three day and five day methanol extracts of *C. aurantifolia* exhibited antibacterial activity on *S. aureus*, *S. epidermidis*, MRSA, *B. cereus*, *Ps. aeruginosa* and *E. coli*. This is consistent with the research of Pathan et al.[16] whose study demonstrates that the hydroalcoholic extract of the *C. aurantifolia* leaf exhibited antibacterial activity on *Klebsiella pneumonia*, *Pseudomonas* sp. and *S. aureus*. This suggests good support to use this plant as a medicinal plant and as a base for the development of new drugs, such as phytomedicine. Preliminary phytochemical screening of the leaf extract of *C. aurantifolia* revealed the presence of carbohydrates, alkaloids, flavonoids, steroids and tannins. This is well known since tannins and saponins are important plant metabolites, which are mainly responsible for antimicrobial activity.[16,17]

In this study, the five day green tea ethanol and five day oolong tea methanol extracts showed almost similar zones of inhibition against all the tested bacteria, except *B. cereus*, which showed the highest activity (25.3 mm) (Figure 1), MIC values of 12.5 and 6.25 µg/ml, respectively, and MBC values of more than 50 µg/ml (Table2). This is consistent with Sharma et al.[6] who stated *Camellia sinensis* (tea) is known for its therapeutic properties (anti-inflammatory, anti-microbial, anti-tumour, anti-oxidative and anti-ageing). Although the antimicrobial properties of green tea have been studied, its role against bacterial strains related to skin infections and mechanism of action is not well understood. We focused on exploring the anti-microbial activity and the basic mechanism of the aqueous green tea leaf extract on the selected bacterial strains. *S. epidermidis, Micrococcus luteus, Brevibacterium linens*, *Ps. fluorescens* and *B. subtilis* were found to be sensitive to green tea extract via the disc diffusion assay (zone of inhibition $\geq$ 7 mm). The MIC was determined via a nitro blue tetrazolium (NBT) assay (0.156–0.313

Table 3. Antibacterial activity of some medicinal plant extracts against bacterial species tested by drop plate technique (10 µL approximately 1.5×10^8 CFU/ml of bacteria)

Medicinal plant	Extracts	Bacteria					
		Sa	*Se*	MRSA	*Bc*	*Ec*	*Ps*
A. marmelos	Water	n	n	n	n	n	n
	3 day Ethanol	+	+	+	+	+	+
	5 day Ethanol	+	+	+	+	+	+
	3 day Methanol	0	0	+	0	+	+
	5 day Methanol	+	+	+	0	+	+
C. aurantifolia	Water	+	+	+	0	+	+
	3 day Ethanol	0	0	0	0	0	0
	5 day Ethanol	0	0	0	0	0	0
	3 day Methanol	0	0	0	0	0	0
	5 day Methanol	0	0	0	0	0	0
C. tinctorius	Water	n	n	n	n	n	n
	3 day Ethanol	+	+	+	0	+	+
	5 day Ethanol	+	+	+	0	+	+
	3 day Methanol	0	+	+	+	+	0
	5 day Methanol	+	0	+	0	+	+
M. alba	Water	n	n	n	n	n	n
	3 day Ethanol	+	+	+	+	+	+
	5 day Ethanol	+	+	+	+	+	+
	3 day Methanol	n	n	n	n	n	n
	5 day Methanol	n	n	n	n	n	n
P. longum	Water	n	n	n	n	n	n
	3 day Ethanol	0	0	0	0	0	0
	5 day Ethanol	0	0	0	0	+	+
	3 day Methanol	0	0	0	0	0	0
	5 day Methanol	+	+	+	0	+	+
P. sarmentosum	Water	n	n	n	n	n	n
	3 day Ethanol	+	+	+	0	+	+
	5 day Ethanol	+	+	+	0	+	+
	3 day Methanol	0	0	0	0	+	+
	5 day Methanol	0	0	0	+	0	0
S. grandiflora	Water	+	0	+	0	+	0
	3 day Ethanol	0	0	0	0	0	0
	5 day Ethanol	0	0	+	0	+	+
	3 day Methanol	0	0	+	0	+	0
	5 day Methanol	+	+	+	0	+	+
green tea	Water	0	0	0	0	0	0
	3 day Ethanol	0	0	0	0	0	0
	5 day Ethanol	0	0	0	0	0	0
	3 day Methanol	0	0	0	0	0	0
	5 day Methanol	0	0	0	0	0	0
oolong tea	Water	0	0	0	0	0	0
	3 day Ethanol	0	0	0	0	0	0
	5 day Ethanol	0	0	0	0	0	0
	3 day Methanol	0	0	0	0	0	0
	5 day Methanol	0	0	0	0	0	0

n = not tested because medicinal plant extract had no inhibition zone for bacteria; + = growth of bacteria; and 0 = no growth of bacteria

mg/ml). Moreover, the aqueous extract was found to not be toxic to the Vero cell-line up to a concentration of 500 µg/ml. The study of Sasakia et al.[18] found that the activity originated from a monomeric polyphenolrich fraction, and it was stronger than that of pure polyphenols. Moreover, some combinations of monomeric polyphenols showed the highest level of antibacterial activity. These results suggest that the antibacterial activity of the oolong tea extract is caused by a synergistic effect of the monomeric polyphenols, which can easily bind to proteins.

In this study, the three day methanol extract of *P. sarmentosum* showed activity against MRSA, *S. epidermidis, S. aueus, B. cereus, Ps. aeruginosa* and *E. coli* for antimicrobial compounds. *P. sarmentosum* inhibited the growth of *S. epidermidis, S. aueus, B. cereus,*

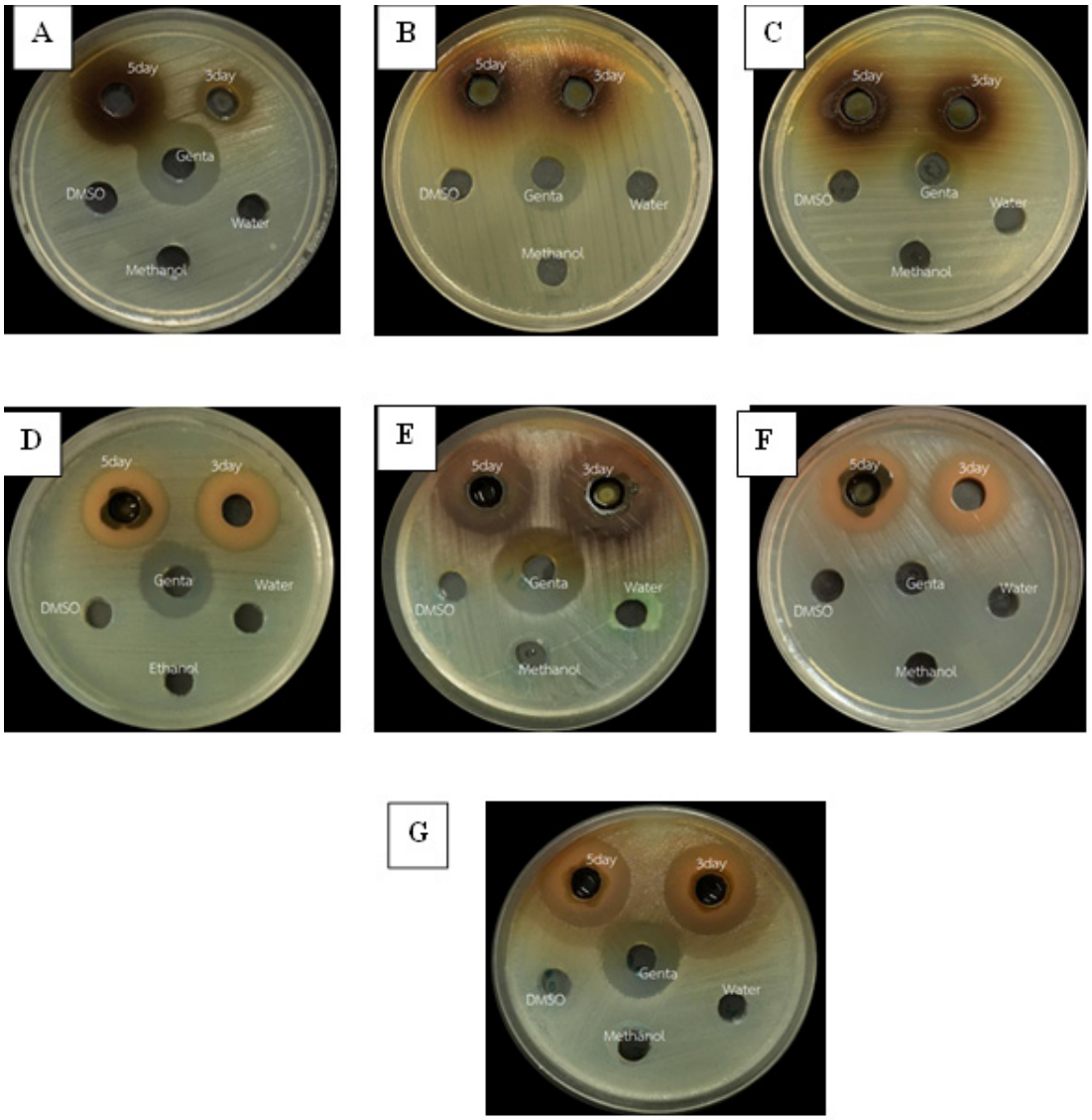

Fig. 1. Antibacterial activity of medicinal plant extracts with (A) methanol extract of *A. marmelos* against *S. epidermidis,* (B) methanol extract of *C. aurantifolia* against *E. coli,* (C) methanol extract of *C. aurantifolia* against *Ps. aeruginosa,* (D) methanol extract of green tea against *B. cereus,* (E) methanol extract of green tea against *S. aureus,* (F) methanol extract of green tea against MRSA and (G) methanol extract of oolong tea against *B. cereus*

Ps. aeruginosa and *E. coli* with inhibition zones of 21.0, 19.3, 18.7, 18.7, 15.0 and 12.7 mm, respectively. This is consistent with Fernandez et al.[4] where the crude methanolic extract of *P. sarmentosum* showed activity against MRSA, *E. coli*, *Vibrio cholera* and *S. pneumonia.* Plant based products have been effectively proven for their utilization as sources of antimicrobial compounds. *P. sarmentosum* inhibited the growth of MRSA with an inhibition zone of 10.0 mm.

Drop plate technique

This study relieved that the nine tested medicinal plant extracts possessed potential antibacterial activity against *S. aureus* DMST4212, *B. cereus* DMST5040, *S. epidermidis* DMST518, *E. coli* ATCC25922, MRSA DMST20625 and *Ps. aeruginosa* DMST4739 (Table 3). When tested by the drop plate technique, the three day ethanol extract and three day methanol extract of *P. longum*; water extract, three day and five day ethanol extracts and three day and five day methanol extracts of green tea and oolong tea; three day and five day ethanol extracts and three day and five day methanol extracts of *C. aurantifolia;* and three day ethanol extract of *S. grandiflora* inhibited the growth of the bacteria (Table 3 and Fig. 2). This is consistent with Kumar et al.[19] whose study showed the green tea extract had antimicrobial activity by the drop plate technique and found that the green tea extract can inhibit the growth of *Staphylococcus* spp., *Streptococci* spp., *Pseudomonas* spp., *Proteus* spp., *Bacillus* spp. and *E. coli*.

In this study, the three day and five day ethanol extracts of *A. marmelos* and *M. alba* did not inhibit the growth of six species of bacteria (Table 3 and Fig. 2). The five day ethanol extract of *P. longum* and five day methanol extract of *P. sarmentosum* can inhibit the growth of gram positive bacteria, but did not inhibit the growth of gram negative bacteria (Table 3). The water

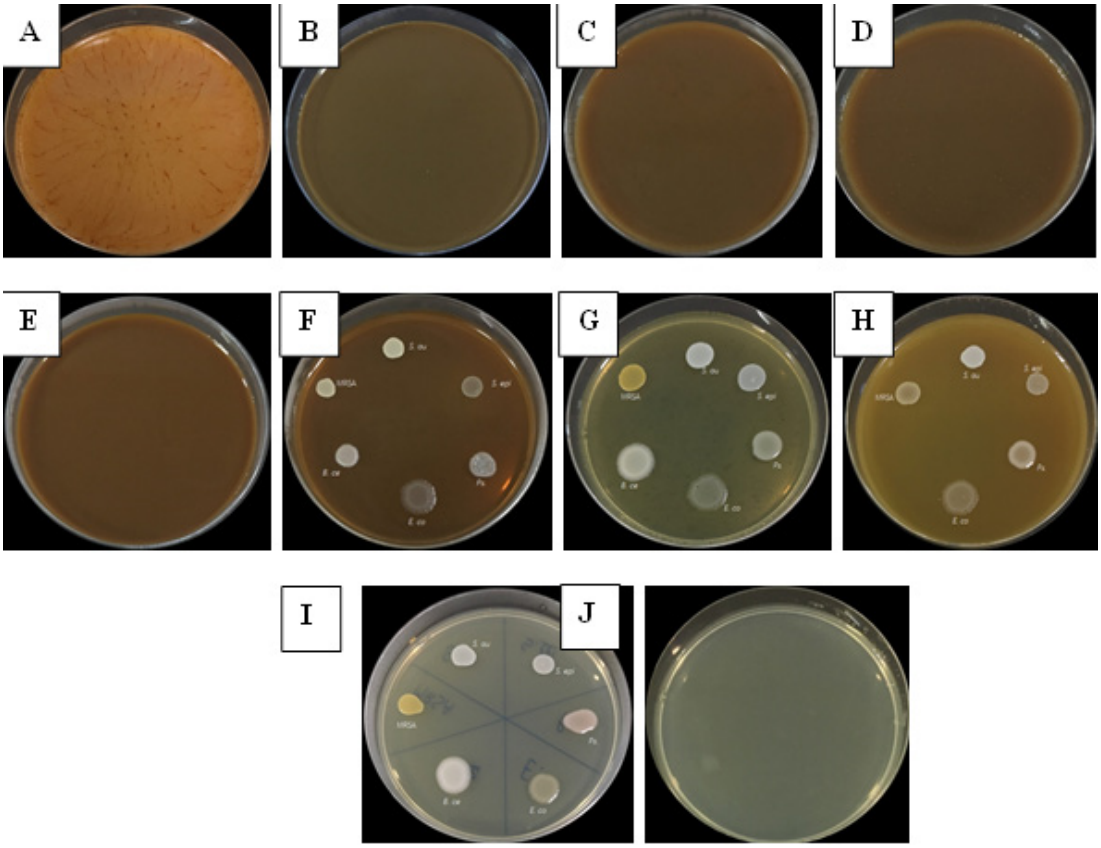

Fig. 2. Antibacterial activity of medicinal plant extracts by drop plate technique with three day ethanol extracts of *P. longum* (A), green tea (B), oolong tea (C), *C. aurantifolia* (D), *S. grandiflora* (E) and *M. alba* (G) and five day ethanol extracts of *A. marmelos* (F) and *C. tinctorius* (H) as well as negative control (I) and positive control (J)

extract of *P. longum*, *A. marmelos*, *C. tinctorius*, *P. sarmentosum* and *M. alba* as well as three day and five day methanol extracts of *M. alba* did not produce inhibition zones in the bacteria (Table 3).

Plants are important sources of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the antibacterial activity assay. Many reports are available on antibacterial properties. Some of these observations have helped in identifying the active principle responsible for such activities and in the development of drugs for therapeutic use in human beings. The results of the present investigation clearly indicates that the antibacterial and antifungal activity varies with the species of plant and plant material used. Thus, the study ascertains the value of plants used in ayurveda, which could be of considerable interest to the development of new drugs.[20]

This work has shown the antibacterial properties of nine medicinal plant extracts that exhibit the highest antibacterial activity against *S. aureus*, *S. epidermidis*, MRSA, *B. cereus*, *E. coli* and *Ps. aeruginosa*. This work has shown that it would be possible to develop antimicrobial properties, because the use of antibiotics is often toxic with many side effects and are expensive. This work has shown that medicinal plant extracts can be added to the culture medium as antibacterial inhibitors for unwanted bacteria, so the preferred bacteria can be grown on the culture medium. This can also be used as the basis for future use.

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