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ARTICLE

Antimicrobial Activity of *Thespesia populnea* (L.) Extracts Against Clinical Isolates of Methicillin-Resistant *Staphylococcus aureus* (MRSA)

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Abstract: *Thespesia populnea* L. (Family: Malvaceae) has been identified as a promising antimicrobial agent in the field of complementary medicine since time immemorial. To date, the antimicrobial activity of *T. populnea* against clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) has not been investigated. Here, our objective was to determine the antimicrobial activity of leaf extracts of *T. populnea* against clinical isolates of MRSA. Aqueous, ethanol and acetone leaf extracts of *T. populnea* were prepared separately following the ultrasound-assisted extraction method (40 kHz, 37 ^{0} C, 30 minutes). The prepared extracts were tested against clinical isolates of MRSA. Bacterial susceptibility was tested using the disk diffusion assay and broth microdilution method. The activity index (AI), relative percentage inhibition (RPI), minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were estimated. Vancomycin was used as the positive control. The ethanol and acetone leaf extracts of *T. populnea* showed significant dose-dependent inhibitory zones ($p < 0.05$) signifying potent antimicrobial activity against MRSA isolates, while the best activity was shown by the acetone extract. Inhibitory zones have not appeared for the aqueous extract. Acetone extract of *T. populnea* showed the highest AI and RPI at 0.765 and 58.67%, respectively, and the values were significantly lower ($p < 0.05$) than that of the vancomycin. The MIC and MBC of the acetone extract of *T. populnea* against MRSA were 0.75 mg/mL. In conclusion, the results revealed that the acetone leaf extract of *T. populnea* exerted antimicrobial activity against clinical isolates of MRSA and could be considered a leading herbal source for the development of new antimicrobial agents.

Keywords: Antimicrobial activity, Clinical isolates, Methicillin-resistant *Staphylococcus aureus, Thespesia populnea* leaves.

Introduction

Antimicrobial resistance has become one of the major public health burdens in the 21st century and threatens the effective prevention and treatment of a variety of infections[1]. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a prevalent antibiotic-resistant organism that could trigger a variety of organ-specific infections[2]. It is the most common on the skin and subcutaneous tissues, followed by invasive infections such as osteomyelitis, meningitis, pneumonia, lung abscess and empyema[3]. The main reason for the resistance

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of MRSA to antibiotics is the presence of the mecA gene sequence, which is identified to generate transpeptidase that lowers the affinity of the organism to bind to antibiotics[4]. In recent decades, the infection rate of MRSA has increased rapidly due to the evolution of bacteria and the abuse of well-developed antibiotics[5]. In fact, *S. aureus* has a remarkable ability to acquire antibiotic resistance, thereby creating implications for treatment options for this pathogen. Among the currently available antibiotics, vancomycin has long been considered the best medicine for the treatment of MRSA infections[6]. The resistant mechanism of vancomycin is due to the specific binding of vancomycin to the terminal D-alanyl-D-alanine moieties of the small peptides in the bacterial cell wall. This inhibits the elongation, cross-linking of bacterial cell wall peptidoglycans and, thereby, repressing cell wall synthesis leading to bacterial death[7]. Although several synthetic antimicrobial agents targeting MRSA have been used in the clinical setting, these drugs are expensive and possess several adverse reactions such as allergy, fever, hemorrhage, ulcers, liver and kidney dysfunctions[8]. Therefore, the development of new anti-MRSA agents that could be effectively used in the management of MRSA is important.

Thespesia populnea L. (Family: Malvaceae), is a shrub commonly distributed in coastal thickets in Sri Lanka, India and Thailand (Figure 1). The shrub is called 'Tulip tree' in English and 'Suriya' or 'Gansuriya' in the local setting. *T. populnea* has been identified as a promising agent in the arena of Sri Lankan traditional and complementary medicine since time immemorial[9]. The anti-inflammatory, antioxidant, anti-hyperglycemic, analgesic, antipyretic and hepatoprotective activities of different extracts, fractions and isolated compounds of *T. populnea* have been scientifically proven[10–13]. Furthermore, the antimicrobial activity of the leaves, bark, root and stem of *T. populnea* has been reported[14–17]. Importantly, several extracts of *T. populnea* leaves[18,19], root[20] and stem bark[21] have shown antibacterial activity against *S. aureus*. However, to date, the antimicrobial activity of *T. populnea* against clinical isolates of MRSA has not been investigated. Based on the disclosure stated above we hypothesized that the extracts of *T. populnea* leaves would exert antimicrobial activity against clinical isolates of MRSA. Herein, the present study was designed to determine the antimicrobial activity of aqueous, ethanol and acetone leaf extracts of *T. populnea*, to estimate MIC and MBC of the selected extracts against clinical isolates of MRSA and to compare its antimicrobial activity with the well-known antibiotic, vancomycin.

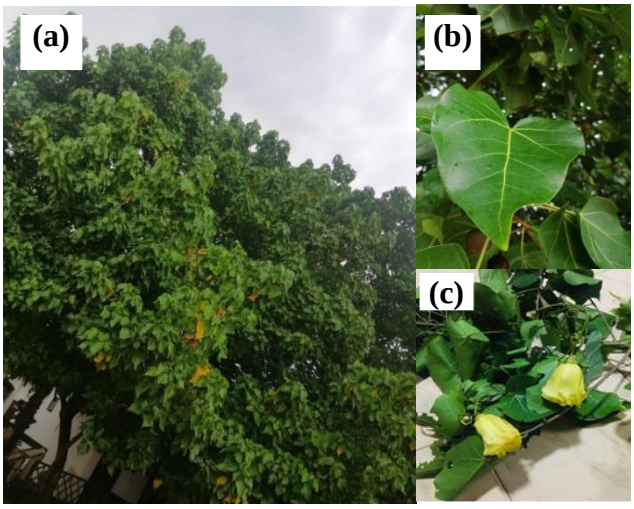

Figure 1. (a) *T. populnea* in natural habitat, (b) Bunch of *T. populnea* leaves, (c) Bunch of *T. populnea* leaves with flowers[9].

Materials and Methods

Vancomycin hydrochloride and all other chemicals used in the present study were of analytical grade (Sigma-Aldrich, USA). A sonicator (GFL 3005, Russia) was used in the extraction of dry leaves of *T. populnea* in the selected solvents, acetone, ethanol and water. Rotary evaporator (Buchi, B480, Germany) and freeze dryer (Telstor LyoBeta 4 ps) were used in

concentrating and freeze-drying the plant extracts, respectively.

The Leaves of *T. populnea* were collected from the Gampaha district, Western Province, Sri Lanka from January – April 2022. The botanical identity was confirmed by the descriptions given by Jayaweera[9] and authenticated at the National Herbarium of the Royal Botanical Gardens, Peradeniya, Sri Lanka. The voucher sample was preserved in the mini herbarium, Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Sri Lanka (MLS/2022/18). The leaves of *T. populnea* were macroscopically examined for foreign matter, such as contaminants, molds, insects and undesirable material. The collected leaves were washed 2–3 times thoroughly with running tap water to remove dust, foreign particles and other extraneous matter. The washed leaves were oven-dried at 40 ^{0} C until a constant weight was reached, ground to a coarse powder and stored in a sterilized airtight container at 4 ^{0} C until use.

Acetone, absolute ethanol and aqueous leaf extracts of *T. populnea* were prepared separately using an ultrasound-assisted extraction method (40 kHz, 37 ^{0} C, 30 minutes). The prepared acetone and ethanol extracts were filtered separately under gravity. The aqueous extract was filtered under vacuum pressure. The acetone and ethanol filtrates were dried separately using a rotary evaporator at 27 ^{0} C, while the aqueous extract was dried by freeze drying (–70 °C). The percentage yield of the dried extracts was determined relative to the weight of the dried leaves of *T. populnea*. Each prepared extract was stored in sterile airtight containers at 4 ^{0} C for future use. The dried masses were separately dissolved in a minimum amount of 20% dimethyl sulfoxide (DMSO), and the working stock solution was prepared by re-dissolving in 20% DMSO to yield the final concentration of 300 mg/mL of each extract.

Test Organisms

A total of ten MRSA isolates identified using morphological and biochemical tests[22] were collected from the Microbiology laboratory, Teaching Hospital, Karapitiya, Sri Lanka, and were subcultured onto blood agar plates. Identification of the isolates was confirmed again by Gram stain and biochemical tests; positive catalase test and positive slide and tube coagulase tests. Antibiotic susceptibility was tested using Clinical Laboratory Standard Institute (CLSI) methods[22]. Overnight-incubated colonies of MRSA were used to prepare 0.5 McFarland turbidity-compatible suspensions in normal saline and were inoculated onto Mueller–Hinton agar (MHA) plates. Vancomycin (30 μg/disc) was placed in each of the MHA plates, and the plates were incubated at 35 ^{0} C ± 2 for 24 hours. The zone diameter around each vancomycin disc was measured and classified as sensitive or resistant based on the CLSI. Zone diameters of 25 mm were considered sensitive, while those ≤ 24 mm were considered resistant. The confirmed colonies were used in the determination of the antimicrobial activity of *T. populnea* leaf extracts.

Antimicrobial Activity

The disk diffusion method was followed to determine the antimicrobial activity. The crude extracts (300 mg/mL) and 10- and 100-fold dilutions were prepared and dissolved in 20% DMSO. The bacteria suspensions were adjusted to a density of 1×10^8 CFU/mL inoculum using 0.5 McFarland turbidity standards. Each standardized inoculum was inoculated onto MHA plates. Blank filter paper discs (Whatman No. 1, diameter = 6 mm) were impregnated with 10 μL of each leaf extract of *T. populnea* and placed on inoculated MHA plates. Vancomycin (30 μg/disc) disk was used as a positive control against MRSA, and a 20% DMSO-soaked paper disk was used as a negative control. The prepared plates were incubated at 35 ^{0} C ± 2 for 24 hours. Any areas of clearance around the discs were considered susceptible to the substances contained in the disc. The diameters of the inhibitory zones were measured in millimeters using a vernier caliper.

The activity index (AI) of the aqueous, ethanol and acetone extracts of the leaves of *T. populnea* was calculated using the following formula[23]:

$$AI = \frac{(Inhibition\ Zone)_{sample}}{(Inhibition\ Zone)_{standard}}$$

Relative percentage inhibition (RPI) was calculated for each extract using the following formula [24]:

$$RPI = 100 \times \frac{x-y}{z-y}$$

where x = total inhibition area of the test extract, y = total inhibition area of the solvent, and z = total inhibition area of the standard. formula.

The broth microdilution method was used to determine the minimum inhibitory concentration (MIC) of the aqueous, ethanol and acetone extracts of the leaves of *T. populnea* against different isolates of MRSA[25]. Stock solutions of the aqueous, ethanol and acetone extracts were prepared with concentrations of 600, 60 and 12 mg/mL, respectively, using 20% DMSO as a solvent. Two-fold serial dilutions of each extract of *T. populnea* were separately prepared in 20% DMSO. The prepared plant extracts (150 µL) were added to each well of a 96-well microtiter plate. Bacterial inoculum was prepared in sterile normal saline, and the turbidity was adjusted to approximately 0.5 McFarland standards. Then 500 µL of the prepared bacterial inoculum was diluted in 4.50 mL of normal sterile saline to achieve 1:10 dilution, and 10 µL of it was added to each well, including positive and negative control wells. Vancomycin (2 µg/mL) and 20% DMSO were used as positive and negative controls, respectively. The microtiter plates were incubated at 35 ^{0} C ± 2 for 24 hours. The mean value of the lowest concentrations of each triplicate, which did not show turbidity, was considered as the MIC[26] of the particular plant extract against the tested organism. Approximately 5 µL from the cleared wells were inoculated onto blood agar plates and incubated at 35 ^{0} C ± 2 for 24 hours. The mean value of the lowest concentrations of triplicate, which did not show growth after incubation, was taken as the minimum bactericidal concentration (MBC) of the relevant plant extract against the tested organism[27].

Statistical Analysis

The disk diffusion method and estimation of AI, RPI, MIC and MBC were carried out in triplicate. The mean and the standard deviation of the triplicate measurements were calculated, and data were analyzed using one-way ANOVA followed by Tukey to compare the mean between groups. Mean values between the ethanol and acetone extracts at the same concentration were compared using the independent sample t-test. $p < 0.05$ was considered statistically significant. All analyses were performed using SPSS 25.0.

Results

The findings of the present study spotlighted the antimicrobial activity of aqueous, ethanol and acetone leaf extracts of *T. populnea* against MRSA isolates. Different percentage yields obtained for the aqueous, ethanol and acetone extracts of *T. populnea* leaves are summarized in Table 1.

Table 1. Dry weight and percentage yield of different leaf extracts of *T. populnea*.

Dry weight of powdered material of *T. populnea* leaves (g)	Extracting solvent	Dry weight of extracted material (g)	Percentage yield (%)
30.0	Water	3.40	11.33
60.0	Ethanol	4.50	7.50
30.0	Acetone	3.70	12.33

Disk Diffusion Test

From the three leaf extracts of *T. populnea*, inhibitory zones were observed only for the ethanol and acetone extracts. None of the concentrations of the aqueous extracts showed inhibitory zones. The ethanol leaf extract of *T. populnea* showed inhibitory zones against three out of the ten isolates of MRSA at a concentration of 3 mg/mL, while the acetone extract showed inhibitory zones against all ten isolates at the same concentration (Table 2). The acetone extract proved to be more effective than the ethanol extract, resulting in comparatively large zones of inhibition (Table 2). All inhibitory zones resulting from the extracts against MRSA isolates differed significantly ($p < 0.05$) from that of vancomycin. Negative control (20% DMSO) did not result in inhibitory zones against MRSA. The ethanol extract at a concentration of 300 mg/dL showed significantly higher inhibitory zone diameters against the clinical isolates of MRSA compared to concentrations of

3 and 30 mg/dL. The ethanol extract at concentrations of 3 and 30 mg/dL showed no significant differences in inhibitory zone diameters ($p > 0.05$) against MRSA isolates. Both the ethanol and acetone extracts at the concentration of 3 mg/dL showed no significant differences in inhibitory zone diameters ($p > 0.05$) against the 6th MRSA isolate. Both the ethanol and acetone extracts at concentrations of 30 mg/dL showed no significant differences in inhibitory zone diameters ($p > 0.05$) against four MRSA isolates, as shown in Table 2. Furthermore, both ethanol and acetone extracts at a concentration of 300 mg/dL showed no significant differences in inhibitory zone diameters ($p > 0.05$) against six MRSA isolates. The acetone extract showed significant dose-dependent inhibitory zone diameters ($p < 0.05$) against MRSA (Table 2).

Table 2. Antimicrobial activity of the different extracts of *T. populnea* against the clinically isolated MRSA.

MRSA isolates	Mean diameter of the inhibition zone (mm)						
	Ethanol extract (mg/mL)			Acetone extract (mg/mL)			Vancomycin (µg/mL)
	3	30	300	3	30	300	30
1	-	7.73 ± 0.57	10.40 ± 0.60[d]	7.43 ± 0.50	9.76 ± 0.54	11.08 ± 1.01[d]	19.19 ± 1.63
2	-	8.14 ± 0.53	9.09 ± 0.90	8.10 ± 0.87	9.42 ± 0.58	11.08 ± 0.95	17.31 ± 0.40
3	-	10.43 ± 0.62[c]	10.75 ± 0.57	8.09 ± 0.90	9.74 ± 0.52[c]	13.02 ± 0.20	17.05 ± 0.89
4	-	8.41 ± 0.62[c]	9.39 ± 0.61[d]	8.73 ± 0.54	9.06 ± 0.35[c]	10.77 ± 0.51[d]	18.11 ± 0.05
5	-	10.47 ± 0.62[c]	12.46 ± 0.54[d]	8.09 ± 1.00	11.13 ± 0.07[c]	12.42 ± 0.54[d]	18.09 ± 1.00
6	7.73 ± 0.54[a,b]	10.41 ± 0.57[a,c]	11.09 ± 0.90	7.41 ± 1.54[b]	10.05 ± 0.99[c]	10.09 ± 0.97	18.42 ± 0.50
7	6.40 ± 0.52[a]	9.05 ± 0.05[a]	10.78 ± 0.56	9.17 ± 0.98	10.43 ± 0.62	3.41 ± 0.59	21.07 ± 0.82
8	8.07 ± 0.70[a]	8.47 ± 0.56[a]	10.73 ± 0.64[d]	10.43 ± 0.62	11.05 ± 0.92	12.77 ± 0.55[d]	20.96 ± 2.15
9	-	8.45 ± 0.62	11.09 ± 0.95[d]	8.47 ± 0.53	10.09 ± 0.05	11.75 ± 0.58[d]	17.22 ± 0.33
10	-	8.11 ± 1.01	10.11 ± 0.98[d]	8.14 ± 0.34	10.74 ± 0.62	11.06 ± 1.00[d]	20.72 ± 0.52

The diameter of the inhibitory zones is represented as mean ± SD. Vancomycin was used as the positive control. – denotes no measurable diameter. [a] denotes non-significant differences between the ethanol extract concentrations of 3 and 30 mg/dL. [b] denotes non-significant differences between the ethanol and acetone extracts at the concentrations of 3 mg/dL. [c] denotes non-significant differences between the ethanol and acetone extracts at the concentrations of 30 mg/dL. [d] denotes non-significant differences between the ethanol and acetone extracts at a concentration of 300 mg/dL. $p < 0.05$ is considered statistically significant.

Tables 3 and 4 show that the acetone extract exhibits the highest AI (0.765) and RPI (58.67) values against the MRSA isolates. A one-sided relationship was observed between the concentration of plant extracts and AI and RPI, indicating a dose-dependent antimicrobial activity. However, the AI and RPI values of all extracts were significantly lower ($p < 0.05$) than those of vancomycin.

Broth Dilution Method

The MRSA isolates were more susceptible to the acetone extract with the lowest MIC of 0.10 mg/mL (Figure 3). The acetone extract at a concentration of 0.10 mg/mL inhibited 30% of the MRSA isolates. The MIC values of the ethanol leaf extract of *T. populnea* ranged from 0.47–15.00 mg/mL, while for the acetone extract it ranged from 0.10–0.75 mg/mL. Similarly, the acetone extract showed the lowest MBC value at 0.10 mg/mL against the MRSA isolates (Figure 2). The aqueous extract had the highest MIC and MBC values against the tested isolates (MIC > 100 mg/mL, MBC ≥ 300 mg/mL).

Discussion

In the present study, the determination of the antibacterial activity of aqueous, ethanol and acetone leaf extracts of *T. populnea* against clinical isolates of MRSA was carried out. A higher extraction yield was obtained by aqueous and acetone extraction compared to ethanol extraction. The disk diffusion method was

carried out in the present study as a preliminary the aqueous, ethanol and acetone leaf extracts of *T. populnea* against MRSA isolates. Here, the disk diffusion method was followed since it has several advantages, such as being inexpensive, and flexible and it allows visibility of growth, correct inoculum and other abnormalities[28]. In the assessment of the antimicrobial activity of screening test to confirm the inhibitory effects of several plant extracts against MRSA isolates, the disk diffusion method has been frequently used[25,29,30]. In the present study, vancomycin was used as the positive control; it was used in several previous studies in which antimicrobial activity was tested against MRSA isolates or strains[25,31,32].

Table 3. Activity index (AI) of the different concentrations of *T. populnea* leaf extracts.

MRSA isolates	AI of the different concentrations of extracts					
	Ethanol extract (mg/mL)			Acetone extract (mg/mL)		
	3	30	300	3	30	300
1	-	0.402 ± 0.060[b]	0.543 ± 0.095[c]	0.388 ± 0.071	0.508 ± 0.076[b]	0.575 ± 0.077[c]
2	-	0.476 ± 0.003	0.531 ± 0.006	0.474 ± 0.006	0.551 ± 0.034	0.648 ± 0.056
3	-	0.613 ± 0.049[b]	0.631 ± 0.024	0.475 ± 0.026	0.571 ± 0.017[b]	0.765 ± 0.046
4	-	0.464 ± 0.034[b]	0.518 ± 0.034	0.481 ± 0.030	0.500 ± 0.002[b]	0.594 ± 0.028
5	-	0.580 ± 0.033[b]	0.690 ± 0.029[c]	0.448 ± 0.056	0.617 ± 0.005[b]	0.688 ± 0.029[c]
6	0.368 ± 0.041[a]	0.555 ± 0.047[b]	0.591 ± 0.021[c]	0.394 ± 0.074[a]	0.535 ± 0.053[b]	0.537 ± 0.053[c]
7	0.324 ± 0.021	0.431 ± 0.017[b]	0.514 ± 0.047	0.438 ± 0.066	0.497 ± 0.050[b]	0.639 ± 0.055
8	0.472 ± 0.004	0.429 ± 0.022	0.544 ± 0.017	0.529 ± 0.026	0.561 ± 0.046	0.648 ± 0.043
9	-	0.494 ± 0.034	0.649 ± 0.059[c]	0.495 ± 0.029	0.590 ± 0.001	0.688 ± 0.035[c]
10	-	0.391 ± 0.039	0.488 ± 0.036[c]	0.393 ± 0.011	0.518 ± 0.015	0.534 ± 0.035[c]

AI: Activity index. AI is represented as mean ± SD. [a] denotes non-significant differences between the ethanol and acetone extracts at a concentration of 3 mg/dL. [b] denotes non-significant differences between the ethanol and acetone extracts at a concentration of 30 mg/dL. [c] denotes non-significant differences between the ethanol and acetone extracts at a concentration of 300 mg/dL. $p < 0.05$ is considered statistically significant.

Table 4. Relative percentage inhibition (RPI) of the different concentrations of *T. populnea* leaf extracts.

MRSA isolates	RPI of the different concentrations of extracts					
	Ethanol extract (mg/mL)			Acetone extract (mg/mL)		
	3	30	300	3	30	300
1	-	16.42 ± 5.01[b]	30.05 ± 10.81[c]	15.37 ± 5.81	26.18 ± 8.04[b]	33.45 ± 8.90[c]
2	-	22.66 ± 0.31	28.24 ± 0.60	22.44 ± 0.52	30.42 ± 3.80	42.20 ± 7.24
3	-	37.69 ± 5.94[b]	39.83 ± 3.07	22.65 ± 2.48	32.65 ± 1.92[b]	58.67 ± 7.06
4	-	21.58 ± 3.20[b]	26.93 ± 3.54	23.24 ± 2.86	24.98 ± 0.23[b]	35.34 ± 3.33
5	-	33.71 ± 3.94[b]	47.72 ± 4.00[c]	20.29 ± 5.05	38.02 ± 0.64[b]	47.41 ± 3.99[c]
6	17.05 ± 2.87[a]	30.95 ± 5.34[b]	34.97 ± 2.56[c]	15.87 ± 6.05[a]	28.83 ± 5.59[b]	29.06 ± 5.55[c]
7	9.41 ± 2.37	18.58 ± 1.47[b]	26.53 ± 4.69	19.45 ± 5.82	24.86 ± 5.12[b]	41.00 ± 7.13
8	16.75 ± 1.03	18.47 ± 1.90	29.59 ± 1.86	27.98 ± 2.76	31.56 ± 5.10	42.14 ± 5.47
9	-	24.49 ± 3.45	42.38 ± 7.73[c]	24.58 ± 2.91	34.87 ± 0.16	47.36 ± 4.70[c]
10	-	15.38 ± 3.10	23.86 ± 3.56[c]	15.48 ± 0.89	26.89 ± 1.55	28.55 ± 3.72[c]

RPI: Relative percentage inhibition. RPI is represented as mean ± SD. [a] denotes non-significant differences between the ethanol and acetone extracts at a concentration of 3 mg/dL. [b] denotes non-significant differences between the ethanol and

acetone extracts at a concentration of 30 mg/dL. [c] denotes non-significant differences between the ethanol and acetone extracts at a concentration of 300 mg/dL. $p < 0.05$ is considered statistically significant.

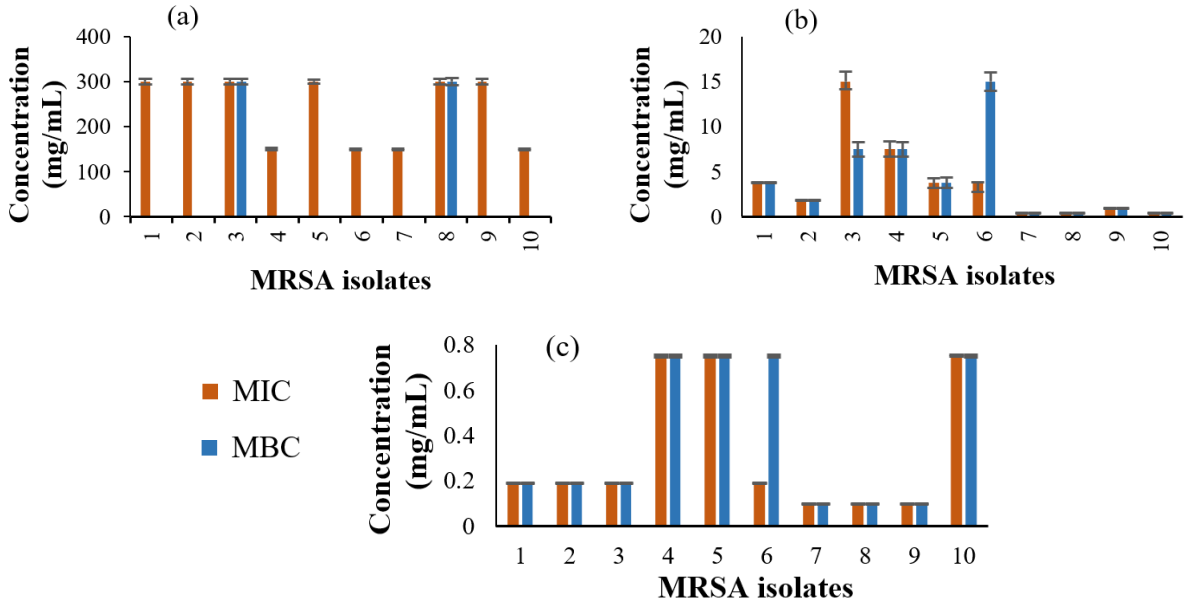

Figure 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of (a) aqueous extract (b) ethanol extract (c) acetone extract of *T. populnea* leaves against the ten isolates of MRSA.

According to the present study highlights, the ethanol and acetone leaf extracts of *T. populnea* showed potent antimicrobial activity against *S. aureus*, as evidenced through the disk diffusion method; the results are in line with previous reports[18]. The topical gel prepared from the alcoholic extract of *T. populnea* showed remarkable inhibitory zones on MHA plates against *S. aureus*, and therefore, it was recommended to use in the fast healing of wounds[33]. Importantly, phenolic compounds present in *T. populnea* may contribute to antimicrobial activity and restrict the metabolism of bacteria, interact with cell components, and thus, may reduce the fast growth of bacteria[34,35]. Furthermore, the disk diffusion method in the current study implicated that the aqueous leaf extract of *T. populnea* had no antibacterial activity against clinical isolates of MRSA. According to a previous study, the aqueous root extract of *T. populnea* exerted antibacterial activity against *S. aureus*, however, the activity was lower than that of the ethanol extract[20].

The findings of the present study also highlighted the dose-dependent antimicrobial activity of both the ethanol and acetone leaf extracts of *T. populnea* against clinical isolates of MRSA. In a previous study, the ethanol root extract of *T. populnea* showed dose-dependent antimicrobial activity; MIC of the ethanol extract was there reported to be 10 µg/mL for *S. aureus*[20]. Based on the findings of the present study, the acetone extract was more potent than the ethanol leaf extract of *T. populnea* against the MRSA isolates. It showed the highest AI and RPI values. These results were further supported by the lowest values of MIC and MBC observed. The acetone extract might exert its antimicrobial activity by altering the key pathogenesis, including the inhibition of protein and nucleic acid synthesis and altering the integrity of the cell membrane[36]. Even though, the aqueous and ethanol extracts did not show antimicrobial activity against MRSA isolates, except against three MRSA isolates in the disk diffusion method, those extracts had MIC and MBC values in the broth microdilution method. This could be attributed to the fact that the broth microdilution method is more sensitive than the disk diffusion method for screening the antimicrobial properties of plant extracts[37]. The properties of the plant extracts, such as pH, volatility, solubility and diffusion in agar medium, influence the results of the disk diffusion method. On the other hand, these properties play no role in the broth microdilution method[37]. Indeed plant extracts, as a mixture of secondary metabolites, are not expected to

diffuse easily in the disc diffusion method, affecting thus the result[37].

The antimicrobial activity of the secondary metabolites isolated from *T. populnea* has not been reported yet. However, the antimicrobial potential of some of the compounds such as lupeol, β-sitosterol, benzoic acid, methyl salicylate, capric acid, pentadecanoic acid, kaempferol, hexadecenoic acid and oleic acid (Figure 2, **1-9**), that are also present in *T. populnea*, was reported[38–43]. Future research studies will focus on the determination of the antimicrobial activity of the compounds isolated from the bioactive extracts of *T. populnea*, investigate their structure-activity relationships, and address their pharmacokinetics.

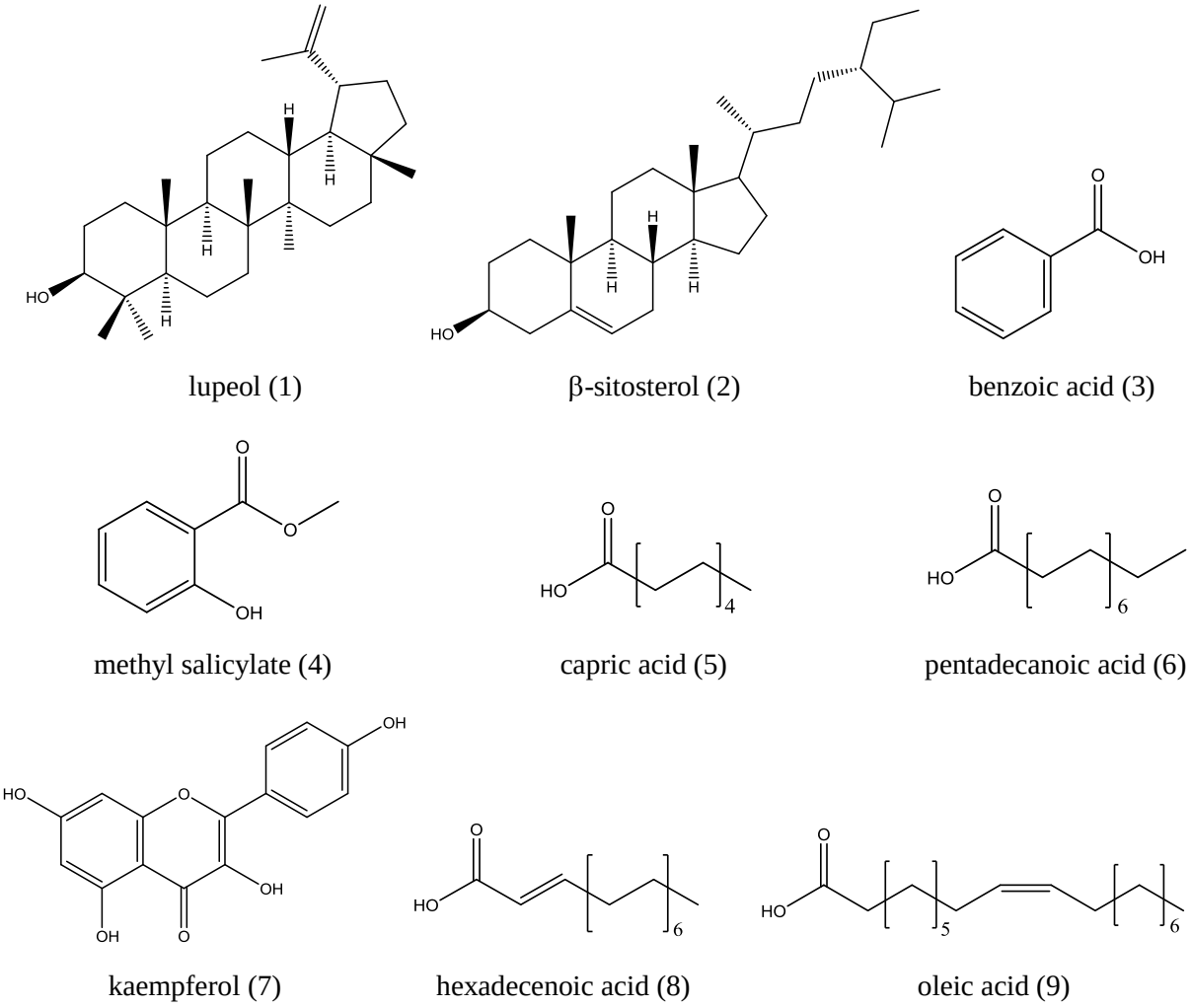

Figure 3. Potent antimicrobial compounds (1 – 9) present in the leaf of *T. populnea*.

Conclusion

The present study provides the first scientific evidence for the antimicrobial activity of aqueous, ethanol and acetone leaf extracts of *T. populnea* against clinical isolates of MRSA. The ethanol and acetone extracts showed remarkable antimicrobial activity, with the acetone extract being the most potent having the highest AI and RPI values and the lowest MIC and MBC values (0.75 mg/mL). In conclusion, the acetone extract of *T. populnea* leaves could be considered a potential herbal source for the development of novel antimicrobial agents against MRSA.

Ethical Consideration

Ethical approval was granted from the Ethical Review Committee, Faculty of Allied Health Sciences, University of Ruhuna, Sri Lanka (2021.09.33).

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