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RESEARCH ARTICLE OPEN ACCESS

Antibacterial and Antibiofilm Properties of Medicinal Plant Extracts against Multi Drug Resistant *Staphylococcus* Species and Non Fermenter Bacteria

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Abstract

Antimicrobial resistance to the pathogenic microorganism has been characterized as a public health emergency both in the community and in hospitals. That is why; we need to find alternatives, which could be used as antibacterial agents. Therefore aim of this study is to determine the antibacterial and antibiofilm properties of 4 plant extracts Clove (*Syzygium aromaticum*), Tea (*Camellia sinensis*), Garlic (*Allium sativum*), coriander (*Coriandrum sativum*).Antibacterial properties of plant extracts at different concentrations (50, 25, 12.5, 6.25 mg/mL) were tested against Multi Drug Resistance biofilm producing *Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter baumannii, Staphylococcus epidermidis* and *Staphylococcus saprophyticus* using the agar well diffusion method.Minimum Bactericidal Concentration (MBC) and antibiofilm properties of the plant extracts were determined using the tube dilution method and modified crystal violet assay, respectively. Total of 180 clinical isolates were screened for their MDR Pattern. Out of these, 72 were MDR isolates. These MDR isolates were categorized into weak, moderate and strong biofilm producers. Fourteen, Forty nine and nine were weak, moderate and strong biofilm producers, respectively. Out of the 4 plant extracts, *Syzygium aromaticum* and *Camellia sinensis* were found to be more effective with maximum zone of inhibition (20 – 25 mm), MBC 6.25 mg/ml and biofilm reduction of more than 50% compared to *Allium sativum* and *Coriandrum sativum*. All medicinal plant extracts were effective at different concentrations against the biofilm producing MDR isolates but *Syzygium aromaticum* and *Camellia sinensis* showed maximum antibacterial and antibiofilm activity.

Keywords: Antibacterial, Antibiofilm activity, Multi Drug Resistant Bacteria, Plant extracts

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INTRODUCTION

Multi Drug Resistant (MDR) bacteria causing infectious diseases are a major public health problem globally. Human health, environment as well as ecosystem are equally suffering because of the excessive usage of antibiotics which result in development of multi drug resistance among pathogenic bacteria. Various reports have shown drug resistance to pathogenic bacteria[1,2]. Hospitals and the communities worldwide have witnessed rapid increasing of antibiotic resistance among pathogenic bacteria contributing in increased morbidity, mortality, and cost of health-care[3,4]. High resistance to antibiotics can be due to an important virulence factor known as bacterial biofilm which may be responsible for persistent chronic and recurrent infections. Bacterial biofilm get easily attached on to various living and nonliving solid surfaces, medical devices such as valves and catheters by forming a matrix itself[5]. Therefore, the diffusion of antibiotics is hampered because of the establishment of biofilm, which results in the physiological changes in the growth mode and the low metabolic rate of inner layers of bacteria[6,7]. Biofilm mediated infections needs to be treated through new strategies. In this context, a renewed interest has focused on the use of medicinal plants which are natural substances, rich in secondary metabolites and are well known for their antimicrobial properties[8]. Awareness about the importance of medicinal plants has been increased in the recent years despite the advances made in the field of science and research, as these medicinal plants contain certain active biological compound (phenolics,essentialoils, terpenoids, alkaloids, lectins, polypeptides, polyacetylenes) which has shown to have antibacterial properties[9,10].

Antimicrobial resistance to the drugs used against pathogenic microorganism has been characterized as a public health emergency both in the community and hospitals. Therefore the use of Medicinal plants has been brought into consideration and is studied intensively by various researchers to know their antimicrobial activity. Researchers have also revealed the important components like eugenol in clove,catechins in tea, allicin in garlic and phytoconstituents in leaves of coriander which act as a vital source of pharmacological effects[11,12,13,14]. Extracts of plants contain mixtures of these components and others such as alkaloids, polyphenols and terpenoids, which are known for their antioxidant, antidiabetic, antiviral, anti-inflammatory antifungal and antimicrobial properties. So, considering the importance of medicinal plants as an antibacterial agents, current study was done to evaluate the antibacterial and antibiofilm activities of the four plants *Syzygium aromaticum, Camellia sinensis, Allium sativum* and *Coriandrum sativum*.

MATERIAL AND METHODS

Bacterial strains

All clinically isolated *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* from various specimens were screened for their Drug Resistance status according to CLSI guidelines – CLSI M100-S22, 2012[15]. Multi drug resistant isolates were further tested for biofilm production and categorized into 3 groups - strong, moderate and weak biofilm producers[16]. Referral ATCC Bacterial strains of the similar isolates that have been previously characterized in Microbiology laboratory of SGT Medical College, Hospital and Research Institute, Gurugram were simultaneously tested in triplicates for antibacterial activity and single testing for biofilm inhibition assay.

Collection and certification of medicinal plants

Syzygium aromaticum - UHF herbarium no. 13632, *Camellia sinensis* - 13633, *Allium sativum* - 13590 and *Coriandrum sativum* - 13634 were obtained and certified from Department of Forestry, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh.

Plant Extract Preparation

The methanolic extracts of the above mentioned plants were prepared. Flower buds (*Syzygium aromaticum*), dried leaves (*Camellia sinensis*, *Coriandrum sativum*) and bulb part (*Allium sativum*) of plants were crushed to powder and soaked into 50ml of methanol. Further, it was continuously boiled for 3 minutes for 3 times, with a gap of 2 minutes interval between each boiling time. The extract or supernatant was collected, subjected to centrifugation for 5minutes at

3600g until clear supernatant was obtained. The supernatant was then filtered using 0.2 um filter (Micropore filters), and stored at 4°C until further use[17].

Antimicrobial activity by using Agar well diffusion method

Sterile petri dish plates containing 20 ml Muller Hinton agar were prepared. Fresh culture suspensions (0.5 McFarland unit) of isolated pathogenic bacteria were swabbed on the respective plates. Sterile gel puncher was used to make wells over the agar plates in which plant extracts were added at various concentrations (50, 25, 12.5 & 6.25 mg/mL). These plates were further incubated for 24 hours at 37°C. After incubation,the diameter of inhibitory zones around each disc were measured in mm and recorded[17,18].

Determination of Minimum Bactericidal Concentration (MBC)

MBC is defined as the concentration producing a 99.9% reduction in colony forming units (CFU) number in the initial inoculum. Serial two-fold dilutions of the plant extracts were made at concentration of 50, 25, 12.5 and 6.25 mg/mL to which 100uL of microorganism suspension at a final density of 10^5cells/ml were added. The tubes were incubated at 37°C for 24 h. The tubes after 24 h of incubation were sub-cultured on Mueller Hinton agar and the bacterial growth was observed on the very next day. MBC was determined as the lowest concentration of plant extract that failed to yield any bacterial growth in the subcultures[19].

Determination of Biofilm Formation by bacterial isolate using modified crystal violet assay

Sterile 96-well tissue culture plates were used to which 50 µl of Mueller–Hinton broth per well was added. Fresh bacterial suspensions (1.0 McFarland) were made and 50µl were added to the wells and incubated for 48 hours at 37°C. To check for the biofilm formation, contents from the wells were removed by washing with 200µl normal saline after which 200 µl of 0.1% crystal violet stain was added and incubated again for 20 minutes. Then, each well was thoroughly washed with deionized water and later the wells were added with 200 µl of 96% ethanol. Optical density (OD) of the adherent bacteria was calculated using ELISA reader at 630 nm. Formation of biofilm was calculated using the formula.

OD of bacteria= [(OD growth control – OD sample) / OD growth control] × 100.

Strains were classified as follows[20]:

OD ≤ ODc= No biofilm producer

ODc< OD ≤ 2 × ODc= Weak biofilm producer

2 × ODc< OD ≤ 4 × ODc= Moderate biofilm producer

4 × ODc< OD= Strong biofilm producer.

ODc: Optical density of growth control

Determination of Anti Biofilm Activity of plant extracts using modified crystal violet assay

Sterile 96-well tissue culture plates were used to which 50 µl of Mueller–Hinton broth was added to each well. Two-fold serial dilutions of plant extract were made in the tissue culture plates. Final concentrations to be tested were 50, 25, 12.5 and 6.25 mg/mL. Fresh bacterial suspensions (1.0 McFarland turbidity standard matched) were made and 50 µl was added to the wells containing plant extract at different concentrations. Bacteria without plant extract was used as growth control. After 24 hrs of incubation modified crystal violet assay was performed as described above. The percentage of biofilm inhibition was calculated by using the following formula:

[(OD growth control – OD sample) / OD growth control] × 100.

The biofilm inhibition concentration (BIC50) was defined as the lowest concentration of extracts that showed 50% inhibition on the biofilm formation[20].

RESULTS

Out of 180 clinical isolates screened, 72 were MDR isolates. These MDR isolates were categorized into weak, moderate and strong biofilm producers.

Out of 72 MDR isolates, 14 weak, 49 moderate and 9 were strong biofilm producers. Weak biofilm producers were excluded. So total 58 biofilm producers were considered for the study as described in Table 1.

Extracts of the plants (*Syzygium aromaticum*, *Camellia sinensis*, *Coriandrum sativum* and *Allium sativum*) were tested for their antibiofilm and antimicrobial properties at

Table 1. biofilm production by clinical MDR bacterial isolates

Bacterial Isolates	Number of Bacteria Isolates	Strong Biofilm Producers	Moderate Biofilm Producers	Weak Biofilm Producers
Staphylococcus aureus	23	3	15	5
Pseudomonas aeruginosa	28	4	16	8
Acinetobacter baumannii	8	2	5	1
Staphylococcus epidermidis	8	Nil	8	Nil
Staphylococcus saprophyticus	5	Nil	5	Nil

Table 2. Antibacterial and antibiofilm activity of Syzygium aromaticum

Bacterial Isolates	Concentrations (mg/mL)	Zone of Inhibition (Mean ± SD)	MBC (mg/mL)	Biofilm Reduction (Mean ± SD)
Staphylococcus aureus (18 isolates)	50	23.6 ± 1.20 mm	6.25	65 ± 0.024%
	25	22.2 ± 1.38 mm		57 ± 0.045%
	12.5	22.6 ± 1.19 mm		54 ± 0.027%
	6.25	20.7 ± 1.32 mm		57 ± 0.030%
Staphylococcus aureus (ATCC 25923)	50	23.7 ± 0.58 mm	6.25	63%
	25	21.0 ± 1.00mm		59%
	12.5	21.7 ± 1.53mm		55%
	6.25	20.7 ± 1.58mm		58%
Pseudomonas aeruginosa (20 isolates)	50	23.2 ± 1.31 mm	6.25	54 ± 0.020%
	25	24.6 ± 1.36 mm		52 ± 0.025%
	12.5	23.3 ± 1.26 mm		53 ± 0.023%
	6.25	20.0 ± 2.28 mm		52± 0.035%
Pseudomonas aeruginosa (ATCC 27853)	50	23.3 ± 1.53mm	6.25	55%
	25	24.3 ± 0.58mm		51%
	12.5	22.3 ± 1.15mm		57%
	6.25	20.3 ± 1.53mm		54%
Acinetobacter baumannii (07 isolates)	50	24.1 ± 2.11 mm	6.25	53 ± 0.023%
	25	22.9 ± 1.34mm		55 ± 2 %
	12.5	22.7 ± 1.11 mm		54 ± 0.020%
	6.25	15.3 ± 1.79 mm		50 ± 0.028%
Acinetobacter baumannii (ATCC 19606)	50	24.7 ± 1.53mm	6.25	57%
	25	22.7 ± 0.58mm		54%
	12.5	22.0 ± 2.00mm		53%
	6.25	16.0 ± 1.00mm		51%
Staphylococcus epidermidis (08 isolates)	50	21.8 ± 1.83 mm	6.25	65 ± 0.027%
	25	25.0 ± 1.31 mm		55 ± 0.021%
	12.5	22.6 ± 1.41 mm		55 ± 0.022%
	6.25	21.6 ± 2.20 mm		50 ± 0.032%
Staphylococcus epidermidis (ATCC 12228)	50	21.7 ± 0.58mm	6.25	66%
	25	24.7 ± 1.53mm		57%
	12.5	23.0 ± 1.00mm		54%
	6.25	21.3 ± 1.15mm		52%
Staphylococcus saprophyticus (05 isolates)	50	22.2 ± 1.30 mm	6.25	62 ± 0.020%
	25	24.8 ± 1.30 mm		53 ± 0.024%
	12.5	23.0 ± 1.58 mm		52 ± 0.018%
	6.25	21.6 ± 1.14 mm		52 ± 0.011%
Staphylococcus saprophyticus (ATCC 15305)	50	23.0 ± 1.00mm	6.25	65%
	25	23.7 ± 1.53mm		55%
	12.5	22.7 ± 0.58mm		53%
	6.25	21.0 ± 1.00mm		54%

concentration of 50, 25, 12.5 and 6.25mg/mL on 58MDR biofilm producers. Among them *Syzygium aromaticum* and *Camellia sinensis* were found to be more effective as compared to *Allium sativum* and *Coriandrum sativum* against the tested bacterial isolates.

Syzygium aromaticum showed maximum zone of inhibition (25.0 ± 1.31mm) against *Staphylococcus epidermidis* at 25mg/mL concentration and minimum zone of inhibition (15.3 ± 1.79mm) against *Acinetobacter baumannii* at 6.25mg/mL concentration. MBC was 6.25mg/mL for each MDR bacteria. Antibiofilm inhibition was more than 50% for all the concentrations as described in Table 2.

Camellia sinensis was effective against each bacteria at different concentrations with maximum zone of inhibition (25.4 ± 1.14mm)

Table 3. Antibacterial and antibiofilm activity of *Camellia sinensis*

Bacterial Isolates	Concentrations (mg/mL)	Zone of Inhibition (Mean ± SD)	MBC (mg/mL)	Biofilm Reduction (Mean ± SD)
Staphylococcus aureus (18 isolates)	50	25.1 ± 1.59 mm	6.25	73 ±0.023%
	25	23.9 ± 1.35 mm		71±0.020%
	12.5	21.8 ± 1.28 mm		62 ±0.027%
	6.25	15.0 ± 1.30 mm		57 ±0.035%
Staphylococcus aureus (ATCC 25923)	50	24.6 ± 1.15mm	6.25	75%
	25	22.0 ± 1.00mm		70%
	12.5	21.3 ± 1.15mm		65%
	6.25	17.6 ± 1.53mm		54%
Pseudomonas aeruginosa (20 isolates)	50	20.3 ± 1.49 mm	6.25	73 ±0.023%
	25	16.0 ± 1.17 mm		71 ±0.032%
	12.5	15.2 ± 1.28 mm		64 ±0.022%
	6.25	18.1 ± 1.28 mm		61 ±0.024%
Pseudomonas aeruginosa (ATCC 27853)	50	20.3 ±1.15mm	6.25	72%
	25	17.0 ±1.00mm		69%
	12.5	15.6±0.58mm		66%
	6.25	15.3 ± 1.53mm		59%
Acinetobacter baumannii (07 isolates)	50	25.1 ± 1.46 mm	6.25	71 ± 0.013%
	25	24.7 ± 1.50 mm		73 ± 0.019%
	12.5	20.1 ± 1.35 mm		66 ± 0.026%
	6.25	24.7 ± 1.11 mm		63 ± 0.021%
Acinetobacter baumannii (ATCC 19606)	50	25.3 ± 0.58mm	6.25	73%
	25	24.2 ± 1.28 mm		74%
	12.5	23.9 ± 1.64 mm		62%
	6.25	23.7 ± 1.16 mm		65%
Staphylococcus epidermidis (08 isolates)	50	25.3 ± 1.19 mm	6.25	63 ± 0.021%
	25	24.2 ± 1.28 mm		62 ± 0.015%
	12.5	23.9 ± 1.64 mm		57 ± 0.017%
	6.25	23.7 ± 1.16 mm		53 ± 0.020%
Staphylococcus epidermidis (ATCC 12228)	50	25.3 ±0.58mm	6.25	66%
	25	23.7 ± 1.50mm		64%
	12.5	23.0 ± 1.00mm		54%
	6.25	20.0 ± 1.73mm		55%
Staphylococcus saprophyticus (05 isolates)	50	25.4 ± 1.14 mm	6.25	75 ± 0.020%
	25	24.8 ± 1.10mm		70 ± 2.0%
	12.5	22.0 ± 1.58mm		61 ± 0.017%
	6.25	23.6 ± 1.14 mm		60 ± 0.015%
Staphylococcus saprophyticus (ATCC 15305)	50	24.6 ± 1.53mm	6.25	71%
	25	25.0 ± 1.00mm		73%
	12.5	22.3 ± 1.15mm		64%
	6.25	21.0 ± 2.00mm		63%

against *Staphylococcus saprophyticus* at 50mg/mL concentration and minimum zone of inhibition (15.0 ± 1.30 mm) against *Staphylococcus aureus* at 6.25mg/ml concentration. MBC was 6.25 mg/mL. Antibiofilm reduction was more than 50% for each isolate at all concentrations as described in Table 3.

A. sativum did not show any antibacterial activity against *S. epidermidis* and *Staphylococcus saprophyticus* with no zone of inhibition but has shown maximum zone of inhibition (18. 9 ± 1.61mm) against *Pseudomonas aeruginosa* at 50mg/mL and minimum zone of inhibition (14.8 ± 1.38 mm) against *Staphylococcus aureus* at 12.5 mg/mL concentration. MBC came out to be 12.5 mg/mL and reduction in biofilm formation was less than 50% (range between 20-43%) for all concentrations as described in table 4.

Table 4. Antibacterial and antibiofilm activity of *Allium sativum*

Bacterial Isolates	Concentrations (mg/mL)	Zone of Inhibition (Mean ± SD)	MBC (mg/mL)	Biofilm Reduction (Mean ± SD)
Staphylococcus aureus (18 isolates)	50	14.9 ± 1.62 mm	12.5	31 ± 0.039%
	25	17.0 ± 1.32 mm		22 ± 0.025%
	12.5	14.8 ± 1.38 mm		19 ± 0.027%
	6.25	15.9 ± 1.39 mm		21 ± 0.017%
Staphylococcus aureus (ATCC 25923)	50	16.6 ±1.15mm	12.5	35%
	25	17.0 ± 2.00mm		25%
	12.5	16.0 ± 1.00mm		22%
	6.25	15.3 ±0.58mm		22%
Pseudomonas aeruginosa (20 isolates)	50	18. 9± 1.61mm	12.5	32 ± 0.022%
	25	-		33 ± 0.029%
	12.5	-		19 ± 0.030%
	6.25	-		20 ± 2.0%
Pseudomonas aeruginosa (ATCC 27853)	50	19.3mm	12.5	31%
	25	-		35%
	12.5	-		24%
	6.25	-		18%
Acinetobacter baumannii (07 isolates)	50	14.9 ± 1.35 mm	12.5	25 ± 0.052%
	25	16.7 ± 1.11 mm		22 ± 0.025%
	12.5	15.9 ± 1.34 mm		24 ± 0.045%
	6.25	14.9 ± 2.11 mm		22 ± 0.047%
Acinetobacter baumannii (ATCC 19606)	50	16.3 ±0.58mm	12.5	24%
	25	15.3 ±1.20mm		25%
	12.5	15.3 ± 1.50mm		23%
	6.25	13.0± 1.00mm		20%
Staphylococcus epidermidis (08 isolates)	50	-	12.5	34 ±0.086%
	25	-		13 ± 0.012%
	12.5	-		36 ± 0.101%
	6.25	-		21 ± 0.062%
Staphylococcus epidermidis (ATCC 12228)	50	-	12.5	37%
	25	-		16%
	12.5	-		32%
	6.25	-		26%
Staphylococcus saprophyticus (05 isolates)	50	-	12.5	43 ± 0.028%
	25	-		41 ± 0.064%
	12.5	-		36 ± 0.030%
	6.25	-		20 ± 0.105%
Staphylococcus saprophyticus (ATCC 15305)	50	-	12.5	47%
	25	-		44%
	12.5	-		33%
	6.25	-		24%

Coriandrum sativum has shown maximum zone of inhibition (17.4 ± 1.27mm) against *Acinetobacter baumannii* at 50mg/mL concentration and minimum zone of inhibition (9.80 ± 1.25 mm) against *Staphylococcus epidermidis* at 6.25mg/mL concentration. MBC was varying for each bacterial isolate ranging from 12.5-50 mg/mL. Biofilm reduction of all bacteria were less than 50% for all extract concentrations as described in table 5.

Table 5. Antibacterial and antibiofilm activity of *Coriandrum sativum*

Bacterial Isolates	Concentrations (mg/mL)	Zone of Inhibition (Mean ± SD)	MBC (mg/mL)	Biofilm Reduction (Mean ± SD)
Staphylococcus aureus (18 isolates)	50	15.9 ± 1.55 mm	12.5	42 ± 0.066%
	25	15.6± 1. 50 mm		44 ± 0.027%
	12.5	14.6 ± 1.14 mm		36 ± 0.103%
	6.25	15.7 ± 1.32 mm		38 ± 0.048 %
Staphylococcus aureus (ATCC 25923)	50	17.0 ± 1.00mm	12.5	45%
	25	16.3 ±0.58mm		46%
	12.5	14.6 ±0.58mm		38%
	6.25	13.3±1.53mm		35%
Pseudomonas aeruginosa (20 isolates)	50	14. 8 ± 1.71 mm	25.0	42 ± 0.040%
	25	12. 2 ± 1.86 mm		39 ± 0.115%
	12.5	14.6 ± 1.69 mm		39 ± 0.058%
	6.25	11.9 ± 1.37 mm		27 ± 0.106%
Pseudomonas aeruginosa (ATCC 27853)	50	15.3 ±0.58mm	25.0	40%
	25	13.0 ± 1.00mm		41%
	12.5	13.6±1.53mm		37%
	6.25	10.6±1.53mm		30%
Acinetobacter baumannii (07 isolates)	50	17.4 ± 1.27 mm	25.0	42 ± 0.040%
	25	15.6 ± 1.51 mm		39 ± 0.115%
	12.5	16.7 ± 1.49 mm		39 ± 0.058%
	6.25	16.8 ± 1.46 mm		27 ± 0.106%
Acinetobacter baumannii (ATCC 19606)	50	17.6 ±0.58mm	25.0	39%
	25	16.0±1.00mm		41%
	12.5	15.3±1.15mm		43%
	6.25	14.6±1.53mm		31%
Staphylococcus epidermidis (08 isolates)	50	14.8 ± 1.49 mm	50.0	34 ± 0.086%
	25	11.1 ± 1.46 mm		13 ± 0.012%
	12.5	13.0 ± 1.31 mm		36 ± 0.101%
	6.25	9.80 ± 1.25 mm		21 ± 0.062%
Staphylococcus epidermidis (ATCC 12228)	50	14.6 ±0.58mm	50.0	37%
	25	12.6±0.58mm		16%
	12.5	13.0±1.00mm		33%
	6.25	10.6±1.53mm		24%
Staphylococcus saprophyticus (05 isolates)	50	15.2 ± 1.48 mm	50.0	43 ± 0.028%
	25	13.2 ± 1.30 mm		41 ± 0.064%
	12.5	13.0 ± 1.48 mm		36 ± 0.030%
	6.25	12.8 ± 1.64 mm		20 ± 0.105%
Staphylococcus saprophyticus (ATCC 15305)	50	15.3 ±1.53mm	50.0	45%
	25	13.6 ±0.58mm		44%
	12.5	12.00±1.00m		34%
	6.25	10.3±1.53mm		26%

DISCUSSION

This study was aimed to detect antibacterial and antibiofilm properties of four medicinal plant extracts. As we know, multi drug resistance against most commonly used chemical drugs is a highly faced problem nowadays and it is a matter of concern. Therefore, our area of interest is more focused on natural products that can be

used as an alternative to the antimicrobials.

Syzygium aromaticum has shown maximum zone of inhibition at 50mg/mL concentration against *Staphylococcus aureus* (23.6 ± 1.20mm), *Pseudomonas aeruginosa* (24.6 ± 1.36mm), *Acinetobacter baumannii* (24.1 ± 2.11mm), *Staphylococcus epidermidis* (25 ± 1.31mm) and *Staphylococcus saprophyticus* (24.8 ±1.3mm) at 50 and 25 mg/mL concentration. Similar results were shown by Anita *et al*.[21] who revealed inhibition zone of 28mm for *Staphylococcus aureus* and 30mm for *Pseudomonas aeruginosa* at 25 mg/ml concentration. Another Study done by Neelima *et al*., has also shown almost similar results where zone of inhibition by *Syzygium aromaticum* against *Pseudomonas aeruginosa* was between 15-30 mm at different concentrations(25, 50, 100, 200µg/ml)[22]. Liaqat *et al*.[23] has reported MBC value (20mg/mL) of clove against *E. coli* whereas another study done by Mahajan *et al*.[24] has shown MBC against *Staphylococcus aureus* and *Pseudomonas aeruginosa* ranging between 6.25 – 25mg/ml, which is slightly higher to our study in which MBC came out to be 6.25 mg/mL against all biofilm producing MDR isolates. In the present study, biofilm reduction was more than 50% at each concentration of clove extracts (50, 25, 12.5, 6.25 mg/mL). Significant biofilm reduction by clove at different concentrations has also been reported by other authors too[25,26]. These results show that the methanolic extract of clove is effective against biofilm producing MDR isolates.

C. sinensis also proved its antibacterial and antibiofilm activity at all studied concentrations. It has shown highest zone of inhibition (25.1 ± 1.59mm) at 50mg/ml concentration against *Staphylococcus aureus*, *Staphylococcus epidermidis* (25.3 ± 1.19mm) *Staphylococcus saprophyticus* (25.4 ±1.14mm), *Acinetobacter baumannii* (25.1 ± 1.46) and *Pseudomonas aeruginosa* (20.3 ± 1.49mm). Compared to our study, Mehta *et al*.[12] has shown highest zone of inhibition (15mm) at 50 mg/ml against MDR *Pseudomonas aeruginosa* and *E. coli*, and 10mm against *Staphylococcus aureus*. Another study by Archana *et al*.[27] has shown zone of inhibition of 16mm, 12mm against *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively at different concentrations ranging from 20-100µl. These results were in concordance to our study. Liaqat *et al*.[26] has also shown in his study that methanolic extract of *Camellia sinensis* was effective in reducing the biofilm formation at concentrations from 5-45mg/ml and their range of MBC was 20-40mg/ml against MDR isolates whereas in our study the MBC value of *Camellia sinensis* was 6.25mg/ml and biofilm inhibition was significantly more than 50% for all concentrations. Study done by Fakheri[28] also gave MBC value of 2.5mg/ml against *Staphylococcus aureus* and 1.25mg/ml for *Staphylococcus saprophyticus* which is quite similar to our study. This proves that *Camellia sinensis* does possess antibacterial property. Bacterial susceptibility to *Camellia sinensis* extract is because of the known bactericidal effect of epigallocatechin-gallate (polyphenolic fractions of catechin component of *Camellia sinensis*) which is attributed to membrane perturbation[12].

Allium sativum has shown decent results with zone of inhibitions between 18. 9 ± 1.61 to 14.8 ± 1.38mm against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Acinetobacter baumannii* at different concentrations. It did not show any activity against *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*. Other study done by Mohsenipou *et al*.[29] has shown no zone of inhibition by *Allium sativum* extract except against *Bacillus cereus* (8 mm) whereas Lekshmi *et al*.[30] has mentioned the zone diameter of 13.8±0.29mm against *Staphylococcus aureus* which relates to our study. In the present study, Biofilm inhibition was less than 50% (20-30%) at each concentration whereas Lekshmi *et al*., has mentioned more than 50% biofilm inhibition in which in contrast to the present study. Another study by Shams *et al*., has shown the concordant results to the study as reduction in biofilm was moderate which is less than 50%[31]. Mohsenipou *et al*., have shown the MBC ranged between 2.5–5.0 mg/ml against *Staphylococcus aureus* and *Pseudomonas aeruginosa* which is comparatively lower to our study where MBC was 12.5mg/ml[29]. Al-Bayati stated that presence of higher content of organo-sulphur compounds and thiosulfate compound (Allicin) in the *Allium sativum* are responsible for its antibacterial effects. Variation in the inhibitory zone in different bacterial isolates are may be due to permeability of allicin and other components of *Allium sativum* to the bacteria[32].

Coriandrum sativum has shown maximum antibacterial activity against *Acinetobacter baumannii* with zone of inhibition 17.4 ± 1.27 to 15.6 ± 1.51mm and minimum against *Staphylococcus epidermidis* with zone of inhibition 14.8 ± 1.49 to 9.8 ± 1.25 mm at all concentrations. Study done by Rathabai has shown comparatively less zone of inhibition of 9.90±0.10mm and 12.17±0.29mm against *Pseudomonas aeruginosa* and *Staphylococcus aureus* by methanolic extract of Coriandrum at higher concentration of (1gm/ml)[33]. Another study done by Bakhet *et al.* showed that the extract of *C. sativum* when used in different concentrations (100, 50 and 10%) has shown inhibition zones of 13-11mm for *Staphylococcus aureus*, 13mm for *E. coli*, 9-7mm for *Pseudomonas aeruginosa* which is quite close to our study[34]. MBC in our study was found to be 12.5mg/ml for *Staphylococcus aureus* but for *Acinetobacter baumanni* and *Pseudomonas aeruginosa*, the MBC was 25mg/ml. Similar findings were shown by Alireza *et al*.[35] who reported the MBC value of 25mg/ml against *Staphylococcus aureus* and 50mg/mL for *Pseudomonas aeruginosa*. Our study has showed that *Coriandrum sativum* did not have much effect on inhibiting the biofilm and it was supported by study done by Bezalwar *et al*.[36] and Abraham *et al*.[37] as their research revealed no effect of coriander extract on biofilm inhibition. These results showed that the *Coriandrum sativum* was not efficient in reducing biofilm but have certain antibacterial properties when used at higher concentration range. The difference in the antimicrobial properties of these herbs to the bacterial strains is may be due to different bio-reactive substances present in extracts with different processing techniques.

CONCLUSION

In this study, all the four plant extracts have shown their effectiveness against the multidrug-resistant bacteria but overall *Syzygium aromaticum* and *Camellia sinensis* were found to be better than *Allium sativum* and *Coriandrum sativum*.

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CONFLICT OF INTEREST

All authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed above have made a substantial, direct and intellectual contribution to the work and approved it for publication.

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None.

DATA AVAILABILITY

All datasets analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any study with human participants or animals performed by any Authors.

REFERENCES

1. Faujdar SS, Bisht D, Sharma A. Antibacterial activity of *Syzygium aromaticum* (clove) against uropathogens producing ESBL, MBL, and AmpC beta-lactamase: Are we close to getting a new antibacterial agent? *J Family Med Prim Care.* 2020; **9**(1):180-186. PMID: 32110587; PMCID: PMC7014883. https://doi.org/10.4103/jfmpc.jfmpc_908_19
2. Nitisha Malik, DakshinaBisht and Sameer Singh Faujdar. Extended Spectrum β Lactamases and Metallo β-Lactamases Production in *Klebsiella pneumoniae* Isolates Causing Pneumonia in Rural Population of Uttar Pradesh. *Int.J.Curr.Microbiol.App.Sci*. 2019; **8**(06): 1732- 1738. https://doi.org/10.20546/ijcmas.2019.806.207
3. Jastaniah SD. The antimicrobial activity of some plant extracts, commonly used by Saudi people, against multidrug resistant bacteria. *Life Sci J.* 2014; **11**(8):78–84.
4. Mill Robertson FC, Onyeka CI, Tay SCK, Walana W. *In vitro* antimicrobial activity of antibact, and herbal medicinal productt against standard and clonicalbacterila isolates. *J Med Plants Res*. 2015; **9**(11): 370–8. https://doi.org/10.5897/JMPR2015.5758
5. S.S. Grant and D.T. Hung. Persistent bacterial infections, antibiotic tolerance, and the oxidative stress response. *Virulence* 2013; **4**(4): 273–283. https://doi.org/10.4161/viru.23987
6. T.B. Rasmussen and M. Givskov.Quorum-sensing inhibitors as anti-pathogenic drugs. *International J Med Microbiol.,* 2006; **296**(2-3), 149–161. https://doi.

org/10.1016/j.ijmm.2006.02.005

7. Sun F, Qu F, Ling Y, Mao P, Xia P, Chen H, et al. Biofilm-associated infections: antibiotic resistance and novel therapeutic strategies. *Future Microbiology* 2013; **8**(7): 877–886. https://doi.org/10.2217/fmb.13.58
8. L. C. Simoes, M. Simoes, andM. J. Vieira. Biofilm interactions between distinct bacterial genera isolated from drinking water. *Applied and Environmental Microbiology* 2007; **73**(19): 6192–6200. https://doi.org/10.1128/AEM.00837-07
9. Mickiene R, Bakutis B, Baliukoniene V. Antimicrobial activity of two essential oils. *Ann Agric Environ Med* 2011; **18**: 139-144.
10. Prashant KR, Dolly J, Singh KR, Gupta KR, Watal G. Glycemic properties of *Trichosanthes dioica* leaves. *Pharm Biol* 2008; **46**(12): 894-899. https://doi.org/10.1080/13880200802370167
11. Nagwa A. Abdallah, Rafat Z. Abd El –Rahman, Arega K. Amer , Lamia I . M. Ismaeel. Antibacterial Activity of Some Plant Extracts on The Treatment of Surgical Wounds Infection. *Egypt. J. Exp. Biol.* (Bot.) 2013; **9**(1): 115 – 124.
12. Abhishek Mehta, Gaurav Saxena and Abin Mani. Comparative Analysis of Antibacterial Activity of Aqueous, Ethanolic, Methanolic and Acetone Extracts of Commercial Green Tea and Black Tea against Standard Bacterial Strains. *Int.J.Curr.Microbiol.App. Sci.* 2016;**5**(11): 145-152. https://doi.org/10.20546/ijcmas.2016.511.017
13. Ninyio, Nathaniel Nyakaat Fadason, Tayaza Bala and Maidawa, Godwin Zakariya. Anti-Biofilm Effect of *Allium sativum* Extract on Clinical Isolates of *Staphylococcus aureus*. *Nigerian Journal of Microbiology* 2016; **30**(2): 3494-3500.
14. Ankita Verma, Deepali Agarwal, Jyotsna Dhanik, Neelam Arya and Vivekanand. Antibacterial Efficacy and Cluster Analysis of Genotypic Extracts of Coriandrum Leaves and Seeds against Human Pathogenic Bacterial Strains. *Int.J.Curr.Microbiol.App.Sci.* 2018; **7**(05): 2727-2736. https://doi.org/10.20546/ijcmas.2018.705.316
15. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect*. 2012; **18**: 268–81. https://doi.org/10.1111/j.1469-0691.2011.03570.x
16. O'toole, G.A., Kolter, R. Initiation of biofilm formation in Pseudomonas fluorescens WCS365 proceeds via multiple, convergent signalling pathways: a genetic analysis. *Mol. Microbiol* 1998; **28**, 449-461. https://doi.org/10.1046/j.1365-2958.1998.00797.x
17. Syed H. Abidi, Khalid Ahmed, Sikander K. Sherwani and Shahana U. Kazm.Reduction and removal of *Pseudomonas aeruginosa* biofilm by naturalagents. *International Journal of Chemical and Pharmaceutical Sciences* 2014; **5**(1): 28-34.
18. Shakti Rath, Debasmita Dubey, Mahesh C Sahu, Nagen K Debata, Rabindra N Padhy. Surveillance of multidrug resistance of 6 uropathogens in a teaching hospital and in vitro control by 25 ethnomedicinal plants used by an aborigine of India. *Asian Pacific Journal of Tropical Biomedicine* 2012; S818-S829. https://doi.org/10.1016/S2221-1691(12)60319-0
19. JD N'guessan, M R Dinzedi, N Guessennd, A Coulibaly, M Dosso, A J Djaman,et al. Antibacterial activity of the aqueous extract of Thonningiasanguinea against Extended-Spectrum-β- Lactamases (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* strains. *Tropical Journal of Pharmaceutical Research,* September 2007; **6**(3): 779-783. https://doi.org/10.4314/tjpr.v6i3.14659
20. MilosNikolic, Sava Vasic, JelenaDurdevic, OlgicaStefanovic and Ljiljanacomic. Antibacterial and Anti-biofilm activity of ginger (*Zingiber officinale* (roscoe)) ethanolic extract: *Kragujevac J. Sci.*2014; **36**: 129-136. https://doi.org/10.5937/KgJSci1436129N
21. Anita Dua, Gaurav Garg, Sushil Nagar, Ritu Mahajan. Methanol extract of clove (*Syzygium aromaticum* Linn.) damages cells and inhibits growth of enteropathogens. *Journal of Innovative Biology* 2014; **1**(4): 200-205.
22. Neelima Christopher, Rajesh Ramachandran and KhaleelAhamedThaha. Anti Biofilm and Anti Plasmid Activites of *Syzygium aromaticum* and *Kaempheria galanga* against *Pseudomonas aeruginosa*. *Int.J.Curr. Microbiol.App.Sci.* 2017; **6**(1): 859-865. https://doi.org/10.20546/ijcmas.2017.601.101
23. Iram Liaqat, Najma Arshad, Muhammad Arshad, Safdar Ali Mirza, Nazish Mazhar Ali and AmmaraShoukat. Antimicrobial Activity of Some Medicinal Plants Extracts Against Food Industry Isolates. *Pakistan J. Zool.* 2017; **49**(2), 523-530. https://doi.org/10.17582/journal.pjz/2017.49.2.523.530
24. Dinesh Maharjan, Anjana Singh, Binod Lekhak, Shaila Basnyat, Lekhnath S. Gautam. Study on Antibacterial Activity of Common Spices. *Nepal Journal of Science and Technology* 2011; **12**: 312-317. https://doi.org/10.3126/njst.v12i0.6518
25. N. S. Chavan, R. D. Phadtare, and T. B. Chavan. Effect of aqueous extracts of different medicinal plants on control of *Streptococcus mutans. Int. J. Curr. Microbiol. App. Sci* 2015; **4**(4): 1072-1081.
26. I Liaqat, Q Pervaiz, S Jamil Bukhsh, SI Ahmed and N Jahan. Investigation of Bactericidal Effects of Medicinal Plant Extracts on Clinical Isolates and Monitoring Their Biofilm Forming Potential. *Pak Vet J.,* 2016; **36**(2): 159-164.
27. S. Archana and Jayanthi Abraham. Comparative analysis of antimicrobial activity of leaf extracts from fresh green tea, commercial green tea and black tea on pathogens. *Journal of Applied Pharmaceutical Science* 2011; **01**(08): 149-152.
28. Barat Ali Fakheri, Samaneh Bagheri, Nafiseh Mahdi Nezhad. Comparison of Antimicrobial and Antioxidant Activities of Four Different Tea Extracts. *Med Microbiol Infec Dis*, 2015, **3** (3-4): 57-61.
29. Zeinab Mohsenipour, Mehdi Hassanshahian. The Effects of *Allium sativum* Extracts on Biofilm Formation and Activities of Six Pathogenic Bacteria. *Jundishapur J Microbiol*. 2015; **8**(8): e18971. https://doi.org/10.5812/jjm.18971v2
30. Packia Lekshmi N. C. J., Viveka S., Jeeva S. and Raja Brindha J. Efficacy of crude extracts of *Allium sativum* and *Allium cepa* against human pathogens. *Adv. Appl.*

Sci. Res. 2015; **6**(1):72-78.

31. Shams S., Mehdipour A., Kermani S., Ghorbani H., Ragolia S., Anti-Biofilm Activity of *Punica granatum, Ricinus communis,* and *Allium sativum* Plant Extracts on *Streptococcus mutans*. *Infection Epidemiology and Microbiology*. 2018; **4**(2):67-72.
32. Shatha Al Bayati. Antibacterial Effect of Ethanolic Extract of *Allium sativum* on Biofilm Forming *Staphylococcus aureus* which Cause Folliculitis. *Int. J. Curr. Microbiol.App.Sci*. 2018;**7**(01): 1904-1913. https://doi.org/10.20546/ijcmas.2018.701.229
33. V. Rathabai and Kanimozhi. D. Evaluation of Anti Microbial Activity of *Coriandrum sativum*. *International Journal of Scientific Research and Reviews* 2012; **1**(3), 01-10.
34. Amel O. Bakhiet,Sabna D. Mohammed ,Samia, M.A. El Badwi , Warda, S. Abdel Gadir, Abdel-GadirH.Alkhatim and S.E.I. Adam. Antimicrobial Activity of *Petroselinum sativum* and *Coriandrum sativum* Seeds. *Research Journal of Microbiology* 2006; **1**: 346-352. https://doi.org/10.3923/jm.2006.346.352
35. Vasiee Alireza, Tabatabaei Yazdi Farideh, Mortazavi Seyed Ali. The Antibacterial Activity of Coriandrum (*Coriandrum sativum*) On Pathogenic Microorganisms "*In vitro*". *Iranian Journal of Infectious Diseases And Tropical Medicine* 2016; **20** (71): 59-66.
36. Bezalwar P. M, Shuddhalwar P. P. A Quest of Anti-Biofilm Activity of *Zingiber officinale* Root and *Coriandrum sativum* Seed Extract Against Clinical Isolates of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *International Journal of Science and Research* 2015; **4**(4): 2581-84.
37. Karlapudi P. Abraham et al. Investigation of the Potential Antibiofilm Activities of Plant Extracts. *Int J Pharm PharmSci.,* 2012; **4** (4): 282-285.