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 5 minutes at
3600g until clear supernatant was obtained. The supernatant was then filtered using 0.2 um filter (Micropore filters), and stored at 4⁰C until further use.

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 37⁰C. After incubation, the diameter of inhibitory zones around each disc were measured in mm and recorded.

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 100µL of microorganism suspension at a final density of 10⁵ cells/ml were added. The tubes were incubated at 37⁰C 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.

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 was added. 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:

\[ \frac{([OD\ growth\ control - OD\ sample])}{OD\ growth\ control} \times 100. \]

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

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 ± 1.00 mm |                | 59%                      |
|                            | 12.5                   | 21.7 ± 1.53 mm |                | 55%                      |
|                            | 6.25                   | 20.7 ± 1.58 mm |                | 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 ± 1.53 mm | 6.25 | 55%                      |
|                            | 25                     | 24.3 ± 0.58 mm |                | 51%                      |
|                            | 12.5                   | 22.3 ± 1.15 mm |                | 57%                      |
|                            | 6.25                   | 20.3 ± 1.53 mm |                | 54%                      |
| Acinetobacter baumannii (07 isolates) | 50                     | 24.1 ± 2.11 mm | 6.25 | 53 ± 0.023% |
|                            | 25                     | 22.9 ± 1.34 mm |                | 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 ± 1.53 mm | 6.25 | 57%                      |
|                            | 25                     | 22.7 ± 0.58 mm |                | 54%                      |
|                            | 12.5                   | 22.0 ± 2.00 mm |                | 53%                      |
|                            | 6.25                   | 16.0 ± 1.00 mm |                | 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 ± 0.58 mm | 6.25 | 66%                      |
|                            | 25                     | 24.7 ± 1.53 mm |                | 57%                      |
|                            | 12.5                   | 23.0 ± 1.00 mm |                | 54%                      |
|                            | 6.25                   | 21.3 ± 1.15 mm |                | 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 ± 1.00 mm | 6.25 | 65%                      |
|                            | 25                     | 23.7 ± 1.53 mm |                | 55%                      |
|                            | 12.5                   | 22.7 ± 0.58 mm |                | 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) in Table 3.

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 ± 1.49mm                   | 6.25         | 73 ± 0.023%                  |
|                                  | 25                     | 16.0 ± 1.17mm                   |              | 71 ± 0.032%                  |
|                                  | 12.5                   | 15.2 ± 1.28mm                   |              | 64 ± 0.022%                  |
|                                  | 6.25                   | 18.1 ± 1.28mm                   |              | 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 ± 1.46mm                   | 6.25         | 71 ± 0.013%                  |
|                                  | 25                     | 24.7 ± 1.50mm                   |              | 73 ± 0.019%                  |
|                                  | 12.5                   | 20.1 ± 1.35mm                   |              | 66 ± 0.026%                  |
|                                  | 6.25                   | 24.7 ± 1.11mm                   |              | 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 ± 1.19mm                   | 6.25         | 63 ± 0.021%                  |
|                                  | 25                     | 24.2 ± 1.28mm                   |              | 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) | 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 ± 1.61 mm) 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*  | 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*  | 50                     | 16.6 ± 1.15 mm                 | 12.5        | 35%                         |
| (ATCC 25923)             | 25                     | 17.0 ± 2.00 mm                 | 25%         |
|                          | 12.5                   | 16.0 ± 1.00 mm                 | 22%         |
|                          | 6.25                   | 15.9 ± 0.58 mm                 | 22%         |
| *Pseudomonas aeruginosa* | 50                     | 18.9 ± 1.61 mm                 | 12.5        | 32 ± 0.022%                 |
| (20 isolates)            | 25                     | -                              | 33 ± 0.029% |
|                          | 12.5                   | -                              | 19 ± 0.030% |
|                          | 6.25                   | -                              | 20 ± 2.0%   |
| *Pseudomonas aeruginosa* | 50                     | 19.3 mm                        | 12.5        | 31%                         |
| (ATCC 27853)             | 25                     | -                              | 35%         |
|                          | 12.5                   | -                              | 24%         |
|                          | 6.25                   | -                              | 18%         |
| *Acinetobacter baumannii*| 50                     | 14.9 ± 1.35 mm                 | 12.5        | 25 ± 0.052%                 |
| (07 isolates)            | 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*| 50                     | 16.3 ± 0.58 mm                 | 12.5        | 24%                         |
| (ATCC 19606)             | 25                     | 15.3 ± 1.20 mm                 | 25%         |
|                          | 12.5                   | 15.3 ± 1.50 mm                 | 23%         |
|                          | 6.25                   | 13.0 ± 1.00 mm                 | 20%         |
| *Staphylococcus epidermidis* | 50                     | -                              | 12.5        | 34 ± 0.086%                 |
| (08 isolates)            | 25                     | -                              | 13 ± 0.012% |
|                          | 12.5                   | -                              | 36 ± 0.101% |
|                          | 6.25                   | -                              | 21 ± 0.062% |
| *Staphylococcus epidermidis* | 50                     | -                              | 12.5        | 37%                         |
| (ATCC 12228)             | 25                     | -                              | 16%         |
|                          | 12.5                   | -                              | 32%         |
|                          | 6.25                   | -                              | 26%         |
| *Staphylococcus saprophyticus* | 50                     | -                              | 12.5        | 43 ± 0.028%                 |
| (05 isolates)            | 25                     | -                              | 41 ± 0.064% |
|                          | 12.5                   | -                              | 36 ± 0.030% |
|                          | 6.25                   | -                              | 20 ± 0.105% |
| *Staphylococcus saprophyticus* | 50                     | -                              | 12.5        | 47%                         |
| (ATCC 15305)             | 25                     | -                              | 44%         |
|                          | 12.5                   | -                              | 33%         |
|                          | 6.25                   | -                              | 24%         |
**Coriandrum sativum** has shown maximum zone of inhibition (17.4 ± 1.27 mm) 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) (mm) | MBC (mg/mL) | Biofilm Reduction (Mean ± SD) |
|------------------------|-------------------------|--------------------------------------|-------------|-------------------------------|
| *Staphylococcus aureus* (18 isolates) | 50                      | 15.9 ± 1.55                          | 12.5        | 42 ± 0.066%                   |
|                        | 25                      | 15.6 ± 1.50                          | 44 ± 0.027% |
|                        | 12.5                    | 14.6 ± 1.14                          | 36 ± 0.103% |
|                        | 6.25                    | 15.7 ± 1.32                          | 38 ± 0.048 %|
| *Staphylococcus aureus* (ATCC 25923) | 50                      | 17.0 ± 1.00                          | 12.5        | 45%                           |
|                        | 25                      | 16.3 ± 0.58                          |             |                               |
|                        | 12.5                    | 14.6 ± 0.58                          |             |                               |
|                        | 6.25                    | 13.3 ± 1.53                          |             |                               |
| *Pseudomonas aeruginosa* (20 isolates) | 50                      | 14.8 ± 1.71                          | 25.0        | 42 ± 0.040%                   |
|                        | 25                      | 12.2 ± 1.86                          | 39 ± 0.115% |
|                        | 12.5                    | 14.6 ± 1.69                          | 39 ± 0.058% |
|                        | 6.25                    | 11.9 ± 1.37                          | 27 ± 0.106% |
| *Pseudomonas aeruginosa* (ATCC 27853) | 50                      | 15.3 ± 0.58                          | 25.0        | 40%                           |
|                        | 25                      | 13.0 ± 1.00                          |             |                               |
|                        | 12.5                    | 13.6 ± 1.53                          |             |                               |
|                        | 6.25                    | 10.6 ± 1.53                          |             |                               |
| *Acinetobacter baumannii* (07 isolates) | 50                      | 17.4 ± 1.27                          | 25.0        | 42 ± 0.040%                   |
|                        | 25                      | 15.6 ± 1.51                          | 39 ± 0.115% |
|                        | 12.5                    | 16.7 ± 1.49                          | 39 ± 0.058% |
|                        | 6.25                    | 16.8 ± 1.46                          | 27 ± 0.106% |
| *Acinetobacter baumannii* (ATCC 19606) | 50                      | 17.6 ± 0.58                          | 25.0        | 39%                           |
|                        | 25                      | 16.0 ± 1.00                          |             |                               |
|                        | 12.5                    | 15.8 ± 1.15                          |             |                               |
|                        | 6.25                    | 14.6 ± 1.53                          |             |                               |
| *Staphylococcus epidermidis* (08 isolates) | 50                      | 14.8 ± 1.49                          | 50.0        | 34 ± 0.086%                   |
|                        | 25                      | 11.1 ± 1.46                          | 13 ± 0.012% |
|                        | 12.5                    | 13.0 ± 1.31                          | 36 ± 0.101% |
|                        | 6.25                    | 9.80 ± 1.25                          | 21 ± 0.062% |
| *Staphylococcus epidermidis* (ATCC 12228) | 50                      | 14.6 ± 0.58                          | 50.0        | 37%                           |
|                        | 25                      | 12.6 ± 0.58                          |             |                               |
|                        | 12.5                    | 13.0 ± 1.00                          |             |                               |
|                        | 6.25                    | 10.6 ± 1.53                          |             |                               |
| *Staphylococcus saprophyticus* (05 isolates) | 50                      | 15.2 ± 1.48                          | 50.0        | 43 ± 0.028%                   |
|                        | 25                      | 13.2 ± 1.30                          | 41 ± 0.064% |
|                        | 12.5                    | 13.0 ± 1.48                          | 36 ± 0.030% |
|                        | 6.25                    | 12.8 ± 1.64                          | 20 ± 0.105% |
| *Staphylococcus saprophyticus* (ATCC 15305) | 50                      | 15.3 ± 1.53                          | 50.0        | 45%                           |
|                        | 25                      | 13.6 ± 0.58                          |             |                               |
|                        | 12.5                    | 12.0 ± 1.00                          |             |                               |
|                        | 6.25                    | 10.3 ± 1.53                          |             |                               |

**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, 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 too25,26. 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 Fakheri28 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 perturbation12.

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/ml29. 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 bacteria32.
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)\(^3\). 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\(^4\). MBC in our study was found to be 12.5mg/ml for Staphylococcus aureus but for Acinetobacter baumannii and Pseudomonas aeruginosa, the MBC was 25mg/ml. Similar findings were shown by Alireza et al.\(^5\) 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.\(^6\) and Abraham et al.\(^7\) 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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ETHICS STATEMENT
This article does not contain any study with human participants or animals performed by any Authors.

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