Antibacterial Effects of *Camellia sinensis* and *Achillea millefolium* on Several Antibiotic-resistant Bacteria

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Authors’ contributions

This work was carried out in collaboration between all authors. Authors AA and SAM designed the study and wrote the first draft of the manuscript. Author AA performed the statistical analysis. Author MRK managed the literature searches. Author FK prepared the plant and author DS prepared the bacteria. Author AM prepared the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

**Background and Objectives:** Infectious diseases are among the most known important causes of morbidity and mortality in the world. The ongoing explosion antibiotic-resistant bacteria and side effects of medications, application of assistive methods including the use of medicinal plants for treatment have become particularly important.

**Materials and Methods:** In this study, the ethanol extract of green tea (*Camellia sinensis*) and yarrow flower (*Achillea millefolium*) were prepared by maceration method and its antibacterial effect on four clinical strains of antibiotic resistant bacteria including *Klebsiella pneumoniae, Acinetobacter, Escherichia coli* and *Pseudomonas aeruginosa* were evaluated with microtiter plate method for determining the MIC (Minimum Inhibitory Concentration).
**Results:** The MICs of green tea extract for *Acinetobacter baumannii* was 15.6 mg/ml and 31.25 mg/ml for *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli*. The MIC of *Achillea millefolium* extract for *Acinetobacter baumannii* and *Escherichia coli* was 125 mg/ml and against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* was 250 mg/ml.

**Conclusion:** Considering the antimicrobial effects of green tea and *Achillea* on four strains of resistant bacteria, the extract of this plant can be used in control and treatment of infections caused by these infectious agents.

**Keywords:** *Camellia sinensis*; *Achillea millefolium*; minimum inhibitory concentration; antibiotic-resistant bacteria.

### 1. INTRODUCTION

Emerging infectious diseases imposes a significant financial burden on global economies and health [1]. Increasing resistance to common antibiotics due to irregular use of them, has threatened the public health. To prevent this threat, identification and use of new antibacterial compounds is required [2]. Natural products derived from medicinal plants as major sources of biologically active compounds have been important candidates for production of new chemicals in the pharmacy. There are about 500,000 plant species around the world that only one percent of their phytochemicals have been evaluated, and there is a great potential for the discovery of novel bioactive compounds [3]. Recently, the use of herbal medicines are widely used because of low side effects, low cost, easy access and compatible with human nature [4]. The plants have been used in traditional medicine for thousands of years and even with the advances of modern medicine, products derived from medicinal plants have become the basis for expansion of new chemical drugs in pharmacy [5].

Green tea (*Camellia sinensis*) is one of the most consumed beverage in the world especially Iran [6], increase in the use of green tea is day by day due to its wide range of disease treating potential. Green tea contains protein, amino acids, carbohydrates minerals, trace elements, lipids, vitamins B, C and E, caffeine, theophylline, poly phenolic compounds [7]. Green tea has showed antibacteri activity against a many strain of bacteria, both Gram positive and Gram negative bacteria [8] and showed antiviral effect [7].

*Achillea millefolium* L. belonging to the Family Asteraceae that grows in Asia, Europe, North Africa, North America and Iran [9]. *Achillea* species contains Proazulenes, essential oils, flavonoids, coumarins, terpenoids, sterols, lignans, amino acids and alkamides [10]. *Achillea* species have several effects such as antibacterial, anti-inflammatory, antitumor, anti cirrhosis, anti fever, and useful for curing insomnia, stopping the bleeding and for wound healing [9,11].

The aim of this study is evaluation of antibacterial effects of ethanol extract of *Achillea millefolium* and green tea on several strains of antibiotic-resistant human pathogenic bacteria in the laboratory condition.
2. MATERIALS AND METHODS

2.1 Plant Preparation and Extraction

Yarrow plants in late June, its flowering season, were collected from the region of Sardasht, Lordegan city located in the province of Chahar Mahal and Bakhtari. The plant samples were evaluated and registered as *Achillea wilhelmsii* with herbarium number 19706 by Leila Ghaem Maghami at University of Isfahan.

Flowers were isolated and dried under appropriate conditions, away from light and moisture. Then for optimized extraction of flowers, they were powdered with electric mill.

Green tea was purchased from medicinal plants market of Isfahan.

The dried powder was mixed with 50% ethanol solution. After 72-48 hours it was filtered through Buchner funnel. Then extract was placed in the rotary evaporator (Heidolph Company, Germany) in order to separate the solvent from the extract [12]. Then, they were put in the oven at 40°C to dry completely.

2.2 Bacterial Isolates and Media Preparation

Four antibiotic resistant isolates of bacteria including *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Escherichia coli* and *Pseudomonas aeruginosa* were isolated from patients who referred to Al-Zahra hospital, Isfahan, IRAN.

2.3 Determination of Minimum Inhibitory Concentration (MIC)

The extracts were tested against pathogens by a micro-dilution broth method for determine of minimum inhibitory concentration (MIC). bacteria fresh culture was diluted at tryptose soy broth (TSB: Quelab, Montreal, Canada), the turbidity equal to 0.5 McFarland was prepared and diluted at a ratio of 1 to 100 to obtain turbidity equal to $1 \times 10^6$. Different dilutions (Serial dilutions) extracts were prepared in broth media. Then 100 µl of different dilutions of extract containing 100 µl of bacterial suspension were poured in 96-well polystyrene plates [13]. Also, wells containing 200 µl of broth media and wells containing broth media and bacteria, were considered as negative and positive controls, respectively. Some wells containing 100 µl media and 100 µl from each dilution were considered as turbidity controls. Three replications were considered for each bacteria. Then, the plate surfaces were covered and incubated in incubator for 24 h at 37°C. The growth of bacteria was measured at 630 nm using a micro-plate reader (AWARENESS, Technology INC, Atat fax 2100).

2.4 Statistic Analysis

The data were statistically analyzed (One way ANOVA and Tukey’s post hoc tests) on Graf Pad Prism 5 software (Graph Pad Software Inc., CA, USA). A P-value of less than 0.05 was considered statistically significant.

3. RESULTS

Results of tests for evaluation of sensitivity of bacteria to several antibiotics are shown in Table 1 indicated that this bacteria have resistant to many of examined antibiotics.

In this study, the minimum inhibitory concentrations of green tea and yarrow flower extracts against four strains of antibiotics resistant pathogenic bacteria were investigated and the results are shown in Table 2.

MIC of green tea for *Acinetobacter baumannii* was 15.6 mg/mL which was significantly less than that for the other three bacteria (P<0.05). and for *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa* was 31.25 mg/mL

In the case of yarrow flowers, the highest MIC, 250 mg/ml, was related to *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, which was significantly (P <0/05) higher than two other bacteria. The MIC of green tea on four bacterial strains was less than that of *Achila millefolium* (P <0/05), indicating its higher antibacterial activity.

The inhibitory effects of different concentrations of green tea and yarrow flower (*Achila millefolium*) extracts on the growth of the tested bacteria are shown in Fig. 1. In 500-125 mg/mL concentrations of yarrow flower, the growth of *Escherichia coli* and *Acinetobacter baumannii* decreased >90% and in 500-250 mg/mL of yarrow flower, the growth of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* decreased >90%. In addition, in 500-31.25 mg/mL concentrations of Green tea the growth of *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa* its 500-15.6 mg/mL concentration, the growth of *S. sanguis* decreased >90%.
Table 1. Determination of sensitivity of tested bacteria against common antibiotics

| Klebsiella pneumoniae | Acinetobacter baumannii | Escherichia coli | Pseudomonas aeruginosa |
|----------------------|-------------------------|------------------|------------------------|
| **R**                | **S**                   | **R**            | **S**                  |
| Amikacin             | Colistin                | Amikacin         | Colistin               |
| Gentamicin           | Chloramphenicol         | Gentamicin       | Ciprofloxacin          |
| Ciprofloxacin        | Ciprofloxacin           | Levofoxacin      | Meropenem              |
| Levofoxacin          | Levofoxacin             | Ceftazidime      | Meropenem              |
| Ceftazidime          | Ceftazidime             | Ceftaxime        | Imipenem               |
| Cefotaxime           | Cefotaxime              | Imipenem         | Ceftriaxone            |
| Imipenem             | Imipenem                | Ceftriaxone      | Meropenem              |
| Ceftriaxone          | Ceftriaxone             |                  |                        |
| Meropenem            | Meropenem               |                  |                        |

- **S** = Sensitive
- **R** = Resistant

Table 2. Values of MIC for the four strains of antibiotic resistant pathogenic bacteria

| MIC (mg/ml) | Pseudomonas aeruginosa | Escherichia coli | Acinetobacter baumannii | Klebsiella pneumoniae |
|-------------|------------------------|------------------|-------------------------|------------------------|
| **Camellia sinensis** | 31.25                  | 31.25            | 15.6                    | 31.25                  |
| **Achila millefolium** | 250                    | 125              | 125                     | 250                    |
Achillea millefolium

Green tea

Fig. 1. The effect of various concentrations of ethanol extract of yarrow flowers and green tea on the growth of four strains of antibiotic resistant pathogenic bacteria

Curve X = Various dilution tested extracts
Curve Y = Absorbance at 630 nm

4. DISCUSSION

Recently, there has been a growing interest in the use of herbal medicine in developed and developing countries [14]. Plant medicine has been considered to treat different diseases, especially infectious ones. Today, there is a universal tendency toward utilizing plant drugs instead of chemical drugs. Wide acceptance of the plant drugs in the field of Medical sciences has been observed due to less side effects, as well as appropriate prices [15].

Green tea is the second oldest and largest most consumed drinks in the world [7]. Recent studies suggest that green tea help to reduction in the risk of cardiovascular disease and cancer, help to oral health and have anti-hypertensive effect, body weight control, antibacterial and antivirasic activity, UV protection [16].

Achillea millefolium L. is belongs to the Asteraceae family that is medicinal plant and used in folk medicine against gastrointestinal disorders, lack of appetite [17].
In this study, antimicrobial activity of green tea and yarrow flower on four strains of antibiotic resistant bacteria including *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Escherichia coli* and *Pseudomonas aeruginosa* was evaluated *in vitro*. The results showed that green tea and yarrow flower (*Achillea millefolium*) extracts has antibacterial effects these bacteria.

There are several study regarding to antimicrobial activity of Green tea and yarrow:

Dubey et al showed that the aqueous extract of Green tea have antibacterial effect against Standard Strains *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and clinical isolates of Methicillin Resistant *Staphylococcus aureus* (MRSA) and Multidrug Resistant *Pseudomonas aeruginosa* (MDRPA) [18].

Radjji et al. [19] studied the antimicrobial effects of green tea extract against *Staphylococcus aureus* resistant to mexitilin and multidrug-resistant *Pseudomonas aeruginosa* and showed that the plant extract has a favorable effect on these bacteria and showed MIC of green tea for *S.aureus* and MRSA were 400 µg/ mL, and MIC for *P.aeruginosa* and MDR-*P.Aeruginosa* were 800 µg/ mL, respectively.

Vashvaei et al showed the highest MIC of green tea extract was concentration of 2.5 mg/ml for 8 strains of *Pseudomonas aeruginosa*. And MIC of achiella extract against four strains of *Pseudomonas aeruginosa* was 5 mg/ml [6].

In our study, MIC of green tea extract for *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae* was 31.25 mg/ml and for *Acinetobacter baumannii* was 15.6 mg/ml.

Amjad et al. examined the antimicrobial effect of yarrow leaves and flowers on *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa* and reported that methanol extract of this plant is most effective on *Staphylococcus aureus* and *Bacillus cereus*, and the minimum inhibitory concentrations against them were 25 and 12.5 mg/ml respectively. They also showed that *P. aeruginosa* is resistant against this extract [20].

Saeedi et al. [21] examined the antimicrobial effect of aqueous extracts of yarrow and sage on 10 standard strains of human pathogenic bacteria including *Pseudomonas aeruginosa*, *Shigella shinga*, *Klebsiella pneumonia*, *Salmonella typhi*, *Proteus mirabilis*, *Serratia marcescens*, *Bacillus cereus*, *Enterobacter cloacae*, *Staphylococcus saprophyticus*, *Staphylococcus aureus* and showed that *Staphylococcus aureus* was most sensitive to MIC= 0/62 mg/ml.

In this study, MIC of yarrow flower extract for *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* was 250 mg/ml and for *Escherichia coli* and *Acinetobacter baumannii* was 125 mg/ml.

In our study, it was showed that effect of green tea on growth bacteria was significantly higher than that of yarrow flower (P<0.05). The MIC of green tea for *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Escherichia coli* and *Pseudomonas aeruginosa* were less than those of yarrow flower (P<0.05). However, further investigations are needed to determine cause of the findings.

5. CONCLUSION

Due to the positive effects of two Herbal extracts on hospital bacteria resistant to several common antibiotics, the plants can be used in different pharmaceutical formulation for the prevention and control of infectious diseases associated with these bacteria. It is recommended that additional studies be conducted to establish the optimal effect of the above extracts.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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