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Original research

Inhibitory effect of *Syzygium aromaticum* L. and *Coffea arabica* L. extract on *Staphylococcus aureus* in vitro and comparing it with selected antibiotics

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Abstract

Since antimicrobial resistance is on the rise, especially in vital microscopic organisms responsible for nosocomial contaminations, finding effective and safe anti-bacterial compounds is one of the most significant research areas. As an important bacterial pathogen, *Staphylococcus aureus* is considered one of the leading causes of nosocomial infections. In this study, based on the antimicrobial properties of medicinal plants, the inhibitory and anti-bacterial effects of aqueous extracts of *Syzygium aromaticum* L. and *Coffea arabica* L. on *S. aureus* bacteria were investigated by disk diffusion test. Aqueous extraction was executed, and the inhibitory effect of these plants was examined in the Muller-Hinton agar medium. The results were measured by a caliper. According to the results, clove and coffee aqueous extracts appear effective at creating a zone of inhibition against *S. aureus*. By increasing the concentration of the extract, larger zone of inhibitions, as large as selected antibiotics, are formed against the growth of *S. aureus*. Clove aqueous extract at a concentration of 100 µl exhibited a zone of inhibition of 30 mm. In contrast, coffee extract at the same concentration exhibited a zone of inhibition of 23 mm. Furthermore, the inhibition zones created by the selected antibiotics, tetracycline and vancomycin, were found to be 24 mm and 27 mm, respectively. As a final result, clove and coffee aqueous extracts have a significant antimicrobial effect on *S. aureus*.

Keywords: Staphylococcus aureus, Coffee, Clove, Aqueous extract, Antibiotic

1. Introduction

Medicinal plants have long been known for their medicinal properties. In the past, as people sought out specific herbs to treat ailments, various plants were discovered as edible and medicinal poisons. With the advancement of science and technology, chemical drugs were also produced, and gradually medicinal plants lost their therapeutic value [1]. Due to the increased side effects caused by chemical drugs and the emergence of antibiotic resistance in recent decades, humans have been turning back to medicinal plants, which is why the 21st century is called the "return to nature" era [1].

Despite vast advances in scientific and medical knowledge, infectious diseases are still the leading cause of death worldwide [2]. Antibiotics have been the golden key to treating infectious diseases for a long time [3]. Even though new antibiotics are being synthesized in greater numbers and diversity, antibiotic resistance is rising due to improper use and poses a significant challenge to infection control [4]. Therefore, measures should be taken to resolve this

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issue [5, 6]. *Staphylococcus aureus* is the main cause of nosocomial infections and can cause many human-related infections [7]. Infections caused by *S. aureus* can occur in the blood, in the lower respiratory tract, on the skin, and in soft tissues, as well as in ventilator-associated pneumonia and intravenous catheter-associated bacteremia. This situation has been exacerbated by the increasing occurrence of strains that highly resist different antibiotics [8]. The importance of *S. aureus* as a human pathogen lies not only in its ability to cause a wide range of life-threatening infections but in its ability to develop resistance to antimicrobial agents as well [9-11]. The most well-known compound in coffee is caffeine, which has a wide range of physiological and medicinal properties [12]. Methylxanthine concentration is considerable in coffee. The physiological effects of caffeine include a reduction in sleep and heart muscle stimulation. Trigonelline is an alkaloid biologically derived from the enzymatic methylation of nicotinic acid, which can produce the B-complex vitamin, also known as niacin. Trigonelline inhibits cancer cell invasion in vitro, considering its potential biological activity. In addition, the compound can regenerate dendrites and axons in animal models, suggesting that it may rectify memory. Recently, it has been considered an anti-bacterial compound against *Streptococcus mutans* which causes tooth decay [13-15]. In addition to polyphenols, other natural substances such as trigonelline, caffeine, and alpha-dicarbonyl compounds have shown anti-bacterial activity against *S. mutans*. However, the results of caffeine have been controversial [16]. Caffeinated extracts have shown more anti-bacterial effects than decaffeinated extracts. The coffee extract can inhibit the growth of some microorganisms with a synergistic effect [13, 17]. *Syzygium aromaticum* L., also known as clove, is an aromatic plant with countless benefits that contains biologically active compounds. This plant contains eugenol, caryophyllene, and other substances, and it was initially produced in China. Its essential oil extract exhibits antimicrobial properties against several Gram-positive and Gram-negative organisms, including fungi, because of its oleic acids and lipids [18-20]. Eugenol is the primary substance of clove and is used as an antiseptic in dentistry. Clove has strong antimicrobial properties against *Enterococcus* and *Escherichia coli* and can restrict the growth of resistant bacteria in raw meat [21-23]. This study evaluates the inhibitory effect of *Syzygium aromaticum* L. and *Coffea arabica* L. aqueous extracts on *S. aureus*.

2. Materials and Methods

2.1 Bacteria

The standard strain BAA-976 of *S. aureus* was prepared in the Bacteriology Laboratory of Islamic Azad University in Ilam, Iran (2016), and after cultivation on Muller Hinton Agar (MHA) medium, suspensions with 0.5 to 4 McFarland dilutions were prepared. *S. aureus* was used to examine the anti-bacterial effect of plants. The chosen bacteria were cultured in a liquid LB medium for 24 hours to perform antimicrobial tests. Then, bacteria were cultured twice using the lawn culture method [24, 25].

2.2 Collection and extraction

To prepare aqueous extract of clove and coffee, samples were collected from the research laboratory of the Islamic Azad University of Ilam. Following sterilization, they were ground into powder and stored under optimal conditions. Extraction was done by boiling them in sterile distilled water and filtering them through filter paper. The extracts from both plant samples were obtained by boiling a concentration of 10 grams of plant powder with 100 ml of distilled water for 30 minutes. The resulting liquid was then filtered through a Buchner funnel and a standard filter paper.

2.3 Antimicrobial assay

It is possible to determine the microbial sensitivity using the disk/well diffusion method in agar. The disc diffusion method was used to perform the antimicrobial test, with each sample subjected to three repetitions. Tetracycline (30 µg), chloramphenicol (30 µg), and trimethoprim-sulfamethoxazole (1.25/23.75 µg) antibiotics were used to show microbial sensitivity. *S. aureus* suspension was prepared. The MHA medium was divided into four wells on each plate. After they were cultured, control antibiotics and different concentrations were added to the other two wells of each plate. The order of 10 microliters and repeating up to 100 microliters of clove and coffee plant extracts were poured separately [25]. Coffee herbal extract concentrations from 10, 15, 25, 50, and 75 to 100 microliters were injected into sterile wells created in separate plates. Antibiotic disks were put on

plates after culturing using the lawn method. The same steps were done in the above order for the clove extract. After 30 minutes, to dry out the clove and coffee extracts in the wells, they were placed in the incubator for 24 hours. A diameter measurement was used to determine the results (Figure 1, and 2). The inhibitory zones created by the antibiotics were measured and recorded by calipers, and interpreted based on the Clinical & Laboratory Standards Institute (CLSI) 2021 tables [26].

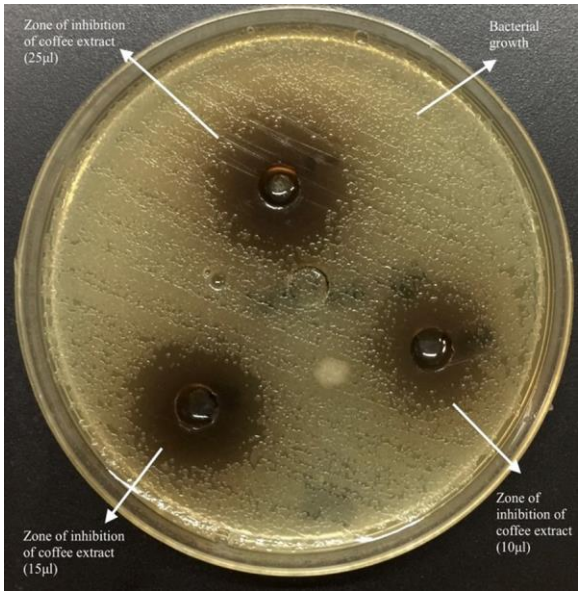

Figure 1. Shows zone of inhibition of the combination of coffee (*Coffea arabica* L.) in the volume of 10, 15 and 25 microliters.

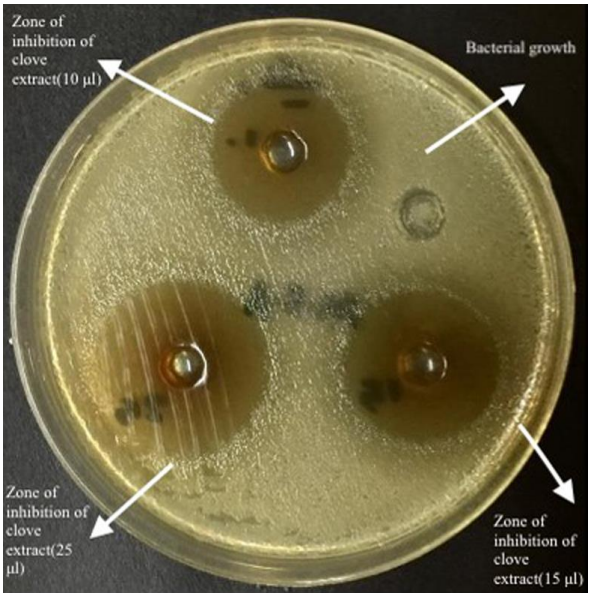

Figure 2. Shows the zone of inhibition of clove (*Syzygium aromaticum* L.) in the volume of 10, 15 and 25 microliters.

3. Results

In this study, tetracycline antibiotic is reported as sensitive to *S. aureus* since it forms a zone of inhibition with a diameter of 27 mm. Similarly, trimethoprim-sulfamethoxazole with a diameter of 30 mm exhibits sensitivity. In addition, chloramphenicol were found to be sensitive, with inhibition zones 33 mm. The average diameter of the zone of inhibition created by clove extract was 25.5 mm, while coffee extract created an average of 18.33 mm (Table 1).

Table 1. The average diameter of the zone of inhibition of *S. aureus* in the presence of coffee and clove extract.

Volume (mL)	Clove (mm)	Coffee (mm)
10	20	15
15	23	16
25	25	17
30	26	17
50	26	19
75	29	20
100	30	23

4. Discussion

This study was conducted to determine the effect of clove and coffee extracts on *S. aureus*. The results of this survey show that the clove and coffee extracts have a significant effect on preventing the growth of this bacteria. Other studies have shown that bacterial infections are a significant health problem worldwide. *S. aureus* is one of the most important pathogens that can cause various infections, from superficial skin infections such as thrush to dangerous diseases such as endocarditis and meningitis. This bacterium is a vital threat in veterinary medicine and the cause of severe diseases in livestock and animals [27]. Thus,

these extracts can be a very effective drug in treating staphylococcal infections. Many scientists and researchers around the world have also succeeded in discovering the anti-bacterial and inhibitory effects of other medicinal plants on this bacterium. Further, Monadi et al. (2015) conducted a study on this bacterium and found that methanolic extracts of four plants, Ziziphus Spina-Christi, Peganum Harmala, Salvia Officinalis, and Quercus Brantii, inhibited the formation of biofilm by *S. aureus*. They reported that methanolic extracts of Salvia Officinalis at the concentration of 350 mg had the greatest inhibitory effect on the formation of biofilms, while other extracts were effective at higher concentrations. Notably, 100 mg of our aqueous clove extract formed a zone of inhibition with a diameter of 30 mm, while only a 25 mm zone of inhibition was reported by Peganum harmala extract at a concentration of 350 ml. As a result of comparing, our clove extract is more effective against *S. aureus* than Peganum harmala [28]. Daglia et al. showed the synergistic effect of caffeine with alpha-dicarbonyl compounds in coffee against the same microorganism. A further study by Almeida et al. found that caffeine at concentrations present in the beverage (0.5 mg/ml to 1.0 mg/ml) temporarily inhibited *Streptococcus mutans* (4h), with higher caffeine concentrations required to achieve stronger and longer inhibitions. These researchers also observed that when coffee extracts were supplemented with caffeine, the anti-bacterial effect of the extract improved, indicating a synergistic effect [29]. In 2018, Ghorbani et al. showed that the highest inhibitory effect against *S. aureus* belonged to the ethanolic extract of the thyme plant in a 500 ml volume with a diameter of 28.33 mm. The inhibitory effect of clove and coffee in volumes of 100 ml and even less has shown a stronger effect on bacterial growth [30]. The results obtained in the study of the inhibitory effect of clove plant and coffee on *S. aureus* were obtained for the first time. In the past years, a group of dental students recorded the inhibitory effect of coffee on *Streptococcus pyogenes* and *S. mutans* and *Streptococcus pneumoniae* [31].

Additionally, Safahani et al. conducted a study on the medicinal plants of Golestan province in Iran against *S. aureus* bacteria. A disc diffusion method showed that Eucalyptus globulus, Punica granatum, and Berberis vulgaris inhibited bacterial growth. It was found in this study that Punica granatum peel extract possessed significant anti-bacterial properties [32]. Moon et al. suggest that clove oil and eugenol could be natural anti-bacterial agents against cariogenic bacteria. It has been demonstrated that clove oil inhibits the activity and growth of oral bacteria because its main compounds, such as ogle and beta-caryophyllene, effectively prevent tooth decay or pathogenic bacteria [22].

In the past, herbal medicines were used to treat most infectious diseases of different origins. Formerly, medicinal plants such as thyme and garlic were used [33, 34]. However, researchers today attempt to identify herbal materials with high efficacy and antimicrobial properties to treat bacterial infections. These plants can be used as natural medicine if their results are standardized to replace the current low-effective antimicrobial medications [32]. It is suggested that the extract of these plants can be used at least as a local antiseptic. The findings of this study can provide a method for replacing herbal medicines with chemical ones. This is imperative in the fight against drug resistance. There is no doubt that herbal medicines can contribute significantly to reducing the use of chemical medicines.

In this study, low volumes of clove extract were found to be effective against *S. aureus*, and using clove extract in combination with coffee could have a significant impact. Also, clove and coffee extracts have anti-bacterial properties and can be used as a cheap and available source for therapeutic use. However, more studies are needed in this regard so that the anti-bacterial effects of these two plants and their compounds can be accurately investigated.

Authors' contributions

All authors contributed equally to all aspect of work. Also, all authors read and approved the final version of article.

Conflict of interests

There is no conflict of interest.

Ethical declarations

The study design approved by local ethic committee of Ilam Islamic Azad University, Ilam, Iran.

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