

The Effect of (Aqueous-Alcoholic) Extracts and Black Pepper Essence on *Vibrio cholerae* and *Staphylococcus aureus*

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Background: plants been used for biological basis of drug substances in thousand years. Some of the plants have inhibitory effects on growth of intestinal infections. Black pepper is the plant that uses as classical medicine in the infections. *Vibrio cholerae* and *Staphylococcus aureus* are two important bacterial agents in the food contamination.

Objectives: In this study, inhibitory effects of (watery_alcoholic) extractions and essence of black pepper were studied against *S. aureus* and *V. cholerae*.

Materials and Methods: Extraction produced by soaking method then for determined antibacterial effects were used cylinder method. For preparation essence 50 gram of powdered black pepper was extracted by soaking, method and effect of essence was studied against the target cultuer by using the cup diffusion method. We also determined the MIC and MBC by micro dilution method.

Results: Essence of black pepper showed good antibacterial effect on this bacteria. MIC of essence on the *V. cholerae* and *S. aureus* was 38 µg/mL and 75 µg/mL, but alcohol and watery extractions didn't have antibacterial effect on these bacteria.

Conclusions: essence of black pepper showed a potent antibacterial activity and therefore, it may be used as an inhibitory extract against *S. aureus* and *V. cholerae* in food industry.

Keywords: Black Pepper; *Staphylococcus aureus*; *Vibrio cholerae*

1. Background

Development of new antimicrobial agents are necessary for several reasons like the emergence and rapid spread of drug resistant in different bacterial pathogens and increasing the spectra of untreatable disease. It is therefore imperative to develop safer and more potent compounds for the future that are not toxic to host. Apart from the drug design, another relatively simpler but extremely useful approach is to screen local medicinal plants in search of suitable antimicrobial substances (1). Cholera, diarrheal disease caused by enteric gram-negative bacteria *Vibrio cholerae*, continues to be a worldwide public health concern. *V. cholera*, is a major health threat in poor nations. It is widely acknowledged as one of the most important water borne pathogen of worldwide economic significance (2). In the other hand *S. aureus* produced five serological distinct enterotoxin that have an effect on the central nervous system that result in severe vomiting. with increasing resistance of bacteria to antibiotics and side effects associated with their application today Using plants with antimicrobial properties are common. Some of these plants, which are added to foods as flavor,

have different properties; they can be used as biological preservatives in order to reduce infections caused by food consumption. Generally, plants have the ability to produce secondary metabolites; about 1200 metabolites have been isolated till now. They are compounds such as aromatics, Phenolics, tannins, Alkaloids and terpenoids. Black pepper is used in Iranian traditional medicine in order to treat many infections. This plant known as piper nigrum, and is native from Vietnam and India; today it is cultivated in most tropical countries. Pepper fruit harvesting begins when it starts turning red and dried in the sun or artificial heat (3). An effective element of black pepper is terpenoids or essential volatile oils that its amount is 1.2 to 3.5 % which contains Diterpene, Sytral and sesquiterpene. Sesquiterpene includes cyclic compound of Chamazulene and this compound is the most important bactericide element of black pepper (2-4). *S. aureus* is an opportunistic pathogen which has an important role in various infectious diseases such as food poisoning. In recent years, the accession of increasing resistance among clinical strains of these bacteria may develop staphylo-

coccal infections at hospitals (5). *V. cholera* serogroup O1_139 is the cause of human cholera and its treatment in the initial stage is fluid and electrolyte replacement and then antibiotics therapy with tetracycline, there is portable resistance plasmids against this drug in *Vibrio cholera* in some endemic areas (6).

2. Objectives

Due to the importance of *S. aureus* and *V. cholera* in clinics and food hygiene, the purpose of this study was to evaluate the effect of extract and essential oil of black pepper on these bacteria.

3. Materials and Methods

For the preparation of aqueous-alcoholic extract, 10 gr of black pepper was added into 10 ml of ethanol and distilled water, and then rotated on incubator shaker gently for 72 hours. To separate the extract from the solvent mixture, Filtration was used. To obtain the essence, 100 gr of black pepper was added to 400 ml of distilled water and the mixture was poured into a balloon joje that placed over flame and then vacuum pump was connected to the balloon distillation apparatus. After 4 to 5 hours, 2cc essence was formed on the rosewater form on the thin glass tube in the distillation apparatus; this essence should be extracted gently by syringe (3). *S. aureus* ATCC25923 was prepared from Boali's reference center and clinical *V. cholerae* strain isolated from a patient with diarrhea illnesses in one of the hospitals in Mashhad for our research. After determining the final identification by biochemical tests, the sensitivity of *V. cholerae* were measured to nalidixic acid, chloramphenicol, tetracycline and erythromycin and for *S. aureus* to penicillin, erythromycin, tetracycline and Vancomycin by the agar diffusion method using the antibiotic standard discs of Padtan Company. Antimicrobial activity of (aqueous-alcoholic) extracts and black pepper essence were tested against *S. aureus* and *V. cholera* according to Ferreira and Meireles procedure (6, 7). To determine the relative minimum concentration that inhibits the growth of bacteria, serial dilution was prepared in MHB, MIC and MBC was determined based on standard procedure. The positive control tube (medium containing bacteria and ethanol without extract) and negative control (medium) were used besides tubes and incubated at 37°C for 24 hours (8, 9). Essence: MIC and MBC in various dilutions of essence was examined by microplate method. At first, essence suspension is prepared as follow:

10 µL of pepper essence + 10 µL of tween 80 + 4.8 cc of alcohol = essence's suspensions. Then 50 µL of Mueller-Hinton broth medium was poured in two rows of seven wells of microplate and then 50 µL of prepared suspension was added to the first wells and a dilution serial was prepared. At the end, 10 µL of suspension of bacteria was added to the all wells, and put them at 37 ° for 24 hours. Positive control: 20 mL of tween 80 +50 µL of medium

+ 4.8 of alcohol +10 µL of bacterial suspension. Negative control: Medium.

4. Results

The results of susceptibility tests showed that the *S. aureus* was resistant to penicillin but was sensitive to other antibiotics; about clinical *V. cholerae* showed sensitivity to nalidixic acid and chloramphenicol but was resistance to tetracycline and erythromycin. No dilution of aqueous-alcoholic extracts inhibits the growth of both bacteria, and turbidity due to bacterial growth was observed in all tubes except the negative control tube. But the pepper essence has shown significant effect on these two Bacteria, so that, in the case of *Vibrio cholera* no growth was observed to the well in 1.6400 (it means MIC equal to 38 (µg/mL) and there was no growth in case of *S. aureus* to well 1.800 (it means MIC = 75 µg/mL). To determine the MBC (minimum bactericidal concentration), a dilution before MIC and MIC dilution itself were cultivated in Moler Hinton Agar from each bacterium. After 24 hours, in the case of *V. cholera* in plates with dilutions of 1/ 3200 no growth was observed, but in the plate with a dilution of 1.6400, 2 colonies and in the case of *S. aureus* on plates with a dilution of 1.400 no growth was observed; and in plate with a dilution of 1.800, 3 colonies were grown up. Thus, the MBC was been in *V. cholera* equals 76 µg/mL and in *S. aureus* equals 150 µg/mL.

5. Discussion

One of the most apparent influences that modern times have brought for people is their realization of going back to natural products. Although the advances brought by technology has made life easier to people, many are still looking for better alternatives that are proven to be more effective in their most natural form (10). As it was clear in the result, Pepper essence showed high antibacterial properties, Pepper extracts had no effect on the bacteria has been tested. The most important antimicrobial in the black pepper is Chamazulene, which with high percentage exist in plant's essence (10-12). However, the existing Chamazulene in the plant is as a precursor Matrisin in the plant. And only through capturing the essence by distillation become Chamazulene, however, there aren't Chamazulene in aqueous-alcoholic extract, which can be reduced antimicrobial effect on the extracts. Essence (plants terpenoids) has effective impact against microbes Compared with plant extracts Of course, this result is also true in relation to plant peppers (13). Since the clinical application of essences and plant extracts is possible in certain circumstances, the use of pepper essence as an antimicrobial compound into invivo conditions requires further investigation of the mechanism of the ingredients of this plant on microorganisms and pharmacological and pharmacokinetic studies (14). *P. nigrum* (black pepper) is decorous climbing vine native to southern thickened at the node. The fruit contain 1% volatile oil, elsewhere in

tropical regions. They have several uses such as they help in pain relief, rheumatism, chills, flu, and fever (15). The biological role of this specie is explained in different experiments that peppercorn and secondary metabolites of *Piper nigrum* can be used as Antiapoptotic, Antibacterial, Anti-Colon toxin, Antidepressant, Antifungal, Anti-diarrheal, Anti-inflammatory and many other effects. Other roles of this specie include protection against diabetes induced oxidative stress (16). This study will serve to become a ready reference for identification of *Piper nigrum* as antimicrobial agent for cure of infections.

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Authors' Contributions

S. Yaslianifard, designed the experiments and first draft of manuscript, S. Yaslianifard and M Mirzaei did the experiments, S M Marashi edit the and written the final draft of manuscript.

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