The Antibacterial Activities of *Piper nigrum* L. Against Mastitis Pathogens and its Antioxidant Activities

Gulten Okmen*, Mustafa Vurkun, Ali Arslan, Olcay Ceylan

Faculty of Science, Department of Biology, Mugla Sitki Kocman University, Kotekli Mugla 48000 TURKEY

ABSTRACT

**Objective / Purpose:** Bacteria causes one of the most common types of chronic mastitis. The most common causative organisms of mastitis include: *Staphylococci*, *Streptococci* and coliforms. The scope of this work was to research the antibacterial effects of *Piper nigrum* extracts against mastitis pathogens, and its antioxidant capacity. **Materials and Methods:** In our study, 2 *Staphylococcus aureus* and 5 Coagulase Negative *Staphylococcus* were used for experiments. Additionally, *Piper nigrum* were collected from Mugla herbalists in Turkey. The plant extracts were tested by disc diffusion assay for antibacterial activity. The antioxidant activities of plant extracts were also determined by ABTS ([2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)]) method. **Results:** The methanol extract of plant showed maximum inhibition zone against 2 bacteria. MIC values of extracts are 6500 μg/mL. The value found in ABTS method is highly effective (75.7%). **Discussion/Conclusion:** As a result, the *in vitro* studies indicate that the extracts of *Piper nigrum* have significant antibacterial and antioxidant activities. In addition to, the plant extracts could be used in treating mastitis caused by the test bacteria.

**Keywords:** Piper, Mastitis, *Staphylococcus*, Antibacterial Activity, Antioxidant Activity.

INTRODUCTION

Mastitis is a complex disease, which is defined as inflammation of parenchyma of mammary glands and is characterized by physical, chemical and usually bacteriological changes in milk and pathological changes in glandular tissues. In this disease, the most common causative organisms are *Staphylococci*, *Streptococci* and coliforms. Coagulase-negative *Staphylococci* (CNS) have been considered to be minor mastitis pathogens, especially in comparison with major pathogens such as *Staphylococcus aureus*. The main reason for this is that mastitis caused by CNS is very mild, and usually remains subclinical. The continuous evolutions of bacterial resistance to currently available antibiotics are increasing problems. Drug-resistant bacteria create additional cases of illness, longer recuperation times and unnecessary deaths that necessitated the search for novel and effective antimicrobial compounds. This situation has forced scientists to search for new antimicrobial substances from various plants which are the good sources of novel antimicrobial chemotherapeutic agents. Because of the concern about the side effects of conventional medicine, the use of natural products as an alternative to conventional treatment in healing and treatment of various diseases has been on the rise in the last few decades. Black pepper or *Piper nigrum* is one of the most popular spice products in oriental countries (mostly in Southeast Asia). *P. nigrum* is a plant of the Piperaceae family, largely used as a flavouring agent in foods. Its characteristic aromatic odour is due to the volatile oils in the cells of the pericap. *Piper nigrum* L. is used to treat asthma, chronic indigestion, colon toxins, obesity, sinus,
congestion, fever, intermittent fever, cold extremities, colic, gastric ailments and diarrhea. Black pepper has been shown to have antimicrobial activity. Both aqueous and Ethanolic extracts of black pepper have been screened for antibacterial activity against a penicillin-G resistant strain of Staphylococcus aureus, Bacillus cereus and B. subtilis. Piperine (1-[1-oxo-2, 4, pentadienyl piperidine) a pungent alkaloid present in black pepper, enhances the bioavailability of various structurally and therapeutically diverse drugs. Larvicidal and anti-cancer activities of Piper nigrum Linn. have been reported.

Reactive oxygen species (ROS) have been implicated in degenerative diseases such as cancer, inflammation, atherosclerosis and aging as also in food deterioration. Restriction on the use of synthetic antioxidants due to their carcinogenic nature has lead to a growing interest in recent years in natural antioxidants of plant origin for application in food industry to combat food deterioration. Spices and herbs are recognized as sources of natural antioxidants and thus play an important role in the chemoprevention of diseases and aging. Many studies have reported that phenolic compounds in spices and herbs significantly contributed to their antioxidant and pharmaceutical properties. Kapoor et al. recently demonstrated that the protective effect of piperine is most likely due to its antioxidant activity. Although a few reports on the antioxidant activity of cultivated P. nigrum are available in the literature.

The aim of the investigation presented in this paper is to evaluate the antibacterial and antioxidant activities of various extracts of Piper nigrum on several mastitis pathogens, as there is a significant lack of information on such activities in literature.

MATERIAL AND METHODS

Plant material

Piper nigrum were obtained from the local herbal market from Mugla in 2016. Taxonomical identification of plant was performed by Dr. Olcay Ceylan from the University of Mugla Sıtkı Koçman, Turkey. In the identification of this spice was used the Flora of Turkey (Herb no: MUH 1045).

The Piper fruits were washed 3 times with running water and sterile distilled water. Then the spice material was air-dried. The fruits were powdered in a laboratory blender. All samples were stock at room temperature, and they were stored at 4°C until required for analysis.

Preparation of extracts

All tests were performed in triplicate and the mean values were given. The air dried and powdered fruits of the spice (50 g) were extracted with solvents using the Soxhlet. In this study, methanol, ethanol and ethyl acetate were used as solvent. After solvents have been evaporate, extracts (250 mg/mL) were kept in small sterile opac bottles under refrigerated conditions until used.

Microorganisms and cultivation

The bacteria obtained from previous studies by Dr. Zafer Cantekin, University of Mustafa Kemal (Project number: 1101 M 0103; Ethics council number: 2010 / 02- 30: 12). Seven bacteria were used in these studies. Two of them were S. aureus, and five of them were Coagulase- Negative Staphylococci (CNS). The bacteria were grown in Mueller-Hinton Broth for 24 h at 37°C (MHB; Merck). The bacteria were identified by conventional biochemical tests.

Determination of antibacterial activity

In this study, the extracts were individually tested against mastitis bacteria. The antibacterial activities of extracts were determined by disc diffusion assay. The bacteria were grown on Mueller-Hinton agar plates (MHA, Merck) at 37°C. The cultures of bacteria set to 0.5 McFarland. Incubations of bacteria were at 37°C for 24 h. The assessment of antibacterial activity was based on measurement of the diameter of the inhibition zones around the discs after 24 h. Methanol, ethanol and ethyl acetate used as negative control. Ampicillin (10µg) antibiotic used as positive control. The extracts were impregnated to discs as 25 mg/mL. All tests were performed in triplicate and the mean values were given.

Determination of minimum inhibitory concentration (MIC)

The other antibacterial activity test is MIC. The MIC value was taken as the lowest concentration that inhibits growth of bacteria after incubation. The broth dilution assay was made according to CLSI standards. The final concentrations of the extracts are 13000, 6500, 3250, 1625, 812.5 µg/mL.

Non- enzymatic antioxidant activity assay

ABTS decolorization assay was used for non- enzymatic antioxidant activity experiments. Main stock liquid include 7 mM ABTS•+ [2,2′-azino-bis-(3-ethylbenothiazoline-6-sulfonic acid)] solution and 2.45 mM potassium persulfate solution. The absorbances were measured at 734 nm by spectrophotometry (Shimadzu UV-1201V, Japan). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Sigma) was used as standard. Results of ABTS scavenging activities were given as mM Trolox equivalents (TE)/g dry mass.
RESULTS

Results of antibacterial activities of different extracts are given in Table 1. The inhibition zones were recorded as mm for all the materials. The all of extracts of Piper nigrum were inhibited S. aureus-18, CNS-32, and CNS-36. The none of extracts of Piper nigrum were not inhibited S. aureus-17, CNS-32 and CNS-33. The highest inhibition zones were shown in S. aureus-18 and CNS-36, and its zone was 10 mm. Ampicillin (10 μg) used as positive control.

MIC values of Piper nigrum are shown Table 2. In this study, 2 bacteria showed lowest sensitivity to various extract of plant (6500 μg/mL). These bacteria are S. aureus-18 and CNS-36 (Table 2).

DISCUSSION

The antimicrobial compounds from plants may inhibit bacteria by a different mechanism than the presently used antibiotics and may have clinical value in treatment of resistant microbial strains. Because of the side effects and bacteria resistance against the antibiotics, the scientist developed new drugs from natural sources such as plants, which have been extensively used as alternative treatment for disease.37,38 This study confirms that the fruits of Piper nigrum possess biological activities.

In the present study, the highest inhibition zone was determined on two bacteria (Table 1). Then one of extracts of Piper nigrum were not inhibited S. aureus-17, CNS-32 and CNS-33 (Table 1). Obasola, et al.39 and Kloos and Bannerman40 reported that CNS pathogens are resistive to most antibiotics. They have multi-resistance genes in plasmids which can be changes and they can spread among the different species covering also S. aureus. Parekh and Chanda41 reported that Piper nigrum was not inhibited S. epidermidis. The mode of action of antimicrobial agents also depends on the type of microorganisms and is mainly related to their cell wall structure and the outer membrane arrangement. The reports supported our results.

All of extracts of Piper nigrum were inhibited S. aureus-18, CNS-32, and CNS-36 (Table 1). Dorman and Deans11 reported that Piper nigrum inhibited S. aureus as 14.5 mm. Reddy et al.42 reported that Piper nigrum suppressed S. aureus as 8-11 mm. Nazia and Perween43 published that P. nigrum inhibited S. aureus as 23 mm inhibition zone. Shan et al.44 determined that P. nigrum repressed S. aureus as 4.6 mm. Karsha and Lakshmi45 shown that Piper nigrum inhibited S. aureus as 14-20 mm. Weerakkody et al.46 found that P. nigrum repressed S. aureus as 5-6 mm inhibition zone. Zarai et a47 reported that inhibition zone of Piper nigrum shown 8-12 mm for S. aureus and 7-14 mm for S. epidermidis. The studies supported our results.

Sethi, et al.48 reported that minimum inhibitory concentration value (MIC) of Crocus sativus against S. aureus was found as 65 mg/mL. Weerakkody et al.46 found that MIC value of P. nigrum as 5 mg/mL. Karsha and Lakshmi45 shown that MIC value of Piper nigrum found as 125 ppm.
In this study, MIC value is 6500 μg/mL, and these data are better than other studies (Table 2). Whereas, Zarai et al.\textsuperscript{27} reported that MIC values of *Piper nigrum* as 312.5 and 1250 μg/mL. The differences in the antimicrobial activities with the reported one may be due to different geographical environment, age of the plant, different method followed for isolation of oil, cultivar type, seasonality etc. According to Alijani,\textsuperscript{et al.}\textsuperscript{52} the extracts of *Piper nigrum* can be considered a weak inhibitor against mastitis pathogens.

Plants play a major role in providing the required antioxidants for the body. Although, traditionally, spices have been used in food preparations to improve flavour and taste.\textsuperscript{30} In our results, the ethyl acetate extract showed 76% inhibition at 250 mg/mL concentration. Whereas other extracts showed different inhibition at same concentration (Table 3). Zarai et al.\textsuperscript{27} reported that the ethanolic extract of *Piper nigrum* showed a high antioxidant activity 65.6% at final concentration of 50 μg/mL. Shan et al.\textsuperscript{24} found that the antioxidant activity of fruit of black pepper as 4.6 mmol TE/100 g DW. Ahmad et al.\textsuperscript{29} determined that callus of *Piper nigrum* have 40% antioxidant activity. Khalaf et al.\textsuperscript{50} reported that *Piper nigrum* inhibited DPPH. The inhibition rate is about 60%. Singh et al.\textsuperscript{25} told that the highest DPPH inhibition found 61% for ether extract. Gülçin\textsuperscript{31} reported that the highest DPPH inhibition of spice found 55% for ethanol extract. Our results are better than these studies. Furthermore, Fogden and Neuberger\textsuperscript{41-55} cited that the active constituents themselves are influenced by a wide range of factors: harvest season, preparation methods, plant species, and location including altitude and climate, and quality control.

**CONCLUSION**

*Piper nigrum* extracts tested in the study were determined to have potential antibacterial activities against *S. aureus* and CNS pathogens isolated from subclinical cow mastitis. Our findings shown that *Piper nigrum* has biological activity. It would be very useful in the finding of novel antibacterial agents. Furthermore, plant extracts have great importance as antioxidant activities. Also, more researches involving more detailed studies to determine which components of spice extracts offer the best biological activity are advised.

**ACKNOWLEDGEMENT**

The authors thank Dr. Zafer Cantekin (Mustafa Kemal University, Turkey) for identification of mastitis pathogens.

**CONFLICT OF INTEREST**

None

**ABBREVIATION USED**

CLSI: Clinical and Laboratory Standards Institute MIC: Minimum inhibitory concentration; ABTS: 2,2’-azino-bis(3-ethyl benzothiazoline-6-sulfonic acid); TE: Trolox equivalent; MHA: Mueller-Hinton Agar; MHB: Mueller-Hinton Agar; DPPH: 2,2-diphenyl-1-picrylhydrazyl; h: hour; mg/mL: milligram/milliliters; μg/mL: microgram/milliliters; nm: nanometer; °C: Celsius degree; mm: millimeter.

**REFERENCES**

Okmen et al.: Biological activities of *Piper nigrum*


Okmen et al.: Biological activities of *Piper nigrum*

**SUMMARY**

- The highest antibacterial activity was determined from methanol extract for *Staphylococcus aureus*-17 and CNS-36. In this study, all of the bacteria were shown sensitivity for 6500 µg/mL concentration. The highest antioxidant activity was shown at ethyl acetate extract. Our results suggest that *Ribes nigrum* has significant antibacterial and antioxidant activity and it could be very useful in the discovery of novel antibacterial and antioxidant agents of plant origin.

---

**PICTORIAL ABSTRACT**

**About Authors**

**Assoc. Prof. D. Gülten Okmen:** She works Mugla Sitki Kocman University in Biology Department. She is member of the European Association of Biotechnology and Biologists Association. She has a lot of scientific meetings and publications. She teaches a lot of courses at the bachelor and graduate degree. She done Ph. D degree at Ankara University, Department of Biology. She is working about Archaeal, Cyanobacterial and Microbial Biotechnology. She also worked as a potential reviewer for various national and international journals.

**Msc. Mustafa Vurkun:** He is MSc.student at Mugla Sitki Kocman University and works about Microbial Biotechnology. He works about Methicillin resistance *Staphylococcus aureus* (MRSA). He has a lot of scientific meetings and article. He is pursuing his MSc. at Mugla Sitki Kocman University.

**MSc. Ali Arslan:** He is MSc.student in the scope of Microbial Biotechnology at Mugla Sitki Kocman University. He works about Archaea. He has a lot of scientific meetings and article. He is pursuing his MSc. at Mugla Sitki Kocman University.

**Ph.D Olcay Ceylan:** He works Mugla Sitki Koçman University in Biology Department. He is working about systematic botanic. He has a lot of scientific meetings and article. He done Ph.D at Mugla Sitki Kocman University in Biology Department.

---

**Cite this article:** Okmen G, Vurkun M, Arslan A, Ceylan O. The Antibacterial Activities of *Piper nigrum* L. Against Mastitis Pathogens and its Antioxidant Activities. Indian J of Pharmaceutical Education and Research. 2017;51(3)Suppl:S170-75.