Benha Veterinary Medical Journal 45 (2023) 196-199

Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

Since 1990

Original Paper

Antibacterial activity of *Spirulina platensis* extract against some bacteria as *Staphylococcus aureus, E. coli and* Klelbsiella species (Invitro) isolated from broilers

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ARTICLE INFO

Keywords

Chicken.
Escherichia coli
Klebsiella
Spirulina platensis
Staphylococcus aureus

Received 29/04/2023
Accepted 18/07/2023
Available On-Line 01/10/2023

ABSTRACT

Forty-five random samples were collected from fresh broiler internal organs lungs, liver and kidney (15 samples each). All samples were screened for Staphylococcus aureus, *Escherichia coli* and Klebsiella. The bacteriological examination revealed the presence of *E. coli* (45/45 isolates), followed by Klebsiella (38/45 isolates) and *S. aureus* (16/45 isolates). The serotyping of *E. coli* (45) typed the strains as O111:H2 (60%), O128:H2 (8.8%), O113:H2 (6.6%), O114:H21 (6.6%), O26:H11 (6.6%) and O91:H12 (4.4%), O124 (2.2%), O103:H4 (2.2%), O55:H7 (2.2%). Biotyping of Klebsiella species (38) revealed that 19/38 (50%) strains were typed as *k. pneumoniae*, *k. oxytoca* 14/38 (36.9 %) and *k. ozaenae* 5/38 (13.1%). The antibacterial activity of *Spirulina platensis* water extract (10, 50, and 100 mg/dl) were tested in-vitro effect against Staphylococcus aureus, *E. coli*, and Klebsiella, using the paper disc diffusion method. *Escherichia coli* serotype O121 and *S. aureus* were the most susceptible strains to *Spirulina platensis* water extract with inhibition zones of (12,14,15mm) and (9,11,13mm) respectively, while minimal inhibitory effects were shown by Klebsiella, whose inhibition zone diameter was (0, 2, and 6 mm). Therefore, *Spirulina platensis* water extract may be useful in various applications and be used as basic knowledge for further investigations.

1. INTRODUCTION

Food-borne diseases are the most serious worldwide health issues. They are the main causes of illness and death in developing nations, claiming the lives of an estimated 2.2 million people each year, the majority of whom are children. (Mensah et al., 2002). Foodborne illness is frequently linked with the consumption of meats and poultry products sold in retail markets around the world. (Vindigni et al., 2007). Chicken flesh is regarded as a major carrier of foodborne pathogens. (Matias et al., 2010). Microorganisms with various virulence factors that give them the power to cause disease are among the bacteria that cause FBDs; among these factors are toxins that can be created in food or once the infection has colonized the digestive tract. Bacteria have been responsible for more than 70% of foodborne transmission fatalities (Hughes et al., 2007). Food-borne diseases caused by Staphylococcus aureus, Bacillus cereus, *E. coli* 0157:H7, and Salmonella enteritidis constitute a major public health concern globally (Isara et al., 2010). *S. aureus* food poisoning is the most common in several nations, which is responsible for up to 41% of food poisoning outbreaks. Although it can affect people of any age, the most prevalent age range is 20 to 49 years old, which can account for up to 48% of cases. The most typically connected food items with *S. aureus* food poisoning are chicken and eggs, cakes, pastas, sauces, and milk and its derivate (Lima et al.,2013)

Chicken flesh is contaminated with faecal organisms, particularly those of the enterobacteriaceae family, which includes Salmonella spp., *E. coli*, Proteus spp., and Klebsiella spp. (Paterson, 2006).Contaminated raw poultry meat is a significant cause of food-related disease in humans around the globe. *S. aureus* isolated from chicken meat samples has recently been found to be resistant to various antibiotics such as penicillin, methicillin (oxacillin), chloramphenicol, and erythromycin, posing a significant danger to consumer health. (Abdalrahman et al., 2015). Klebsiella spp. were the most zoonotic bacteria in the local and imported broiler meat in local markets. They discovered that improper handling of chicken meat results in food-borne microbes, and poultry may be an essential food-borne pathogen reservoir. (Noori and Alwan, 2016).The search for Cyanobacteria with antimicrobial activity has gained popularity in recent years as a result of growing global concern about the worrisome rise in antibiotic-resistant microorganism infection rates. It was discovered that Cyanobacteria extract physiologically active chemicals. Cyanobacteria strains have been found to produce intracellular and extracellular metabolites with a wide range of biological activities, including antibacterial, antifungal, cytotoxic, algaecide, immunosuppressive, and antiviral activities. (Mundt et al.,2001).Spirulina, a blue-green algae, is rapidly becoming a popular health food worldwide. It is a photoautotrophic Cyanobacterium from the class Cyanophyta that is edible, minute, multicellular, filamentous, and alkalophilic. It has a larger cell size for

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simpler growing and harvesting, as well as a cell wall that is easily edible. They have the most protein, vitamins, and nutrients of any single cell protein. They dominate the microflora of alkaline saline waters with pH levels as high as 11.0 and can be found in a wide range of environments, including soils, marches, hot springs, fresh water, ocean, brackish, residential, and industrial wastewaters. (Jensen and Knutsen 1993 and Nicoletti 2016).Spirulina is a Cyanobacterium rich in carotenoid, chlorophyll, phycocyanin, amino acids, minerals, and other useful chemicals (Singh et al., 2005). This microalga is easy to grow and may thrive in both high and low salinity settings. Spirulina contains 60–70% protein, 13.5% carbohydrate, and 4-7% fat, as well as essential amino acids (leucine, isoleucine, and valine), natural pigments (chlorophyll, phycocyanin, and carotenoid), and vitamins A and B12 (Koru, 2012). According to Andrade et al. (2019), Spirulina can be employed in human and animal health supplements due to its high nutritional value. Spirulina growing in re-used Zarrouk medium has polyunsaturated fatty acid levels ranging from 37.58 to 47.49%.*Spirulina platensis* produces a wide variety of bioactive compounds, making it an important source of medications. (Akhtara et al.,2012).This investigation was aimed to demonstrate the antibacterial activity of *Spirulina platensis* water extract against *S. aureus*, *E. coli* and Kelbsiella species isolated from broilers internal organs.

2. MATERIAL AND METHODS

2.1. Sample collection:

Randomly 45 fresh chicken internal organs samples were purchased from various stores. The lungs, the kidneys and liver were sampled 15 times each. Each sample was collected in sterile plastic bags, put in an icebox, and sent to the laboratory as soon as feasible for bacteriological investigation.

2.2. Bacteriological examination (APHA, 2001)

2.2.ISample preparation:

Each sample was homogenised aseptically in a stomacher (Colworth, 400) with 225 ml of 0.1% sterile peptone water for 1.5 minutes before making tenfold serial dilutions.

.2.2.2Bacterial isolation and identification:

The prepared dilution was streaked onto appropriate bacteriological agar for bacterial isolation.

The morphological and biochemical identification of suspected Staphylococci species, *E. coli* and Kelbsiella spp. were done according to Cruickshank et al.,(1975)and Macfadden(2000)

Serological identification of *E. coli* isolates using rapid diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan) for detection of enteropathogenic types, according to (Kok et al., 1996).

2.3.Preparation of Spirulina biomass

Spirulina platensis was cultured in CFTRI media (DXN) and kept at room temperature in natural light by confronting a window. The colony was filtered and washed with acid water after 15 days to eliminate salts. The material was powdered after being dried at 30°C. (Gyenis, et al,.2005).

2.4.Determination of antimicrobial activity of Spirulina biomass

The antibacterial activity was assessed using the paper disc (HI Media) diffusion technique. For uniform distribution of microorganisms, the corresponding bacterial cultures were poured into Mueller Hinton agar (Hi Media Laboratories) plates.

Bacterial inoculums were prepared by adjustion bacteria suspension to 0.5 Mcferland (105 -106 cfu/ml for bacteria) (CLSI,2018).

2.4.1. Antibacterial Assay :

On Mueller Hinton agar media, 0.1 ml of prepared bacterial inoculums (*E. coli*, *Klebsiella pnumoniae*, and *S. aureus*) was cultured. Then 5mm diameter wells were made and filled with100 µl of *S. platensis* water extract at different concentrations (10, 50, and 100 mg/ml). As a positive control, gentamicin (Biogram) (50µl/ml) was used, while distilled water was used as a negative control. The results were recorded and compared to the standard medication as the mean width of the zone of growth inhibition surrounding the well (Kitai et al., 2005). The plates were incubated at 37°C for 24 hours for each bacterium. The zone of inhibition was measured in mm wide wells on each agar plate at the end of the incubation time. (Marasini et al., 2015).

3. RESULTS

3.1.The Bacteriological examination of collected chicken samples (45 samples) revealed the presence of 16 coagulase positive *S. aureus* isolates, 45 *E. coli* isolates and 38 Klebsiella isolates with percentage of 35.6 %, 100% and 84.44% respectively .

The incidence of isolated bacterial species in the collected samples (15 liver samples, 15 lung samples and 15 kidney samples) were shown in table (1).

Table 1 Incidence of *S. aureus, E. coli and Klebsiella* in the collected chicken samples (15 liver samples,15 lung samples, 15 kidney samples)

Organ	Liver		Lung		Kidney	
M.O	+ve	-ve	+ve	-ve	+ve	-ve
S. aureus	5	10	7	8	4	11
E. coli	15	0	15	0	15	0
Klebsiella	13	2	12	3	13	2

3.2. Serological identification of *E. coli* isolates:

The serotyping of (45) isolated *E. coli* showed that, 27/45(60%) strains were typed as O111:H2, O128:H2 4/45(8.8%), O113:H2 3/45(6.6%), O114:H21 3/45 (6.6%), O26:H11 3/45 (6.6%) and O91:H12 2/45 (4.4%), O124 1/45(2.2%), O103:H4 1/45 (2.2%), O55:H7 1/45 (2.2%) as shown in table (2).

Table 2 Serological identification of *E. coli (45)*

Isolated serogroup	No. of isolates	%
O 128:H2	4	8.8
O 124	1	2.2
O 111:H2	27	60
O 91:H21	2	4.4
O 103:H4	1	2.2
O 26:H11	3	6.6
O 114: H21	3	6.6
O 55:H7	1	2.2
O 113:H2	3	6.6
Total	45	100%

The proportion was calculated based on the number of isolates.

3.3. Identification of Klebsiella Isolates:

The isolated Klebsiella spp. From chicken 38 isolates were bio typed as shown in table 3.

Table 3 Bio-typing of *Klebsiella* species isolated from chicken.

Isolated serogroup	No. of isolates	%
Klebsiella pneumoniae	19	50
Klebsiella oxytoca	14	36.9
Klebsiella ozaenae	5	13.1
Total	38	100%

The proportion was calculated based on the number of isolates.

3.4. Determination of antimicrobial activity of *Spirulina platensis* water extract by agar well diffusion method:

The diameter of inhibition zones with different *Spirulina platensis* concentrations was showen in table (4) against different micro-organisms isolated from broilers (*S. aureus, E. coli* and *Klebsiella pnumoniae*).

Table 4. The antimicrobial activity of *Spirulina platensis* extract against *S. aureus, E. coli* and *Klebsiella.*

Microbial strains	Diameter of inhibition zone (mm) by *S. platensis* (Conc.mg/ml)		
	10	50	100
Escherichia coli O2	5	7	10
Escherichia coli O124	4	9	12
Escherichia coli O1	5	7	10
Escherichia coli O125	8	10	11
Escherichia coli O78	9	11	12
Escherichia coli O111	3	5	10
Escherichia coli O113	7	10	12
Escherichia coli O121	12	14	15
Escherichia coli O128	9	10	11
Escherichia coli O55	8	9	11
Escherichia coli O146	7	9	11
Escherichia coli O26	8	9	10
Staphylococcus aureus	9	11	13
Klebsiella pneumoniae	No zone	2	6

4. DISCUSSION

Globally, public health is a major concern. These bacteria are typically spread through contaminated food, and their presence in raw meat and chicken has serious public health implications. (Sousa, 2008). This could be attributed to a combination of factors: low-quality beef carcasses used, bacteria spreading in meat through foodborne illnesses caused by *S. aureus*, *E. coli*, and Klebsiella species, major grinding, poor manufacturing processes, insufficient cleaning and disinfection of both machinery and surfaces, poor personal hygiene, and the use of untrained workers.

S. aureus is also a leading cause of food poisoning and a variety of human illnesses, including pneumonia and postoperative wound infections. (de Boer et al., 2009).

Because *K. pneumoniae* is an opportunistic pathogen, it is responsible for 2%-5% of nosocomial infections, particularly those of the urinary and respiratory tracts, in immunocompromised people. (Podschun and Ullmann ,1998).

In this study the most frequent bacterial contamination found in chicken organs was *E. coli* (100%), followed by Klebsiella (84.4%) and *S. aureus* .(%35.6)

Our findings were consistent with previous studies, as Adegunloye (2006) who reported that poultry served as a dangerous source for some pathogens, acting as a reservoir for pathogens capable of producing enterotoxins, such as *S. aureus*. Kitai et al. (2005) 444 raw chicken meat samples from 145 different supermarkets in 47 prefectures in Japan were examined for *S. aureus* contamination and enterotoxigenicity .*S. aureus* was found in 292 (65.8%) of the samples. These bacterial pathogens in chicken and its products are of public health importance for consumers. (Leloir et al., 2003) .

The results of *E. coli* isolation were nearly similar to those which obtained by Maarouf and Nassif (2008) who collect random samples of frozen maet products from benha city and different villages at Kaliobia Governorate, to evaluate the bacterial quality and the hygienic health hazard of them with some food borne pathogens and Saif (2015) who conducted a study to assess the bacteriological contamination of fresh marketed chicken cuts-up and the risks to public health. The colonial appearance and biochemical profile of the recovered *E. coli* were similar to those previously reported, such as sugar fermentation or enzymatic reactions. (Quinn et al., 2002, and Ezzat et al., 2014).

In this regard, serological identification of 45 isolated *E. coli* recovered from chicken revealed that O111:H2 27/45 (60%), O128:H2 4/45 (8.8%), O113:H2 3/45 (6.6%), O114:H21 3/45 (6.6%), O26:H11 3/45 (6.6%), and O91:H12 2/45 (4.4%), O124 1/45 (2.2%), O103:H4 1/45 (2.2%), and O55:H7 1/45.(%2.2)

Spirulina platensis water extract was tested for antibacterial efficacy against clinical isolates of *S. aureus*, *E. coli* and *Klebsiella pneumoniae*. The extract's effects on the tested isolates varied, with some being more sensitive than others.

According to our results, *Klebsiella pneumoniae* was the most resistant strain to plant extracts, this agreed with Kaushik and Abhishek Chauhan (2008) who showed that, followed by *E. coli* O111, whereas *E. coli* O121 and *S. aureus* were the most susceptible strains to *Spirulina platensis* water extract, respectively.

Antibacterial activity against *S. aureus* and *E. coli*, as well as antifungal activity against A. niger and Candida albicans, was discovered in *S. platensis* extract. This agreed with (Santoyo et al., 2006) who made a liquid extraction of antioxidant and antimicrobial compounds of Spirulina platensis. This extract's primary antioxidant components were identified as zeaxanthin, a myxoxanthophyll-like molecule, and highly polar phenolic compounds. Moreover, antimicrobial activity of different PLE fractions was tested against *S. aureus*, *E. coli*, C. albicans and A. niger.

Furthermore, many studies have been published as antimicrobial agents from microalgae, including Spirulina, which can be considered a rich source of natural antimicrobial agents such as fatty acids, trepenoids, peptieds, polysaccharides and alkaloieds, as reported by Kokou et al., (2012), who found that Spirulina inhibited the growth of six Vibrio strains, making it a good antibacterial agent.

Moreover, Özdemir et al., (2004) investigated the effect of various *S. platensis* extracts on bacteria and discovered that methanol extract is the most effective at inhibiting bacterial growth.

Also, *S. platensis* c-phycocyanin pigment reduced the growth of several bacterial species, including *S. aureus*, *E. coli*, Klebsiella pneumonia, and Pseudomonas aeruginosa. (Sarada et al., 2011; Mohamed and Saber, 2019).

Additionally, Kaushik and Chauhan (2008) showed that the aqueous extracts of *S. platensis* had no inhibitory effect on *K. pneumoniae* but did inhibit *S. aureus*. They reported that the methanolic extract displayed broad-spectrum activity, with the most effective zone of inhibition against *S. aureus*, followed by *E. coli*, *P. aeruginosa* and *S. typhi* .

Another investigation discovered that acetone, ethanol, and diethyl ether extracts are antibacterial against *Klebsiella pneumoniae*, Enterobacter spp., and *E. coli* reference strains. (Kulandaivel et al., 2007).

5. CONCLUSIONS

It concluded that *Klebsiella pneumoniae* was the most resistant strain followed by *Escherichia coli* O111, while *Escherichia coli O121* and *S. aureus* were the most susceptible strains to the water extract of *S. platensis* respectively.

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