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Antimicrobial Properties of a Type of Drug Supplement: Nutrition Bio-Shield Superfood

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***Abstract*—**In this research, a type of drug supplement was synthesized by a green route. This organic biomaterial was named Nutrition Bio-Shield Superfood (NBS). Due to the destructive effects of various infectious diseases, their increasing prevalence and the lack of appropriate medication for treatment, the present study aimed to evaluate antimicrobial properties of the NBS dietary supplement. In the study of the simple effect of concentrations on the inhibitory diameter of the growth of the common bacteria involved in infectious diseases of the human body, the highest diameter of the halo was related to the concentration of 100 mg/ml and the least of them was the concentration of 12.5 mg/ml dietary supplement. In general, the NBS drug supplement increases the level of immunity in human body.

***Keywords*—**Drug supplement, biomaterial, antimicrobial, human body.

I. Introduction

TODAY, herbal remedies and food supplements are widely used around the world because of their availability, relatively low cost, low side effects and effectiveness in the treatment of diseases. Given the adverse effects of chemical drugs and numerous reported side effects, this study aimed to investigate the antimicrobial properties of a healthy and alive drug supplement from wheat. For this purpose, the supplement was synthesized by a green route [1], [2]. This supplement was named Nutrition Bio-Shield Superfood (NBS).

II. Methodology

A. Preparation of Drug Supplement

First stage: The seed genome (set of genes within the nucleus) is often better prepared for activity after winter (hibernation) or a cold shock. Induction of shock to the genome of cells in unfavorable conditions is effective, as well as before creating the optimal conditions. Therefore, the induction of cold shock at this stage of the genome activation process is of utmost importance. In addition, the enzymatic and hormonal system of the plant seed could be exposed to the start conditions for better preparation. At this stage, the seeds are maintained at the temperature of 0 – -5 °C for 10-12 hours (this shock was induced to the genome of cells for the simulation of unfavorable conditions).

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Second stage: At this stage, proper humidity and heat shock are introduced into the genetic system of the seeds so as to provide the favorable conditions for the action of specific enzymes (e.g., nucleases and proteases). At this stage, the genetic system of the nucleus of the seeds begins to synthesize the material, activating the production of protein and vitamins. The material was preserved at the temperature of 20 °C and humidity of 18-25% for 24-48 hours.

Third stage: At this stage, high-precision conditions must be set in order to regulate the pressure, humidity, and acidity, so that the preparation would reach its peak, activating many genes. Moreover, several minerals are placed in the cellular system of the seeds as absorbable ions and biofactors, losing their non-absorbable chemical and molecular state. At this stage, many vitamins are synthesized, leading to the production of group B and ATP vitamins, which are the main sources of energy in the body. At this stage, humidity is 30-40%, temperature is 25 °C, acidity is 8.5 and the duration is 30 hours.

Fourth stage: This stage involves placing the seeds in a conventional fan shelf dryer at the temperature of 30-35 °C for 15-20 hours in order to reach the humidity of 10-15%.

Fifth stage: After drying of the seeds, they are grinded, powdered, and packaged.

B. Antimicrobial Properties

1. Bacterial Strains

In this study, the effect of the NBS drug supplement on some of the common gram-positive and negative bacteria involved in infectious diseases of the human body was investigated. These bacteria include: *Streptococcus mutans, Streptococcus sanguis, Streptococcus salivarius, Streptococcus sobrinus, Escherichia coli, Eikenella corrodens, Pseudomonas aeruginosa, Klebsiella pneumoniae, Candida albicans, Candida glabrata, Candida tropicalis, Acinetobacter calcoaceticus, Streptococcus pyogenes, Staphylococcus aureus, Enterobacter aerogenesis, Citrobacter freundii, Staphylococcus saprophyticus, S. epidermitis, Enterococcus faecalis, Proteus mirabilis, Bacillus cereus, Bacillus subtilis.*

The bacteria were collected from the collection center of the industrial microorganisms of Iran and were transferred to the Microbiology Laboratory of the Green Drug Researchers Knowledge-Based Company in order to investigate the antibacterial effects of the extracts.

2. Dilution of Extracts and Preparation of Discs Containing Extract

In order to evaluate the antimicrobial activity of the dietary

supplement, concentrations of 12.5, 25, 50 and 100 mg/ml were prepared. Then, for the preparation of discs containing the desired extracts, Blanc discs manufactured by Padtan Teb were used to make antibodies. The discs were then placed in tubes containing dilutions of the extracts, and after complete absorption, they placed at 37 °C for 3 to 5 minutes, to completely dry and be ready to be distilled.

3. Antimicrobial Activity Review

The antimicrobial activity of the extracts was evaluated using Disk Diffusion and Minimum Inhibitory Concentration (MIC). In this method, bacteria that were grown in the culture medium were prepared in a physiological serum of 3 × 108 bacteria per ml. Then 50 µl of this suspension was inoculated on the environment of Mueller Hinton-Agar containing 5% of blood. Then the disking was done. The suspension was incubated at 37 °C for 2 days. Then, the plates were examined for the presence of non-growth halo and also standard discs of chlorhexidine, nystatin and cephalexin were used for positive control. Measuring the diameter of the inhibition zone around the discs was done by a millimeter ruler and the results were evaluated. In addition to the discontinuation method, the susceptibility of each strain of the bacteria into the studied extracts was evaluated by dilution method in liquid medium on 96-well round bottom plates. Only the culture medium and the bacterial suspension were added to the first row plates. In the next row, 100 µl of Moeller Hinton nutrient medium was added to 6 wells of plates. To the first well, 100 µl of the concentration of 2 mg/ml of the live-drug supplement was added, and to the sixth well, the concentrations of 2-4-6-8-10-12 mg/ml (provided by dilution method) were added. To each well, 20 µL (equivalent 0.5 McFarland) of bacterial suspension was added. The contents of each well were mixed for 2 minutes by means of a stirrer Plate Reader, and the plates were heated to 35 °C for 24 hours, and the opacity or non-opacity of the wells were evaluated visually. The first venue that showed the least amount of turbidity was determined as the minimum lethal concentration. This experiment was performed in three replicates separately and the average of three replicates for each well was used to determine the MIC.

III. Results and Discussion

Diameters of the non-growth halo due to different concentrations of the drug supplement on some of the common gram-positive and negative bacteria involved in infectious diseases of the human body were listed in Table I. Also, the mean diameters of the growth hole (mm) in the common gram-positive and negative bacteria involved in infectious diseases of the human body with different concentrations of the healthy and alimentary supplement were shown as Fig. 1.

TABLE I
Diameters of the Non-Growth Halo Due to Different Concentrations of Drug Supplement on Some of the Common Gram-Positive and Gram-Negative Bacteria Involved in Infectious Diseases of the Human Body

Row	Bacterial strain	Gram	Concentration				Standard drug	Diameter
			12/5	25	50	100		
1	*Streptococcus mutans*	+	-	7	13	15	Chlorhexidine	15
2	*Streptococcus sanguis*	+	-	-	-	-	Chlorhexidine	17
3	*Streptococcus salivarius*	+	-	-	9	14	Chlorhexidine	16
4	*streptococcus sobrinus*	+	-	-	-	-	Chlorhexidine	14
5	*Escherichia coli*	-	-	12	16	22	Chlorhexidine	13
6	*Eikenella corrodens*	-	-	-	8	16	Chlorhexidine	15
7	*Pseudomonas aerogenosa*	-	-	-	-	-	Chlorhexidine	12
8	*Klebsiella pneumoniae*	-	7	10	14	18	Chlorhexidine	14
9	*Candida albicans*	+	12	14	16	23	Nystatin	31
10	*Candida glabrata*	+	9	12	14	17	Nystatin	26
11	*Candida tropicalis*	+	-	-	15	17	Nystatin	25
12	*Acinetobacter calcoaceticus*	-	-	-	-	-	cephalexin	20
13	*Streptococcus pyogenes*	+	-	9	12	16	cephalexin	19
14	*Staphylococcus aureus*	+	8	12	14	19	cephalexin	21
15	*Enterobacter aerogenesis*	-	-	8	13	17	cephalexin	18
16	*Citrobacter freundii*	-	-	-	-	9	cephalexin	18
17	*Staphylococcus saprophyticus*	+	-	-	9	14	cephalexin	15
18	*S. epidermitis*	+	-	-	9	14	cephalexin	20
19	*Enterococcus faecalis*	+	-	-	-	8	cephalexin	21
20	*Proteus mirabilis*	-	-	-	-	-	cephalexin	17
21	*Bacillus cereus*	+	-	9	13	16	cephalexin	21
22	*Bacillus subtilis*	+	-	-	8	14	cephalexin	24

The minimum concentrations of the NBS supplements into some pathogens of the human body were listed in Table II.

Finally, images related to the diameter measurement of the non-growth halo of the studied drug supplement on some of the common gram-positive and negative bacteria involved in infectious diseases of the human body were shown as Fig. 2.

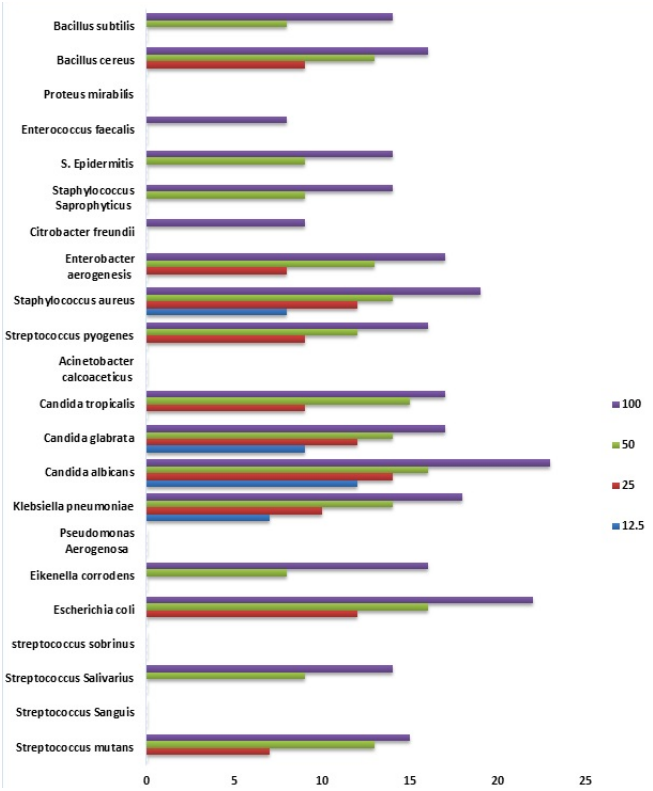

Fig. 1 The mean diameter of the growth hole (mm) in the common gram-positive and negative bacteria involved in infectious diseases of the human body with different concentrations of the NBS healthy and alimentary supplement

TABLE II
THE RESULTS OF THE MIC OF THE DRUG SUPPLEMENTS ON SOME DISEASE-CAUSING PATHOGENS IN THE HUMAN BODY (MIC)

Row	Bacterial strain	MIC
1	*Streptococcus mutans*	8±1
2	*Streptococcus sanguis*	-
3	*Streptococcus salivarius*	10±1
4	*streptococcus sobrinus*	-
5	*Escherichia coli*	6±1
6	*Eikenella corrodens*	8±1
7	*Pseudomonas Aerogenosa*	-
8	*Klebsiella pneumoniae*	6±1
9	*Candida albicans*	4±1
10	*Candida glabrata*	6±1
11	*Candida tropicalis*	8±1
12	*Acinetobacter calcoaceticus*	-
13	*Streptococcus pyogenes*	8±1
14	*Staphylococcus aureus*	4±1
15	*Enterobacter aerogenesis*	8±1
16	*Citrobacter freundii*	-
17	*Staphylococcus saprophyticus*	-
18	*S. epidermitis*	-
19	*Enterococcus faecalis*	-
20	*Proteus mirabilis*	-
21	*Bacillus cereus*	6±1
22	*Bacillus subtilis*	-

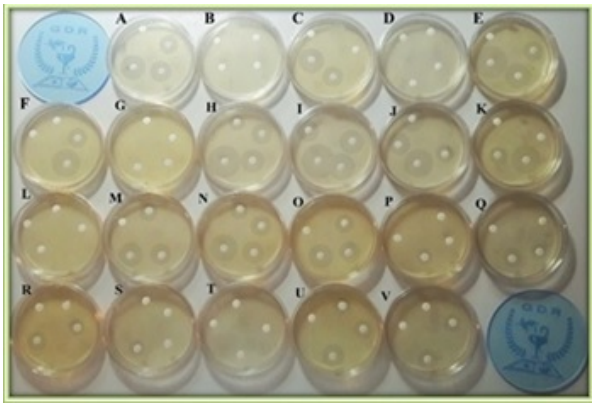

Fig. 2 Images related to the measurement of the diameter of the non-growth halo of the studied drug supplement on some of the common gram-positive and negative bacteria involved in infectious diseases of the human body

The present study, which was performed to evaluate the healthy and viable dietary supplement on the studied organisms, showed that the studied extracts have antibacterial properties [3], [4]. When the extracts were tested on *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Candida albicans* etc., a wider area of growth inhibition with a variety of gram-positive and gram-negative bacteria was obtained. This result is consistent with [3]. These differences in the regions are related to the sensitivity of each organism to the studied supplement [5]-[7]. Factors responsible for the high susceptibility of the bacteria into the supplement have not been well characterized, but may be attributed to the presence of the secondary plant metabolites and the presence of compounds in the pharmaceutical nutritional supplement [8]-[10].

IV. CONCLUSION

After analyzing the antibiotic properties and observing the results, it was found that there was a significant difference between the different concentrations of the healthy dietary supplements in evaluating the inhibiting effect of the bacteria growth. Based on the results obtained, it was found that the concentration of 100 mg/ml drug supplement exhibited comparatively similar results to that of the standard drug. The results obtained from the MIC test showed that the bacteria studied had the highest susceptibility to the concentration of 100 mg/ml of the dietary supplement. Therefore, considering the desired antibacterial effect of the studied drug supplement in this research, it can be concluded that the presence of antimicrobial secondary compounds in the NBS drug supplement can have suitable antibacterial effects for the treatment of various infectious diseases.

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