Antibacterial activity of Ricinus communis against some bacteria isolated from wastewater

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Abstract

This study was conducted at the North Gas Company in Kirkuk during the period from Nov. 2001 - July 2002. Four stations were chosen for sampling collections. The samples chosen from ; station (1) before treatment,and three stations from the treatment unit (production unit ;control unit; and general unit). Four bacterial species (10 isolates for each) staphylococcus aureus;Enterococcus faecalis; Escherichia coli; and Pseudomonas aeruginosa were isolated.Results of the present study showed that minimum inhibitory concentration (MIC) of alcoholic extract for Gram positive bacteria isolates was (4 – 8 mg/ ml), while it was 4 – 16 mg/ ml for Gram negative bacteria.

فعالية المضادات البكتيريه للـ Ricinus communis ضد بعض البكتريا المعزولة من مياه الفضلات

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المستخلص

أجريت الدراسة الحالية في شركة غاز الشمال في كركوك خلال الفترة من تشرين الثاني ٢٠٠١ ولغاية تموز ٢٠٠٢ . تم اختيار اربع محطات لجمع العينات . والعينات جمعت في المحطة الاولى قبل المعالجة ومن ثلاث محطات من وحدة المعالجة (وحدة الانتاج ، وحدة السيطرة ، الوحدة العامة) . عزلت اربعة انواع بكتيرية (عشرة عزلات لكل واحدة) Escherichia coli , Enterococcus faecalis , staphylococcus aureus pseudomonas aeruginosa. أظهرت نتائج الدراسة الحالية إن اقل تركيز مثبط (MIC) للمستخلص الكحولي للعزلات البكتيرية الموجبه هو (4-8 mg/ml) ، بينما كان هذا التركيز(4-16 mg/ml) للعزلات البكتيرية السالبه .

Introduction

Domestic wastewater contains countless numbers of living organisms, most of them are so small to be visible except when viewed under a microscope, which why they are called "microorganisms".Typically,a omestic wastewater prior to entering the treatment plant will contain from 100,000 to 1,000,000 microorganisms per milliliter. These microbes have their origin from two general sources, sanitary wastes and the soil [1]. Both wastewaters and soils contain large numbers of microorganisms, especially bacteria. Generally microorganisms can be regarded as a natural living part of the organic matter found in wastewaters and their presence is most important because they serve as a primary function in degradation of wastes in biological wastewater treatment.In a sense the successful operation of a biological wastewater treatment plant is dependent upon a knowledge of the activities of the microorganisms, especially the bacteria. Efficient treatment then depends on understanding the requirements for optimal growth as well as recognizing unfavorable conditions [2,3].The majority of the microorganisms found in wastewaters are not harmful to man, that is non-pathogenic (do not cause disease), some microorganisms are pathogenic and always are of great concern in wastewater treatment.Among the diseases associated with wastewaters are typhoid fever, dysentery,cholera, and hepatitis [3].Not all bacteria exist in water supplies cause a health hazard to human. Coliform bacteria include 15 species and most do not cause disease. However, a limited number can cause gastroenteritis,most notably entropathogenic E.coli (E.E.C).Other species can combine with fecal streptococcus to cause infection [4]. Large number of coliform bacteria lives in the intestinal tracts of warm and cold blooded animals and is easy to identify during routine water testing. Therefore, they frequently used as indicators to a water supply that may be contaminated from human or animal wastes.Coliform bacteria are carried in feces and waste water and may also be found in groundwater or surface water contaminated by seepage from septic systems or feedlots. If coliform bacteria show up in a water sample, it is possible that disease- causing bacteria, viruses, and parasites from improperly treated wastewater are also present. Disease-causing agents accompany coliform bacteria are a health risk. Generally, health risk is related to transmission of contagious disease, such as typhoid fever, cholera, hepatitis, dysentery, diarrhea, or giardiasis [4,5]. Water pollution exists in many forms. Pollution resulting from fecal contamination is particularly serious problem because of the potential for disease transmission. However, the concentration of pathogens (disease causing bacteria) in natural streams and ground water is generally very small. Testing for these pathogens in routine water analyses is impractical and expensive except in unusual circumstances, analysis must be satisfied with indirect evidence of the presence of pathogens by testing for indicator organisms such as coliform bacteria.The aim of the present study was to evaluate the antibacterial activity of alcoholic and aqueous
extracts of Ricinus communis against four types of bacteria isolated from wastewater (10 isolates for each species).

Materials and methods

The study was conducted at North Gas Company during the period from Nov. 2001 - July 2002.

A.Sampling: Water samples from North Gas Company Water used in the above company is discharge to the community as a wastewater product. Waste water before discard pass in a treatment process .The treatment unit composed of 3 parts:

Production unit

Control unit

General unit

From the above units, four sites were selected as the best situation for the sample collection. The collected samples were transported to the laboratory within two hours and processed as follows: Water samples were enriched in nutrient broth at 37C° for 24 hours .The developed colonies were subcultuered on blood agar, MaConkey agar and incubated at 37 C°for 24 hours. The isolated bacteria *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli., Pseudomonas aeruginosa* (10 isolates for each) were further identified according to [6].*B.Plant extraction*:Parts of the plant were washed with distilled water and oven dried at 37 C° for 10 days [7].In order to obtain plants extracts for evaluation of their antimicrobial activity, the dried parts of the plants were grained in a mortor to fine powder. *Aqueous and alcoholic* extract were obtained following Kady et al. [8].

Results

The results of the present experiments show that there were no antibacterial activities against Staph aureus in the concentrations used in the present study. Even no antibacterial activities were observed with the higher concentrations.The antibacterial activity of Ricinuis communis against Enterococcus facealis isolated from wastewater was illustrated in tables (1). For wastewater isolates, the results show that the MIC of alcoholic and aqueous extracts ranged from 4 – 8 mg/ml.It was found that the MIC of aqueous extract was 4-16mg/ ml for E. coli isolated from wastewater, while the MIC of alcohloic extract was 4 -16 mg/ ml for wastewater isolates (Table 2).The alcoholic and aqueous extracts of Ricinuis communis were tested for their antibacterial activity against Pseudomonas aeruginosa isolated from wastewater (Table 3). The results showed that MIC were ranged from 4 - 16 mg/ ml for alcoholic and aqueous extracts for wastewater isolates.

Table (1): Antibacterial activity of *R. communis* extract against *E. faecalis* isolated from wastewater.

Isolates number	2 mg/ ml		4 mg/ ml		8 mg/ ml	
	Alco.	Aque.	Alco.	Aque.	Alco.	Aque.
1	+	+	+	-	-	-
2	+	+	-	-	-	-
3	+	+	+	+	-	-
4	+	+	+	-	-	-
5	+	+	-	-	-	-
6	+	+	+	+	-	-
7	+	+	+	+	-	-
8	+	+	-	-	-	-
9	+	+	+	-	-	-
10	+	+	-	-	-	-

- + Growth
- - No growth

Table (2): Antibacterial activity of *R.communis* extract against *E.coli* isolated from wastewater.

isolates	2 mg/ ml		4 mg/ ml		8 mg/ ml		16 mg/ ml	
	Alco.	Aque.	Alco.	Aque.	Alco.	Aque.	Alco.	Aque.
1	+	+	+	-	+	+	-	-
2	+	+	+	+	-	-	-	-
3	+	+	+	-	+	-	-	-
4	+	+	+	+	-	-	-	-
5	+	+	+	+	+	+	-	-
6	+	+	-	-	-	-	-	-
7	+	+	+	+	+	-	-	-
8	+	+	+	+	-	-	-	-
9	+	+	+	-	+	-	-	-
10	+	+	+	-	-	-	-	-

- + Growth.
- - No growth.

Table (3): Antibacterial activity of *R. communis* extract against *P. aeruginosa* isolated from wastewaters.

isolates	2 mg/ ml		4 mg/ ml		8 mg/ ml		16 mg/ ml	
	Alco.	Aque.	Alco.	Aque.	Alco.	Aque.	Alco.	Aque.
1	+	+	+	-	+	-	-	-
2	+	+	+	+	+	-	-	-
3	+	+	+	-	-	-	-	-
4	+	+	+	+	-	-	-	-
5	+	+	-	-	-	-	-	-
6	+	+	+	+	+	+	-	-
7	+	+	+	+	+	+	-	-
8	+	+	+	-	-	-	-	-
9	+	+	+	+	+	+	-	-
10	+	+	-	-	-	-	-	-

- + Growth. * - No growth

Discussion

Results of the present study showed that MIC of alcoholic extract for Gram-positive bacteria isolated from wastewater was (4 – 8 mg/ml), while it was 4 -16 mg/ml for Gram negative bacteria. The present results were in agreement with that found by Ferreira et al. [9].*R. communis* is a shrublike herb, stems are 1-4 metres high and belongs to the family Euphorbiaceae. It contains Ricin in its constitutens, Ricin is one of the most thorenghly studied plant toxins. Ricin is produced by the beans of *R. communis*. Many studies have been reported that the antimicrobial activity of *R. communis* seeds is related to the presence of ricin [10, 11, 12]. Ricin is a heterodimeric glycoprotein consisting of an A- chain (RTA, Ricin toxin A-chain , RTA) disulphide-linked to a B-chain (Ricin toxin B-chain , RTB) RTA is an N-glycosidase, responsible for ricin cytotoxicity, that depurinates adenine residue of the rRNA, thus in activating protein synthesis [13]. RTB is agalactor-specific lectin containing three galactose - binding site [12]. Ricins are synthesized in the endosperm cells of maturing seeds, and are stored in an organelle called the "protein body", a vacuolar compartment. When the mature seed germinates, the toxins are destroyed by hydrolysis within a few days. Ricin begins sythesis as a prepropolypeptide that contains both A and B chains. The signal sequence of the Nh3-terminus targets the nascent chain to the endoplasmic reticulum (ER) and is then cleaved off. As the proricin polypeptide elongates, it is N-glycosylated within the lumen of the ER. Protein disulfide isomerases catalyze disulfide bond formation as the proricin molecule folds itself. Proricin undergoes further oligosaccharide modifications within the Golgi complex and then is transported within vesicles to the protein bodies [11].Ricin is not catalytically active until it is proteolytically cleaved by an endopeptidase within the protein bodies. This splits the polypeptide into the A chain and the B chain still linked by a single disulfide bond. Since ricin is inactive until then, the plant avoids poisoning its own ribosomes in case some proricin accidentally passes into the cytosol during synthesis and transport. The ricin a portion of the heterodimer is the enzyme that binds and depurinates a specific adenine of the 28S rRNA. The adenine ring of the ribosome becomes sandwiched between two tyrosine rings in the catalytic cleft of the enzyme and is hydrolyzed by the enzymeís N-glycosidase action. The target adenine is a specific RNA sequence that contains the unusual tetranucleotide loop, GAGA. Ricin is more active against animal than plant ribosomes. [13].

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