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Research Article

The Effect of Cold Aqueous Extract of Lemon Peel against Types of Bacteria Isolated From the Cooling Devices Filters

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Abstract

The antibacterial effect of citrus peel lemon against the bacterial strains obtained from the filters of air conditioners have been selected based on the presence most in these filters such as *Streptococcus, Bacillus spp, Pseudomonas, E coli*. agar well diffusion method used to evaluate antibacterial activity of citrus peels water extract. through the results became clear to us that the cold aqueous extract of lemon peel showed a significant effect on the growth of bacterial species through the diameters of inhibition zone that appeared in all concentrations of the extract (125, 250, 500, 1000) mg/ml. Gram-positive bacteria *Streptococcus spp* were the most affected Where the diameters of inhibition zone (18, 15, 12, 0, 0) mm respectively, while The Gram-negative bacteria *E coli* least affected. The results obtained in this study indicate that citrus lemon peel can be used in the treatment of diseases caused by organisms for the purposes of the pharmaceutical.

Keywords: cold aqueous extract, lemon peel ,cooling devices filters, pathogenic bacteria

الخلاصة

اجريت هذه الدراسة لتحديد تأثير المستخلص المائي البارد لقشور الليمون الحامض ضد الانواع البكتيرية المأخوذة من فلاتر مكيفات الهواء حيث تم اختيار الانواع البكتيرية اعتمادا اعداد تواجدها على هذه الفلاتر ، فكانت الانواع التي تمت دراستها واستخدامها في البحث هي بكتريا (*Streptococcus spp*, *Bacillus spp*, *Pseudomonas aeruginosa*, *E.coli*).
اتبعت طريقة الانتشار بالحفر في اختبار الفعالية البايولوجية للمستخلص المائي لقشور الليمون الحامض ضد الانواع قيد الدراسة ، ومن خلال النتائج اتضح لدينا ان المستخلص المائي البارد لقشور الليمون الحامض اظهر تأثيرا واضحا ضد نمو الانواع البكتيرية اعلاه وذلك من خلال اقطار التثبيط التي ظهرت وفي جميع تراكيز المستخلص (125،250،500،1000) ملغرام/مل.
ومن جهة اخرى كانت بكتريا *Streptococcus spp* الموجبة لصبغة كرام اكثر انواع البكتريا استجابة لتأثير المستخلص حيث كانت اقطار التثبيط (0،0،12،15،18) ملم على التوالي ، فيما اظهرت بكتريا *E.coli* السالبة لصبغة كرام اقل تأثيرا للمستخلص. من خلال النتائج التي تم الحصول عليها نستنتج بانه وبعد اجراء المزيد من الدراسات يمكن استخدام المستخلص المائي البارد لقشر الليمون كنوع من الأدوية والمستحضرات الصيدلانية الناجحة ضد حالات تلوث فلاتر اجهزة التبريد بهذه الانواع من البكتريا.

Introduction

Air is not an appropriate environment for outgrowth of the pathogenic bacteria, any pathogen, that airborne must have originated from a source like humans, animals, plants, soil, food or water [1]. There are numerous sources of biological contamination such as pollutant central heating or cooling systems can become breeding grounds and the distribute these pollutants everywhere the home standing water, water damage materials or damp surfaces can also serve as breeding grounds [2]. From harmless microorganisms, there are many pathogenic species found in filters, the most dangerous bacterial species among which are *Pseudomonas aeruginosa* and *Legionella pneumophila* [3]. As substantiality for fungi, water is a strict demand for bacterial growth. In fact, higher water activities necessitate bacteria than most fungi. The temperature and nutrient demands are mostly met in most indoor environments. Surprisingly, little studies have been conducted on bacterial growth in wet houses [4]. The air amply populated with microorganisms which form so-called bio aerosol is The air inhaled by people.

Bio aerosol is a colloidal comment, created by liquid droplets and particles of solid matter in the air, whose components include or have attached to them viruses, fungal spores and conidia,

bacterial endospores, plant pollen and fragments of plant tissues [5]. To end bacterial outgrowth in the air filters, not only particular interest the conservation and cleaning. Seems it is primary the introduction of antimicrobial agents which prohibit the growth of bacteria in the filtration material, Thus decrease air pollution in the bio-treatment level.

The peel of Citrus fruits is a rich source of flavonoids and many polymethoxylated flavones, which are very rare in other plants [6]. Abundant plants have been used in order to of their antimicrobial advantage, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances (e.g., the phenolic compounds which are part of the essential oils), as well as tannin [7]. Citrus flavonoids have a wide spectrum of biological activity inclusive antibacterial, antifungal, anti-diabetic, anticancer and antiviral activities. Antimicrobial activity of the peel extract is directly concerned with the components that they contain [8]. The citrus peels are wealthy in nutrients and include many phytochemicals, these can be efficiently used as drugs or as food complements. The peel of Citrus fruits is a rich exporter of flavonoid glycosides, coumarin, 5β and γ- sitosterol, glycosides and volatile oils [9]. Medical plant would be the better origin to gain a diversity of drugs as the phytochemical are more specific. Phytochemical show singular platform for structural variety and biological functionality which is necessary for drug discovery [10].

This study aimed in evaluating the effectiveness of cold aqueous extract lemon peel to determine the inhibitory efficacy against many of the bacteria contaminated filters for cooling devices and that citrus lemon peel can be used in the treatment of diseases caused by organisms for the purposes of the pharmaceutical.

Material and Methodologies

Microorganism Isolates

The study was conducted in the city of Baghdad and specifically in the eastern Baghdad area of Palestine Street and include indoor environment like classrooms and science laboratories and many of the homes within this area.

Bacterial isolates follower mitigation dilution method [11]. By taking a certain weight of the sample model up (1g) and put in (9ml) of normal saline solution and left for half an hour, then took him to (1ml) mediated by sucking and laying on the culture media such as blood agar-mediated publisher glass, are process published streaking, then incubated at (37) C° for 24 hours, taking him to different colonies to check or test tinted grams stain and that the distinguish between negative bacteria (G- ve) and gram positive bacteria (G+ ve). Steps detection of bacterial and fungi isolates [12]:

A. Gram Negative Bacteria (G- Ve)

Taking (1ml) of the implant sample, published in the dish containing the culture media Blood agar which is in the middle of the growth of many bacterial species, in the bosom of (37) C° for 24 hours, took a distinct colony Selected colony adoption on the number and percentage of growth and subculture again at the center of a distinguished private Selective media Mac-Conkey agar in order to obtain pure colonies, the incubated in (37) C° for 24 hours, then took one of the colonies for testing them. The differential Biochemical diagnostic testing such as Coagulase test is used to detect these bacteria (G-ve bacteria) [12].

B. Gram Positive Bacteria (G+ Ve)

Taking (1ml) of the implant sample and as we have seen in previous labs, single colonies may be achieved by using the streak plate techniquein on blood agar culture media which is in the middle of the growth of many bacterial species, in the incubated of (37) C° for 24 hours. took a distinct colony and sub-culture at the special culture media such Mannitol salt agar in order to process the diagnosis of bacteria *Staphylococci spp*. Amid another specialist to diagnose the bacteria *Bacillus spp*. It is Nutrient agar, and so as to obtain pure colonies, the dishes were incubated at 37 C° for 24 hours and then tests were complementary to the process of diagnosis and isolation, as happened when negative for bacteria stained gram [12].

C. Diagnosis of Fungus

The isolation, culture, and microscopic examination of molds require the use of suiTable selective media and special microscopic slide techniques. cultural characteristics: *C. albicans* (yeast) can be inoculated by streaking on culture media containing Sabouraud-brain-heart infusion (SABHI) can be made selective for dimorphic

fungi with addition of chloramphenicol (Sabouraud agar or its Emmons version can be made more selective by adding antibiotics) and incubated at 37C° for 24-48 hours and then observed colonies on the media and diagnosis by microscopic examination check the slide to yeast *C.albicans* by placing a drop of (Lacto phenol cotton blue) on clean glass slide by sterile loop, then transfer part of the colony of yeast and blend with the dye, and put the cover glass and examined under a microscope to observe the form of yeast cells

Bacteriological Diagnosis

Diagnosed bacterial isolates depending on Holt *et al.*, (1994) [13].

Morphological Characterization

Diagnosed isolates based on the study of the colonies in terms of shape, size, color and shape of their edges and surfaces and smell and strength and the color, the culture media used such as Nutrient agar and blood agar and MacConkey agar and Mannitol salt agar as well as phenotypic characteristics of bacterial cells included in the form of groupings and on the nature of their interaction with tinted grams Gram stain when preparing slides of bacterial isolates.

Biochemical Tests

It includes the following tests depending on (Atlas *et al.*, 1995) [14].

Catalase Enzyme Production.
Oxidase Enzyme Production.
Haemolysis Test.
Coagulase Enzyme Production.
Methyl Red/Voges-Proskauer (MR/VP)
Indol Test Formation.
Mannitol Fermentation Test.
Simmon's Citrate Agar.
Urease test.
Motility agar.
Nitrate Broth

Preparation of Extract

The fruits of lemon (Citrus lemon) it has been getting of local markets has been taking peel limes and then dried in a dark dry place, then was crushed by electric grinder for obtain lemon peel powder, 100 grams of plant sample powders were steeped in 100ml of cold distilled water and the mixture was then kept in shaker incubator for 24hrs at 35 C^{o} then filtered through filter paper (Whatman no.1) and centrifuged for 10 min at 3000 rpm. The filtrate was then left to evaporate in the incubator at 37 C^{o} for three days The dried powder was weighed and transferred to a sterile universal tube in the refrigerator for later usage, the work of it several concentrations of a progressive (125, 250, 500 and 1000) mg/ml [15]. Agar well diffusion method used according to Baron & Fineglod (1994) and Mahmoud *et al.*, (1989) to determine the antibacterial activity of plant extract [16][17].

Effect of Lemon Peel Water Extract on Bacterial and Fungal Isolate

After preparation of the plant extract and the bacteria, the effect of lemon peel extract test in bacteria studied as spread the microorganisms by sterile spreader glass (0.1)ml of airborne bacteria at the Miller Hinton Agar, and worked holes in the culture media by Cork borer sterile (diameter of 5 mm). put in each hole (10µL) of plant extract solution, in addition to the work of the hole in the center of each dish added to (10µL) of water sterile distilled considered a transaction control, studied the effect of lemon peel extract concentrations (125, 250, 500 and 1000) mg/ml. in the growth of bacteria, the dishes were incubated at temperatures (37) C° for (24) hours after the diameter measuring inhibition zone around each hole in mm.

Results and Discussion

In light of the results obtained it appeared the number of samples for various places specified appears in this study, a house and scientific laboratories and classrooms and types of bacteria in them shows in Table 1 and Figure 1, the effect is obvious to extract lemon on bacteria isolated from filters for heating and cooling which had inhibitory activity on the growth of bacteria as shown in Table 2 and may indicate that the focus in 1000 micro grams perml of the extract is the most efficient concentration which gave the best results in inhibition of bacterial growth by measuring inhibition zone. And it has been found

to be more affected by *Pseudomonas aeruginosa* with all concentrations that agree with [18][19]. In the same concentrations show that affected by the *E. coli* bacteria but the lowest inhibition of the biological effectiveness and that this in line with [9]. And both *Streptococcus spp* and *Bacillus spp* influenced by focusing of the (250-1000) micro grams perml that consistent with [18]. These results agreed with the study conducted by [20] The extract of dried black lemon has shown good antibacterial activity against all of the selected bacteria that have been used in this research with one exception (*Proteus mirabilis*) which did not affected by all dilutions of dried black lemon extract. *Staphylococcus aureus* was the most bacteria that have been affected by this extract, the inhibition zone was 29 mm, and this was the biggest inhibition zone among the other bacteria [20]. and agreed with a nether study conducted by [21] Antibacterial effect of Citrus peel extracts was evaluated against several pathogenic bacteria associated with human and fish infections viz., *Escherichia coli, Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Staphylococcus epidermidis*, *Serratia marcesnces*, *Shigella flexneri*, *Enterobacter amnigenus*, *Salmonella Typhimurium* and *Serratia odorifera*. It was found that ethanol extract showed highly significant inhibition of *E. coli* and *K. pneumonia* (12.6±0.94 mm and 11.6±1.2 mm) whereas methanol extract of *C. sinensis* also showed high zone of inhibition of *S. odorifera* (10.0±2.16 mm). The potential activity of active extracts was assessed and also compared with standard antibiotics through activity index formulation. And Agreed with study showed The citrus peel oils show strong antimicrobial activity, The antimicrobial activity has been checked in terms of MIC by using different solvents against microorganisms [7]. The antimicrobial effects of aqueous extracts of peel and juice from fresh and dried citrus and sweet lemon against 6 Gram-positive and 8 Gram-negative bacterial and one yeast isolates, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Enterococcus faecalis*, *Streptococcus pneumoniae*, *Streptococcus agalactiae, Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella Typhi*, *Proteus spp., Moraxella catarrhalis, Acinetobacter spp*. and *Candida albicans*, all of them were studied. The water extracts of all the materials screened showed various inhibitory effects[8].

Table 1: shows the place of air cooling device and type of bacteria and fungi isolates.

Place the air cooling device	Total No.	*Staph auras* No.	*E coli* No.	*Fungi* No.	*Streptococcus spp* No.	*Candida albicans* No.	*Bacillus spp* No.	*P. aeruginosa No.*
Home	28	3	3	1	5	0	15	1
Science lab.	41	1	6	2	10	1	17	4
Classrooms	23	2	4	1	3	1	6	6
Total	**92 %100**	**6 %6.52**	**13 %14.13**	**4 %4.34**	**18 %19.56**	**2 %2.17**	**38 %41.33**	**11 %11.95**

Table 2: Effect of lemon peel water extract on bacteria and fungi isolates.

Concentration mg/ml / **Bactria types**	**Inhibition zone in(mm)**				
	1000	**500**	**250**	**125**	**control**
Streptococcus spp	18	15	12	0	0
Bacillus spp	17	13	11	10	0
Pseudomonas aeruginosa	18	15	10	10	0
E.coli	15	12	11	0	0

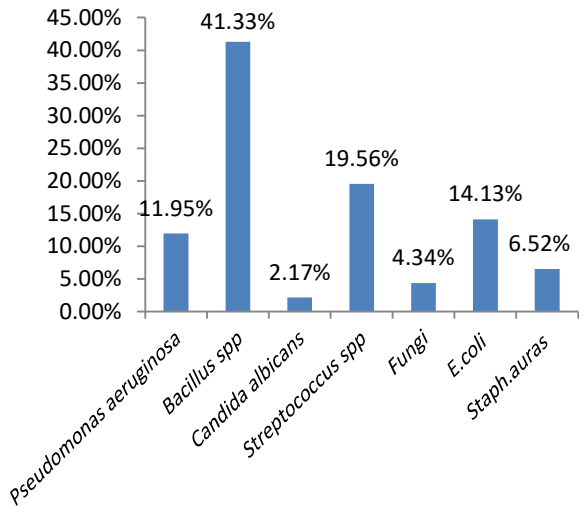

Figure 1: show the ratio for different type of bacteria and fungi in air cooling device.

Conclusion

By the results that appeared to have conclude the filters coolers and air conditioning (cooling devices Filters) contamination of different types of pathogenic bacteria and Lemon peel takes out a wide range of activity against a panel of bacteria responsible for the propagation widespread diseases. These draft extracts open the preference of result new clinically effective antibacterial compounds. On the other hand, *Streptococcus spp* gram positive bacteria (G+ve) more responded of extract from the rest of the other types of gram negative bacteria (G-ve) might be structural composition of bacterial wall lack the bacteria trigger a gram to the layer of the external membrane permeability material to make into the cell than negative bacteria for a gram.

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