



Effect of *Pyracantha coccinea* extract on *Klebsiella spp* that isolated from children with diarrhea

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Abstract: Diarrheal disease is a major cause of child mortality and morbidity in the world, and is often caused by contaminated food and water sources and microbial pathogens. all over the world. This study was designed to effect extract plant *Pyracantha coccinea* in patients diarrhea. sample were obtained from the stool of 120 patients with diarrhea to detect *Klebsiella Spp* from children between the ages of (1 - 15 years) in the period from July 1, 2023 to August 30, 2023 The results of culture showed that 23 samples (56%) were positive to *Klebsiella oxytoca*. and that 18 samples (43%) were positive to *Klebsiella pneumonias*. Kirby disk diffusion method results indicated that *Pyracantha coccinea* extract in all concentrations had the highest effect on bacteria *Klebsiella oxytoca* inhibition percentage against *Klebsiella oxytoca* bacteria was at the zone of inhibition of (10.9 , 9.1 , 3.7) mm while *Klebsiella pneumonias* showed the lowest rate of inhibition *Pyracantha coccinea* extract at (8.3, 7.3 , 2.8) mm , with a p-value <0.05, inhibition be due to the presence of phytochemicals such as phenols, flavonoids, , alkaloids, saponins .

Keywords: Diarrhea , bacteria *Klebsiella Spp* , *Pyracantha coccinea*

تأثير مستخلص *Pyracantha coccinea* على *Klebsiella spp* المعزولة من الأطفال المصابين بالإسهال

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ملخص: يعد مرض الإسهال سبباً رئيسياً لوفيات ومراضة الأطفال في العالم، وغالباً ما يكون سببه تلوث مصادر الغذاء والمياه ومسببات الأمراض الميكروبية. في جميع أنحاء العالم. صممت هذه الدراسة لتأثير مستخلص نبات *Pyracantha coccinea* في مرضى الإسهال. تم الحصول على عينة من براز 120 مريضاً مصابين بالإسهال للكشف عن *Klebsiella Spp* من الأطفال الذين تتراوح أعمارهم بين (1 - 15 سنة) في الفترة من 1 يوليو 2023 إلى 30 أغسطس 2023 وأظهرت نتائج المزرعة أن 23 عينة (56%) كانت إيجابية بالنسبة لـ *Klebsiella oxytoca*. وكانت 18 عينة (43%) موجبة لبكتيريا *Klebsiella pneumonias*. أشارت نتائج طريقة الانتشار على شكل قرص كيربي إلى أن مستخلص *Pyracantha coccinea* بجميع التراكيز كان له أعلى تأثير على بكتيريا *Klebsiella oxytoca* ونسبة التثبيط ضد بكتيريا *Klebsiella oxytoca* كانت في منطقة التثبيط البالغة (10.9 ، 9.1 ، 3.7) ملم بينما أظهرت



Klebsiella pneumonias أقل معدل تثبيط لمستخلص *Pyracantha coccinea* عند (2.8، 7.3، 8.3) ملم، مع قيمة $p < 0.05$ ، ويعود التثبيط إلى وجود مواد كيميائية نباتية مثل الفينولات، الفلافونويدات، قلويدات، الصابونين.

الكلمات المفتاحية: الإسهال، بكتيريا *Klebsiella Spp* ، *Pyracantha coccinea*

1- INTRODUCTION

Diarrhea defines as the passage of three or more frequent of watery or loose stool than normal average for an individual, It is considered the major cause of morbidity and mortality in children under the age of five in developing counties [1]. The infections were resulted by the body micro flora or by the body response to the microbial Actions [2]. One of these dangerous infections for children is diarrhea, A range of parasite, viral, bacterial, and organismal factors can cause diarrhea. Contaminated food and water can transmit infection, as can unhygienic practices that transfer the infection from patient to patient. one of the main bacterial causes of morbidity and mortality in children. *klebsiella*, *Campylobacter* *Salmonella*, *Aeromonas*, *Shigella*, and *Escherichia coli* are among the bacterial pathogens commonly linked to diarrheal disease [3]. There are three primary types of diarrhea, according to clinical and epidemiological perspectives: acute bloody diarrhea, commonly known as dysentery; acute watery diarrhea, which includes cholera and lasts for several hours or days; and persistent diarrhea, which lasts for 14 days or more. Dehydration is the primary cause of diarrheal fatalities. Still, over 90% of dehydration brought on by severe diarrhea [4]. Herbal medicine still widely used in the most developing countries. The medicinal plants effect on microorganisms growth depend on the one of its components, it consist on active compounds like, glycosides, volatile oils, tannins, saponins, resins , alkaloids, stearylates and lipids The plant extracts containing these effective pharmaceutical compounds, [5,6] Including a plant *Pyracantha* is a plant with edible and

medicinal value and widely distributed from eastern Asia to southern Europe but is underutilized and has great potential The extract of *Pyracantha* shows strong biological properties, such as inhibition of tyrosinase activity, anti-oxidative, and tumor-preventive effects [7]

2- METHODOLOGY:

2.1-Sample collection:

A total of 120 samples were collected diarrhea during the period from July (2023/7/1) to the end of August (2023/8/30) and for different age groups (1-15 years) for both genders. Were collected at the Children's Hospital in Kirkuk city



and were identified with the help of a specialist doctor, All information about the affected children was recorded on the questionnaire form which included age, gender, geographical location, the standard of living, and cases of recurrent [8].

2.2-Microscopic examination:

Microscopic examination were classified the bacterial isolates according to the shapes or clusters noted under a microscope, A clean glass slide is prepared, a drop of Normal Sline solution is added, a small amount of excrement is mixed with a wooden stick, the cover is placed, and the examined under a microscope. also learn the positive or negative to gram stain Microscopic examination and according to their staining by gram stain, shape, size of cell arrangement [9].

2.3-Bacterial isolates Identification: Colonies of bacterial isolates were identified, initially with the morphology appearance through notes the color, Consistency and the formed on MacConkey and Nutrient agar medium also according to hemolytic type on blood agar medium. [10].

2.4-Biochemical tests: Biochemical tests also done to detect some biochemical parameters that appropriate for each isolate with using the Vitec-2 compact system [11].

3- preparation of *Pyracantha coccinea* extract:

20 grams of *Pyracantha coccinea* plant seeds were weighed and ground each by using a grinder. The solid material containing the compound was placed inside a tube called a thimble.in the Soxhlet extraction device. A device was installed in a beaker with the use of (ethanol) as solvent, i.e. adding 500 ml of ethanol. At a concentration of 99%, then the condenser is installed It is left for 24 hours to complete the soaking process and to give more scope for extracting the active substance in the sample (12). The solvent is removed after extraction, usually using rotary evaporator at a temperature ranging between (45-50C°) until a dense liquid is obtained, and concentrations different of 5%, 10%, and 20% from it are prepared. It is used to measure its antibacterial activity (13).

4-Evaluation of the effectiveness of minimum inhibitory concentration of *Pyracantha coccinea* on *Klebsiella*:

The bacterial suspensions were prepared for each bacterial isolate, and the suspension was at the age 24 hrs. after incubated at 37 C° for 24 hrs. In comparison with tubes of standard 0.5 McFarland solution, the turbidity of tubes containing bacterial suspensions selected for this study were compared before cultured on Muller-Hinton agar media, the bacterial isolates then cultured on Muller-Hinton agar media, the culture were spread on media by using sterilized cotton swabs, then



appropriate 4 wells were done for each bacterial isolate to conduct the test for extract solution (*Pyracantha coccinea*) and by used the sterilized crock poorer, then about 50 µl of extract solution in the different concentrations in each well were added, and then every cultured plate was incubated at 37C° for 24 hrs. (14).

Statistical analysis:

Data were analyses by (Minitab ver .17) program system, and (ANOVA) test were applied. The means compared by Duncan's multiple range test, under the level of significance at $p < 0.05$

5- RESULTS AND DISCUSSION:

Results: This study included 41 patients with diarrhea, the (Table 1) showed that *Klebsiella oxytoca* recorded highest percentage at (40.78%) among from children patients with a diarrhoea followed by bacterial *Klebsiella pneumonia* with a percentage at (30.26%).

Table 1: bacteria isolated from children with diarrhea

types of isolated bacteria	Number of isolates	%
<i>Klebsiella oxytoca</i>	23	56.09
<i>Klebsiella pneumonia</i>	18	43.90
Total	41	100

in Table (2), the results showed the effect of the difference in the inhibitory activity extract *Pyracantha coccinea* at different concentrations in the bacterial isolate. The 20% concentration of *Pyracantha coccinea* had the greatest effect on bacterial isolate *Klebsiella oxytoca* at the zone of inhibition of (10.9) mm. And then it follows 10% concentration at the zone of inhibition of (9.1) mm. And then it follows 5% concentration at the zone of inhibition of (3.7) mm. while Concentrations of 10% of *Pyracantha coccinea* had the greatest an effect on the bacterial *Klebsiella pneumonia* at the zone of inhibition of (8.3) mm, respectively, And then it follows 20% concentration at the zone of inhibition of (7.3) mm and a concentration of 5% had an less effect on the bacteria *Klebsiella pneumonia* at the zone of inhibition of (2.8) mm with a p -value < 0.05 . The study reached that *Pyracantha coccinea* extract in all concentrations had the highest effect on bacteria *Klebsiella oxytoca*.

Table 2: effect *Pyracantha coccinea* in different concentrations against bacterial *Klebsiella* spp.



Bacteria	<i>Pyracantha coccinea</i>		
	5%	10%	20%
<i>Klebsiella oxytoca</i>	3.7 A	9.1 A	10.9 A
<i>Klebsiella pneumonia</i>	2.8 B	8.3 B	7.3 B
Mean	3.2	9.9	11.2

Discussion: According to the current study's findings, the bacteria *Klebsiella oxytoca* the highest percentage in children patients , And then it follows bacteria *Klebsiella pneumonia* . Our study agreed with the results of the study (Nemchenko etal,2020) in which the percentage of *Klebsiella oxytoca* was (51%). and study agreed also with(Abbas Alzubaidi etal,2020) in which the percentage of *Klebsiella pneumonia* was (40%) , One of the reasons for the diarrhoea was bacteria adhering to the small intestine's cells and growing there. Toxins that they release from their surface cause a serious imbalance in the body's excretion and absorption processes. Furthermore, the intestinal family, which comprises the intestine's natural flora, whenever it is exposed to less than ideal conditions (such as a disorder in the immune system) becomes opportunistic, pathogenic bacteria. Other factors are weak immune systems in children, and ingestion of certain types of contaminated foods, or a decrease in the level of stomach acidity through taking antibiotic medications that suppress stomach acidity. Our study differed from the study (Hashem etal ,2016) in which the percentage (22.5%), and the reason for the may be due to the difference in the sample collection period, the level of health services in hospitals, and the types of diarrhea between the various studies. where he discovered a study conducted by (Sarikurkcu etal , 2015) on the *Pyracantha coccinea*, that the ethanol extract has antibacterial activity because it includes potent compounds that act as barriers against microbes., The extract *Pyracantha coccinea* has was found rich in phenolic, flavonoids, condensed tannins and saponins. Additionally, all the phytochemicals were found in higher amounts in ethanol extract. hese plant extractions in this study can act as an actor to stimulate one compound and reduce the others, it seems the plant's secondary compounds can act with bacteria and sometimes with the same genus of bacteria and sometimes with other types of bacteria (Alajaily, S. M., & Alsoufi, A. S. 2023).

6- CONCLUSIONS



The study found that *Klebsiella spp* is the main reason of diarrhea disease in children. It was found that the ethanol extract of the *Pyracantha coccinea* plant has the most effective effect on the bacteria *Klebsiella oxytoca*.

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