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Inhibitory effects of alcoholic extracts of common mistletoe on some bacterial isolates from different wounds and burning cases

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ABSTRACT :

This study was conducted at the College of Education, Iraqi University, from 2/1/2021 to 2/5/2021, including the collection of three clinical isolates of the bacterial species *Klebsiella pneumoniae, Staphylococcus aureus* and *Acinetobacter baumanii*, from swabs of wounds and burns, which were collected from the Hospital of Medicine City in Baghdad, Iraq. The bacterial samples were cultured on MacConkey agar medium to ensure their purity and incubated in the incubator at a temperature of 37 °C for 24 h. The effects of the cold and hot alcoholic extracts of common mistletoe were tested and examined against *S. aureus, K. pneumoniae* and *A. baumanii* by the agar well diffusion method. In this study we used five different concentrations: 1g, 0.8 g, 0.6 g, 0.4 g, 0.2 g. As the results showed, high inhibitory activity of the hot alcoholic extract was observed against *K. pneumonia* and *A. baumanii*, so that, 1 g, 0.8 g and 0.6 g were the most effective concentrations against bacteria except for *S. aureus, K. pneumoniae* and *A. baumanii* while 0.2 mg and 0.4 mg did not show any effectiveness in inhibiting the growth of bacterial isolates. As for the cold alcoholic extract, it did not show any effect towards any of the three isolates.

Keywords: Alcoholic extract, Bacterial isolates, Common mistletoe.

التأثيرات المثبطة للمستخلصات الكحولية لنبات الدبج على بعض العزلات البكتيرية من مختلف حالات الجروح والحروق

لواء عبد شهاب

مستخلص

أجريت هذه الدراسة في كلية التربية الجامعة العراقية للفترة من 2021/ 1/ 2 ولغاية 2021/ 5/ 2، متضمنة جمع ثلاث عزلات سريرية من الأنواع البكتيرية Klebsiella pneumoniae، Staphylococcus aureus و Acinetobacter baumanii، من مسحات الجروح والحروق التي تم جمعها من مستشفى مدينة الطب في بغداد، العراق. تم زراعة العينات البكتيرية على وسط MacConkey Agar للتأكد من نقائها وحضنت في الحاضنة عند درجة حرارة 37 درجة مئوية لمدة 24 ساعة. تم اختبار ودراسة تأثير المستخلصات الكحولية الباردة والساخنة من نبات الدبج ضد S. aureus وK. pneumoniae وA. baumanii بواسطة طريقة حفر الأجار. في هذه الدراسة استخدمنا خمسة تركيزات مختلفة: 1 جرام، 0.8 جرام، 0.6 جرام، 0.4 جرام، 0.2 جرام. أظهرت النتائج، نشاط تثبيطي عالي للمستخلص الكحولي الساخن ضد K. pneumonia و A. baumanii، بحيث كانت التراكيز 1 جرام، 0.8 جرام، 0.6 جرام هي التراكيز الأكثر فعالية ضد البكتيريا S. aureus, K. pneumoniae وA. baumanii، في حين أن 0.2 ملغم و0.4 ملغم لم تظهر أي فعالية في تثبيط نمو العزلات البكتيرية. أما المستخلص الكحولي البارد فلم يظهر أي تأثير تجاه أي من العزلات الثلاث.

الكلمات المفتاحية: المستخلص الكحولي، العزلات البكتيرية، نبات الدبج.

Short Communication

INTRODUCTION

Common mistletoe is a vascular plant. Its diameter ranges between 25 and 50 cm and may reach 150 cm (Zuber 2004) It is a green plant and perennial. Its branches and leaves are opposite, thick and sometimes short. Its flowers are usually axillary. Fruits are cherry, spherical, whitish, containing one seed, which in turn contain 1 to 3 embryos. It is a unisexual, dioecious plant (Al Wara *et al.* 1993) Its importance arises from the fact that it is a medicinal plant. It has been used since the beginning of the last century for pharmaceutical purposes and has many medicinal benefits in treating cardiovascular diseases, especially high arterial pressure and arteriosclerosis, cancer and joint treatment **(**Jørgensen & Heide 2004**).** Its anti-cancer properties are due to the presence of cytotoxic protein physicotoxins **(**Barberaki & Kintzios 2002). In addition, the homeland of mistletoe is Asia and spread in Europe, Africa, Japan, Siberia, Turkey, Iran, and Amanus (Margaret Loeper 1999). The mistletoe plant is characterized by being one of the economical medicinal plants used in many countries. It has an inhibitory effect on the growth of fungi, as well as being used as a treatment against tuberculosis (Chakravarty 1976).

This study aimed to reveal the effect of alcoholic extract of common mistletoe in inhibiting some bacterial species that cause burns and wounds in human, i.e., *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Acinetobacter baumanii* which were isolated from wounds and burns.

MATERIALS AND METHODS

The procedure for preparing the alcohol-common mistletoe fruit extract

A- Materials and tools included Fruits of common mistletoe, ethanol alcohol, distilled water, beakers, flasks, water bath, cotton and sensitive balance.

B- Method of making the extract of the fruits of (common mistletoe) in hot alcohol. where the concentration of alcohol was 100%, and we prepared alcohol with a concentration of 80%:

1- Fruits of common mistletoe (10 g) were prepared.

2- An aliquot of 40 mL of 100% alcohol was provided, then diluted by adding 10 mL water to prepare 50 mL diluted alcohol.

3- The alcohol was heated in a water bath to reach 78 °C. This is the degree of heating of ethanol.

4- Then 10 g common mistletoe fruits were added to the diluted alcohol. Beaker nozzle was closed tightly with cotton and we mixed it continuously.

5- It was left in a vibrating incubator for 24 h at 25 °C and by a vibrations rate of 150 rpm.

C- Method of making the extract of the fruits of common mistletoe in cold alcohol. The same steps above (1, 2, 4 and 5) but without heating (without the third step).

D- Method for separating the filtrate from the sediment using a centrifugal device: 11 mL of cold alcohol extract was taken, each 5.5 mL in a test tube, as well as 11 mL of hot alcohol extract each 5.5 mL in a test tube. It was placed in a centrifuge and then centrifuged at a speed of 3000 cycle/min, for 15 min. Afterward, the filtrate was separated from the sediment using filter papers. Then, we discarded the residual and took the filtrate.

E- Five different concentrations of common mistletoe extract were prepared in cold and hot alcohol according to the method of Mitscher *et al.* (1972) by adding water to the concentrated common mistletoe solution and obtaining the following five concentrations: 1g, 0.8 g, 0.6 g, 0.4 g and 0.2 g.

Bacterial isolation and identification

Bacterial isolates were evaluated by analysing the general culturing characteristics of the colonies growing on the medium of blood agar, Mannitol salt agar, and McConkey agar, and then the appearance of colony shapes was studied and determined on the basis of haemolysis type, colour, shape, and size, as well as observing other general characteristics such as utilizing mannitol and growth with a high salt concentration., We also used some biochemical tests such as oxidase and catalase tests, then the diagnosis was confirmed using the Vitec system (Alexander *et al.* 2004)

RESULTS AND DISCUSSION

Alcoholic extract from common mistletoe was tested for antibacterial activity against *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Acinetobacter baumanii*, which were isolated from wounds and burns using the agar well diffusion method.

According to Table 1, hot alcohol extract exhibited a high inhibitory effect

against *A. baumanii* and *K. pneumoniae,* depending on the diameter of the inhibition zone. Except for *S. aureus*, the results showed that the concentrations of 1 g were effective against bacteria whose inhibition zone diameter exceeded 16 mm. A concentration of 0.8 g formed an inhibition zone with a diameter of 12 mm, while 0.6 g formed an inhibition zone with a diameter of 10 mm, while 0.4 g and 0.2 g failed to inhibit the bacteria, since exhibited extract resistance. Cold alcohol showed resistance of bacteria to all concentrations and failed to inhibit their growth (Table 2).

Table I. Effectiveness of the hot alcohol extract of common mistletoe against some pathogenic bacterial isolates.

Type of bacteria	1 g	0.8 g	0.6 g	0.4 g	0.2 g
Klebsiella pneumoniae	S	S	S	R	R
Staphylococcus aureus	R	R	R	R	R
Acinetobacter baumannii	S	S	S	R	R

Table II. Effectiveness of the cold alcohol extract of common mistletoe against some pathogenic bacterial isolates.

Type of bacteria	1 g	0.8 g	0.6 g	0.4 g	0.2 g
Klebsiella pneumonia	R	R	R	R	R
staphylococcus aureus	R	R	R	R	R
Acinetobacter baumannii	R	R	R	R	R

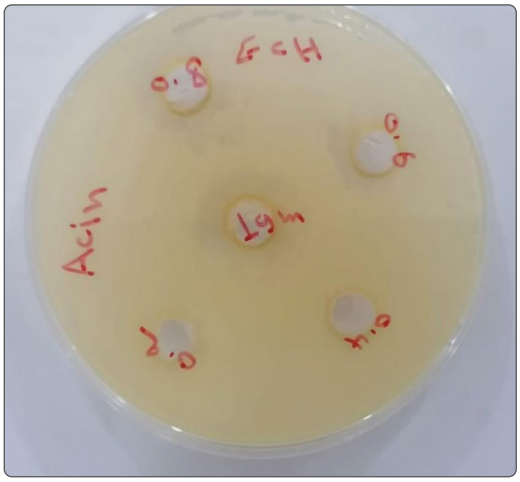

Fig. 1. Inhibition activity of the hot alcohol extract of common mistletoe against *Acinetobacter baumannii*

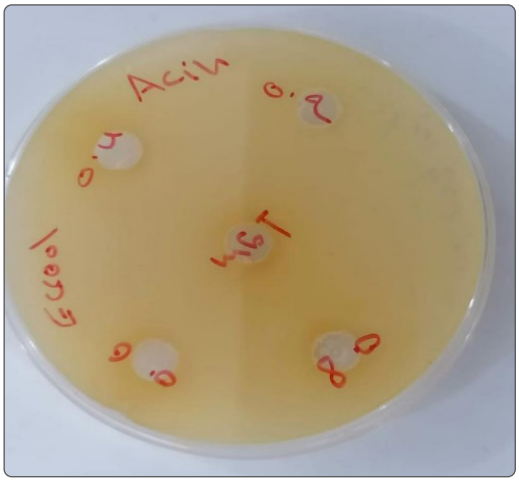

Fig. 2. Inhibition activity of the cold alcohol extract of common mistletoe against *Acinetobacter baumannii*

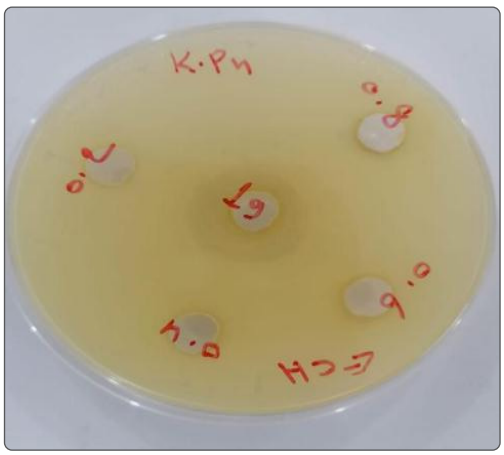

Fig. 3. Inhibiting activity of the hot alcohol extract of common mistletoe against *K. pneumoniae. baumannii*

Fig. 4. Inhibition activity of cold alcohol extract of common mistletoe against *K. pneumoniae. baumannii*

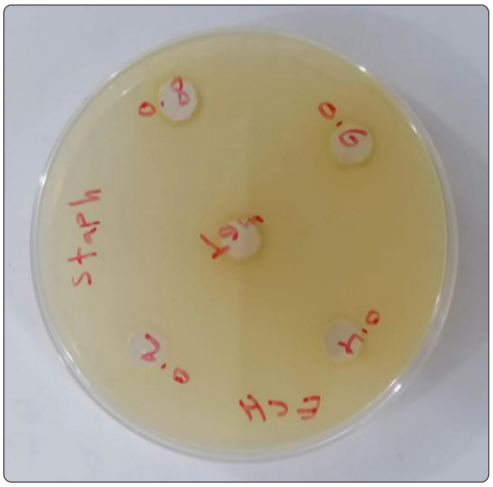

Fig. 5. Inhibition activity of the hot alcohol extract of common mistletoe against *S. aureus*.

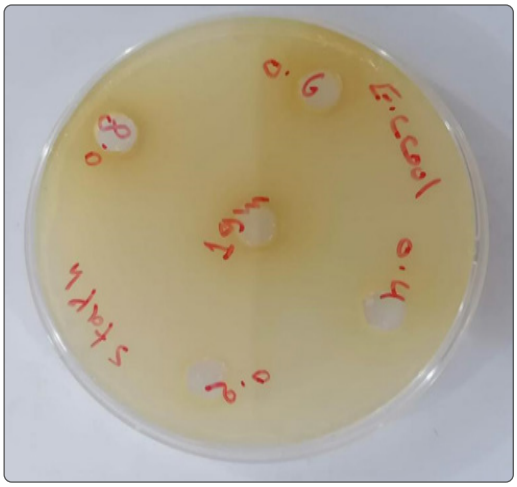

Fig. 6. Inhibition activity of cold alcohol extract of common mistletoe against *S. aureus. baumannii*

In general, it is noted that depending on the concentrations of plant extracts utilized and the quantity of bacteria under investigation, the impact of medicinal plant compounds on microorganisms varies. The results of a single plant extract in many research may be due to differences in the locations and time of the extraction techniques and extract concentrations used in each study (Gur *et al.* 2006)

From the foregoing, we found that the extract of hot alcohol is the most effective on the types of bacteria used in the study. This difference in efficacy may be attributed to the quality and quantity of chemical compounds with efficacy. Inhibition of bacteria in the extracts and the reason may be the polarity of the solvent used in the extraction (Umar *et al.* 2013). In the case of differences between the types of bacte-

ria in sensitivity towards the extracts, there are several studies reporting that Gram-positive bacteria are more enthusiastic about the inhibitory factors (Richter *et al.* 2013). In the case of the growth of Gram-negative bacteria, the components of the cell wall provide a kind of protection for the bacterial cell from these factors, and also, the presence of some protection systems in the negative bacteria that enable them to resist conditions that are not suitable for growth.

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