Plasmid curing by using essential oil of *Rosmarinus officinalis*

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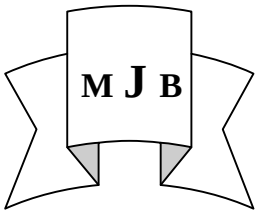

Abstract

Background: *Rosmarinus officinalis* essential oil is a natural product it has been reported to be much safer than antibiotic, and has abroad spectrum activity against gram positive and gram negative stain bacteria. On the other hand it has been reported that the oil has no toxicity and lethality on laboratory white mice therapeutic dose range.

Objective: Used of essential oil of Rosemary in plasmid curing for R-plasmid (antibiotic resistance plasmid).

Methods: Used of minipreparation of plasmid DNA and cured plasmid with essential oil by concentration (100µg/ml), the detection was performed by agarose gel electrophoresis.

Results: The essential oil has an active ability to curing plasmid as it is detected by DNA electrophoresis and lost the phenotype of Antibiotic resistance.

Conclusion: In this study, essential oil of Rosemary used for plasmid curing in active way against *Escherichia coli* pBR322 which contain R-plasmid which is resistant to Ampincilin, Tetracycline and Streptomycin antibiotics this help to used essential oil as cofactor for antibiotics.

Key words: *Rosmarinus officinalis*, essential oil, plasmid curing.

تحييد البلازميد باستعمال مستخلص الزيت الاساسي لنبات اكليل الجبل

الخلاصة

المقدمة: الزيت الاساسي لنبات اكليل الجبل هو منتج طبيعي وقد سجل انه اكثر اماناً من المضادات الحيوية وكذلك له فعاليـــة واسعة الطيف ضد البكتريا السالبة والموجبة لصيغة كزام . فضلاً عن كونه غير سام وغير قاتل للفئران البيض المختبرية ضمن مدى الجرعة العلاجية.

الهدف من البحث : أستعمال الزيت الاساسي لنبات اكليل الجبل (الروزماري) في تحييد بلازميدات المقاومة للمضادات الحيوية.

طرائق العمل: تم استعمال طريقة لعزل الدنا البلازميدي وتحييد البلازميد باستعمال الزيت الاساسي لنبات الروزماري بتركيز (100 مايكروغرام \مل) ومن ثم الكشف باستعمال الترحيل الكهربائي للجل.

النتائج: اظهرت النتائج ان الزيت الاساسي لنبات الروزماري له القابلية على تحييد البلازميدات من خلال الترحيل الكهربائي لدنا البلازميدي والكشف عن فقدان الصفة المظهرية للمقاومة للمضادات الحيوية.

الاستنتاجات: في هذة الدراسة تم استعمال الزيت الاساسي لتحييد البلازميد بشكل فعال ضد بكتريا القولون الحاوية على بلازميد المقاومة للمضادات الحيوية (الامبسلين , التتراساكلين والسترتوماسين) مما يساعد في استعمال الزيت الاساسي كمساعد للمضادات الحيوية.

Introduction

Biological active natural compounds are of interest to pharmaceutical industry for the control of human diseases of microbial origin and for the prevention of lipid peroxidise damage, which has been implicated in several pathological disorders such as Coronary atherosclerosis, Alzheimer's disease cancergenesis as well as in ageing processes[1].

Essential oils are volatile compounds derived from plants secondary metabolism, and may act as phytoprotective agents [2]. Some of these compounds also have antifungal [3], insecticide, anti-inflammatory ,anti-tumorogenic [4] and antibacterial activity which is important for both food preservation and the control of human and plants diseases of microbial origins[5].This aspect assumes particular relevance since an increased resistance of some very dangerous bacterial strains to the most common antibiotics has recently observed[6,7].

Rosemary is a spice and a medicinal herb widely used around the world as a natural antioxidants [8],Its essential oil is being a natural product and it has been reported to be much safer than antibiotics. Another advantage of Rosemary essential oil has been it's a broad spectrum activities against positive and negative gram bacteria [9].

The objective of this study was to use the essential oil in plasmid curing of R-plasmid in bacteria *Escherichia coli* pBR322 which is resistant for Ampincilin (Amp), Tetracycline (Tc)and Streptomycin (Sm) antibiotics. This may help to use the essential oil of Rosemary as a cofactor for antibiotics.

Material and Methods

1-Material :

I. The essential oil of *Rosmarinus officinalis* was obtained as a gift from Khaleel I.R. from Depart. Of biotechnology- College of Sciences– University of Baghdad.

II. The bacterial strain is *Escherichia coli* pBR322 (Amp, Tc, Sm resistant) from Biology Department-College of Medicine-Baghdad University.

2-Plasmid DNA extraction:

Minipreparation of plasmid DNA obtained by alkaline lysis method [11].

3-Plasmid curing by Rosemary essential oil:

E.coli pBR322 was suspended in 5ml of nutrient broth and was added incubated at 37˚c for 24 hrs . Then(0.1 ml) of *Escherichia coli* pBR322 was suspended (9.9ml) nutrient broth which contains (100µg/ml) and incubated at 37˚c for 2 h [10].Serial dilutions were prepared (10^{1} 10^{3}) and samples were spread from each dilution on nutrient agar which containing Amp (100µg/ml) and incubated for (24-48h) at

37˚C . Then many bacterial colonies cultured by tooth picks patching method on nutrient agar contain Amp, Tc, Sm for detection the phenotype

characters or lost them, this method have been repeated for (3 times).

4-Agarose gel electrophoresis:

DNA samples were loaded in electrophoresis on 0.7% agarose [11]. Gel was prepared in TBE [(50mml/l) of Tris-oH, (50mml/l) boric acid, (1mml/l) EDTA]. The gel was examined under UV light (30nm) and photograph was taken.

Results

In the Fig-1- the DNA plasmid pBR322 was in lane -2,3- which extracted by alkaline lysis and the DNA plasmid which treated with essential oil in lane-1-.

Discussion

The essential oil of *Rosmarinus officinalis* (Rosmary) which extradite from leafs is a natural products, the oil has been reported to be much safer than antibiotics [9] and has no toxicity and lethality on sensitive test (LD50) on laboratory white mice on the other hand has a broad spectrum activities against positive and negative strain bacteria (unpublished data).

The *Rosmarinus officinalis* essential oil was shown to contain (4.85%) composition of Bornyl acetate and (3.85%) Camphor. This oil has been reported to have antibacterial activity due to the presence of Camphor[12].

Figure 1 DNA plasmid extraction from *E.coli* pBR322 which treated with essential oil.

1- DNA plasmid extraction from *E.coli* pBR322 which treated with essential oil.

2-3- DNA plasmid extraction from *E.coli* pBR322.

In this plasmid antibiotic resistance, in Fig-1- we can see the DNA plasmid in lane-2, 3- and when used 100µg/ml of essential oil the land-1- there is no band of DNA and lost of the phenotype of antibiotic resistance.

This method was used for sacylic acid by curing of plasmid and sacylic acid has medical importance[10].

In conclusion in this study we used essential oil for plasmid curing as the

active way against the bacteria , this may help to used as cofactor for antibiotics.

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