



Effect of alcoholic extract of rosmarinus against some type of enterobacteriaceae

Ashwak Ibrahim Basheer

Researches & Islamic Studies Center(Mabdaa) , Iraqi University , Bagdad , Iraq

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Corresponding Author:

Name:

Ashwak Ibrahim Basheer

E-mail:

Ashwak902@gmail.com

Tel:

Abstract

Background : In this study the alcoholic extract of rosemary leaves was used to know their inhibition on the growth of some pathogenic bacteria : *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumonia* and *Proteus mirabilis* in Muller agar using different concentrations of this alcoholic extract(200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml). The mean of inhibition zone were measured, and the most effective concentration was on the following bacteria *Salmonella typhi* , *E. coli*, *P. aeruginosa* , *P. mirabilis* and *K. pneumonia* respectively, and when ever the concentration of extract was increasing , the mean of inhibition was increased .

It was concluded from the results of this study and other similar studies that the natural herbs were able to control the pathogenic microorganism which were resistant to antibiotic, in addition to the important role of these herbs to develop an effective drugs which are safe and cheap .

The aim

The main aim of this research was to find out the inhibitory effect of the alcoholic extract of this plant on the following microorganisms:

Escherichia coli, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumonia* and *Proteus mirabilis*.

Methods

The strains which are used in this study have been cultured on Mueller Hinton agar then alcoholic extracts of *Rosmarinus* at concentration 200 mg/ml were added to the agar then the inhibitory effects were considered by measuring the inhibition zone.

Results

This results shows that inhibitory effect of alcoholic extract was more effective on *Salmonella typhimurium* then *E.coli*. *P.aeruginosa*, *P.mirabilis* and *K.pneumonia* respectively.

High effect of the alcoholic extracts of plant *Rosmarinus* on the different type of bacteria investigation of presence of active compounds (Alkaloids, Glycoside, Tannins, Saponine, Resine and Flavonoids) in this plant were carried out.

Introduction

Plants of nature are considered as a source for many drugs (Medicinal Compounds) which have played a dominant role in the improvement of human health since thousand years. Herbal medicine is considered as a source for about 65-80 % of whole population specially in the developing countries for their essential health because of better cultural acceptability, better compatibility with the human body and less side effects [1]. Plant extracts and

essential oils have been studied as important natural anti- microbial agents for years.

The extract of plants or the active constituents have been used as a folk medicine in the treatment of 80 % of the World population according to world health organization. There are over 50 % of drugs which are used clinically as natural products [2]. The plants *Rosmarinus L.* (rosemary) were belong to the family of lamiaceae. This herbal plant which has a green

picked and fragrant leaves is grown in many places of the world. It is used as cosmetic, flavoring food and in the folk medicine. It is considered as a cholera protective medicine, anti-diabetic, anti-ulcerogenic and anti-inflammatory drugs [3].

Rosemary essential oil is an anti-microbial source and its chemical mixture has anti-oxidant properties and anti-microbial effects have been proved in various researches. The constituents of this plant such as caffeic acid, carnosol, carnosic acid, ursolic acid and rosmarinic acid have been studied during decades for example phenolic acid has been found in a large quantities [4]. Enterobacteriaceae are distributed worldwide and found in flowering plants, water, eggs, meats, soil, fruits, vegetables, tree, grains and in animal by the insect then to the man [5]. Enterobacteriaceae are gram-negative, facultative, anaerobes, oxidase-negative, catalase positive. The pathogenic bacteria include *Escherichia coli*, *Enterobacter spp*, *Klebsiella spp*, *Proteus spp*, *Salmonella spp*, *Shigella spp* [6]. Along with using antibiotics and anti-microbial drugs, it is being seen the increasing prevalence of antibiotic resistant strains of Enterobacteriaceae especially in infection. Although the production of antibiotics increases day by day but resistance in bacteria has created a major problem worldwide [7]. There are a lot of multi drugs which have been considered as resistant for pathogenic strains of Enterobacteriaceae infection. Some strains of Enterobacteriaceae have produced an extended spectrum of β -lactamase which have been used in the treatment of anti-microbial infection in hospital [8]. The increasing resistance to such drugs has been necessary to find an alternative safe drugs and effective factor to treat such bacterial infection. Many researches have confirmed that different pharmaceutical activities are using not only chemical ingredients but also many metabolites of plant origin such as glucosides, phenolic acids, flavonoids, coumarins and essential oils. These are an alternative interesting natural products of plants used for synthesis of preparations which have strong anti-microbial activity [9], and their compounds are used for treatment of hospital infections in advanced countries.

Materials and Methods

1- Plant extraction :

The fresh leaves of Rosemary were collected from local markets and crushed to powder. A 100 grams of leaves weighted and poured into sterilized conical flask. Then one liter of ethanol (70%) was added until herbs compounds were dissolved. Conical flask containing alcohol and herb powder were placed for 24h at room temperature, then centrifuged at 3000 rpm for 15min. The extract was filtered by filter paper (Whitman NO.1) and preserved in sterilized

dishes in incubator at 40 °C for 3days, then stored in refrigerator for further use [10].

One gram of extract was dissolved in 5 ml sterile distilled water. Thus 200 mg/ml of stock was obtained as a standard concentration of 25, 50, 100 mg/ml.

2- Antimicrobial extraction :

The pathogenic strains of Enterobacteriaceae were obtained from Microbiology Department at Tikrit University. Strains of Enterobacteriaceae were collected in (2016) from laboratory of Microbiology and cultured on nutrient broth then incubated at 37°C for 24h. Muller-Hinton agar was prepared, poured in Petri dishes, wells have been done by cork borer. Each petridish was cultured with certain bacteria by diffusion method, then plant extract (few drops) have been added to each well on the surface of agar, petridishes have left to dry and liquids of well have diffused through culture media around the wells then put inverted mode in the incubator [11].

Results have been read after the period of incubation. The positive results were estimated by the appearance of clear zone around the well, the diameter of the halo of growth inhibition was measured and expressed in mm, so the negative results means showed no inhibition and appearance of growth bacteria.

Results and Discussion

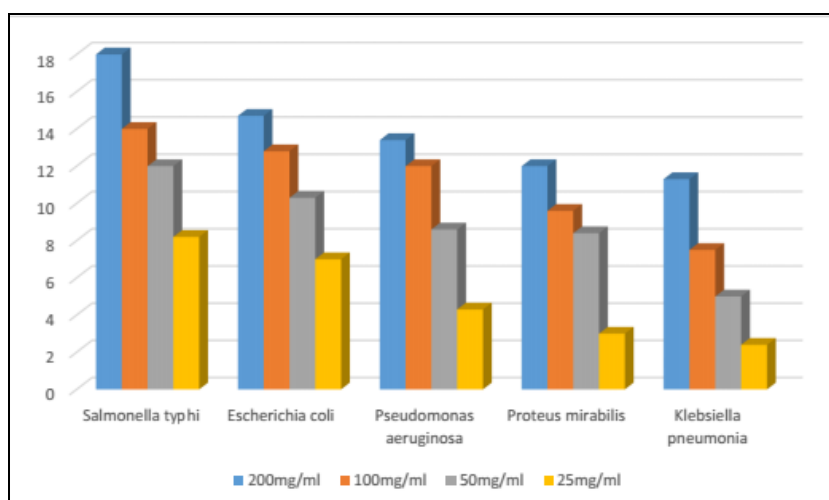
The treatment by plants and their extracts are much more safer on the human health because the antibiotics became source of other diseases such as allergy, in addition to the resistance of most of microorganisms to the chemical antibiotics, this has been substituted by medicinal plants specially of antioxidants activity and of inhibition effect for the growth of bacteria such as the flavonoids. The important characteristic which is responsible for the antimicrobial action of medicinal plants has including hydrophobic components that allow the participation of lipids from the bacterial cell membrane, which disturbs cell structures and make them more permeable [12].

The results of antibacterial activity of alcoholic extract have indicated that the alcoholic extract of rosemary has a significant inhibition on all studied Enterobacteriaceae. The activity of alcoholic extract showed that it is dependent on its concentration.

The diameter of halo of growth inhibition was measured which was ranged between (2.4 – 18) mm for all used concentrations. Results indicated that:

1- All studied bacteria were sensitive to alcoholic extract of rosemary.

2- Figure-1 showed that there was a differences between the effect factor of used concentration in the growth of bacteria, the maximum inhibition was (200) mg/ml of the concentration of extract.



The means of inhibition zones of alcoholic extract of rosemary on some bacteriaceae

3- The alcoholic extract has a very high inhibition at the concentration of (200) mg/ml for *Salmonella* which has a diameter inhibition zone of (18) mm, and the lowest inhibition effect was at the concentration of (25) mg/ml for the *Klebsiella* which was (2.4) mm. All microbiological results obtained during the study showed that alcoholic extract has a very significant antibacterial activity, when *Salmonella* has inhibition

zone of (18) mm at (200) mg/ml concentration of extract, then *Escherichia coli* has (14.7) mm, (13.4) mm for *Pseudomonas* and (12) mm for *Proteus mirabilis*, the less inhibition zone of the same concentration (200) mg/ml was (11.3) mm for *Klebsiella Pneumoniae*, as shown in the following table.

(Table-1) The means of inhibition zones of alcoholic extract of rosemary on some bacteriaceae

25 Mg/ml	50 Mg/ml	100 Mg/ml	200 Mg/ml	Extract concentration
8.2 mm	12 mm	14 mm	18 mm	Salmonella typhi
7 mm	10.3 mm	12.8 mm	14.7 mm	Escherichia coli
4.3 mm	8.6 mm	12 mm	13.4 mm	Pseudomonas aeruginosa
3 mm	8 mm	9.6 mm	12 mm	Proteus mirabilis
2.4 mm	5 mm	7.5 mm	11.3 mm	Klebsiella pneumonia

It is been concluded from the results of this study that the alcoholic extract of rosemary has different modes of action and exhibited stronger biological activity against Enterobacteriaceae and the alcoholic extract has an effect on the growth of gram negative bacteria, this was in agreement with the study [13]. The plant extract contains active gradients such as glycosides, alkaloids, flavonoids, coumarin, tannins and saponins which inhibit the enzymes responsible of basal metabolic reactions by their non specific interactions with the proteins leading to denaturation of proteins and inhibiting the growth of microorganisms [14], and [15]. The activity of alcoholic extract of rosemary may be due to the effect of extract on the permeability of cell membrane and the action of bacteria, the activity of all extracts of this plant were contained phenols which have inhibition on the synthesis of cell walls and cell membranes, nucleic acid and protein, as well as on the metabolism of nucleic acid and on the gram positive and negative bacteria and other active ingredients such as tannins, flavonoids which have wide range inhibition on bacteria and fungi [16].

Several medicinal plants have been used as potential anti-microbial activity instead of chemo therapeutics

and antibiotic methods due to resistance of microorganism for such drugs and the failure of those methods exhibited by the pathogenic microbial infectious agents, so the mode of action of the metabolic compounds of rosemary extract, with multiple targets of action, prevents the development of new virulence factors for bacteria compared to synthetic drugs [17].

Therefore many studies have showed that alcoholic extract possess useful properties for the human health to prevent and treat infectious diseases because of the adaptive resistance which may be have some inhibitory concentration of antibiotics during therapy: cells do not die, but become more resilient [18].

The alcoholic extract proved to inhibit multidrug bacterial strain formation in addition to be anti-fungal, anti-viral and anti-inflammatory agent to treat and prevent pathological changes [19].

Conclusions

The results showed that the alcoholic extract of rosemary has significant inhibition on all studied Enterobacteriaceae and the activity of the extract was increased with the increase in concentration.

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تأثير المستخلص الكحولي لنبات اكليل الجبل في بعض أنواع البكتيريا المعوية

اشواق ابراهيم بشير

مركز البحوث والدراسات الاسلامية (مبدأ) ، الجامعة العراقية ، بغداد ، العراق

المخلص

في هذه الدراسة استخدمنا المستخلص الكحولي لأوراق نبات اكليل الجبل لمعرفة مدى تأثيره في تثبيط نمو بعض انواع البكتيريا الممرضة: *Escherichia coli*, *pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumonia* and *Proteus mirabilis* من خلال زراعتها في وسط Muller agar ومن ثم استخدام المستخلص الكحولي لأوراق النبات بتركيزات مختلفة 200 ملغم/مل، 100 ملغم/مل، 50 ملغم/مل، 25 ملغم/مل وبعدها تم قياس معدلات تثبيط فوجد الترتيب التالي للبكتيريا الأكثر تأثراً بالمستخلص *Salmonella typhi* تليها *Escherichia coli*, *P. aeruginosa*, *P. mirabilis* و *K.pneumonas* وكلما ازداد تركيز المستخلص ازداد معدلات التثبيط. من خلال نتائج بحثنا هذا ومن بقية الدراسات المشابهة له نستنتج ان الاعشاب الطبيعية ممكن ان تكون مصدرا لمنتجات قادرة على السيطرة على الجراثيم الممرضة المقاومة للمضادات الحيوية، اضافة الى الدور المهم لهذه الاعشاب في تطوير الادوية المؤثرة والامنة والرخيصة الثمن.