Effect of Methanolic Extract of Myrtus Communis on Some Pathogenic Bacteria

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Received on: 3/5/2012& Accepted on: 15/8/2012

ABSTRACT

In this study the antibacterial activity of the methanolic extract of Myrtus communis was investigated against Escherichia coli, Klebsiella pneumonia, Pseudomonas aerogenosa and Staphylococcus aureus by agar disc diffusion. Serial concentrations of the extract were tested (1, 2, 3, 4 and 5mg/ml). Most of the extract concentrations showed a relatively high antibacterial activity against all the tested bacteria. The concentration 5mg/ml of plant extracted has the maximum antibacterial activity against all bacterial isolates. Antimicrobial activity of Myrtus communis extract was compared with a number of antibiotics like: Ampicilin (AM10), Tetracyline (TE30), Erythromycin (E15), Carbnicilin (PY100) and Gentamycin (CN10) using antibiogram test. Antibacterial activity of latest concentration (5mg/ml) of Myrtus communis was better than that to Gentamicin against Klebsiella pneumoniae, Escherichia coli and Pseudomonas aerogenosa.

Key words: Biological Effect, Myrtus communis, Pathogenic Bacteria

تأثير المستخلص الميثانولي لنبات الآس على بعض أنواع البكتريا الممرضة

الخلاصة

تم في هذه الدراسة التحري عن الفعالية التضادية للمستخلص الميثانولي لنبات الآس ضد بكتريا Escherichia coli, Klebsiella pneumonia, Pseudomonas aerogenosa and Staphylococcus aureus باستخدام طريقة الانتشار. سلسلة من التراكيز للمستخلص تم اختبارها

https://doi.org/10.30684/etj.31.2B.1
2412-0758/University of Technology-Iraq, Baghdad, Iraq

(١، ٢، ٣، ٤ و ٥ملغم/مل). معظم التراكيز المحضرة من المستخلص اظهرت فعالية تضادية للأنواع البكتيرية قيد الدراسة. من بين التراكيز المحضرة من المستخلص الميثانولي كان التركيز الأخير ٥ملغم/مل هو الأكثر فعالية حيث أعطى أكبر منطقة تثبيط. وقد تم مقارنة الفعالية التثبيطية للمستخلص الميثانولي بعدد من المضادات الحيوية (الامبيسيلين١٠ مايكروغرام، تتراسايكلين٣٠مايكروغرام، الأرثرومايسين ١٥مايكروغرام،كاربينيسيلين ١٠٠مايكروغرام وجنتامايسين ١٠مايكروغرام) المعروفة بقدرتها على تثبيط البكتريا قيد الدراسة. أعطى التركيز الأخير (٥ملغم/مل) من المستخلص الميثانولي لنبات الآس فعالية تضادية أعلى من المضاد الحيوي الجنتامايسين ضد الأنواع Klebsiella pneumoniae, Escherichia coli and Pseudomonas aerogenosa .

INTRODUCTION

Traditional systems of medicine continue to be widely practised on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments[1]. Global estimates indicate that 80% of about 4 billion population cannot afford the products of the Western Pharmaceutical Industry and have to rely upon the use of traditional medicines which are mainly derived from plant material; this fact is well documented in the inventory of medicinal plants, listing over 20,000 species [2]. In spite of the overwhelming influences and our dependence on modern medicine and tremendous advances in synthetic drugs, a large segment of the world population still likes drugs from plants [3]. In many of the developing countries the use of plant drugs is increasing because modern life saving drugs are beyond the reach of three quarters of the third world's population although many such countries spend 40-50% of their total wealth 4 on drugs and health care [4]. As a part of the strategy to reduce the financial burden on developing countries, it is obvious that an increased use of plant drugs will be followed in the future [5].

Myrtus communis (Myriaceae) is an evergreen sclerophyll shrub or small tree, the leaves emit an aromatic and refreshing smell somewhat reminiscent of myrrh or eucalyptus; the taste is very intensive, quite unpleasant and strongly bitter. The plant grows abundantly in the North Western to Eastern Mediterranean [6]. Myrtle is a spice Wnding no wide application because of its bitterness, despite the pleasant odor. Its culinary importance is limited to the region of origin. Foods Xavored with the smoke of myrtle are common in rural areas of Italy or Sardinia [7]. The most important constituents of myrtle oil are myrtenol, myrtenol acetate, limonene, linalool, _-pinene, 1,8-cineole, _-caryophyllenein addition to p-cymene, geraniol, nerol and the phenylpropanoid, methyleugenol [8]. However, there is considerable variability in the composition of oil from different locations [9]. In folk medicine, the fruit of this plant is used in the treatment of many types of infectious disease, including diarrhea and bloody diarrhea, and the leaves are used as antiseptic and anti-inflammatory agent, and as a mouthwash, for the treatment of candidacies [10, 11]. So in this study we investigated the antibacterial properties of methanolic extract of myrtus communis against some pathogenic bacteria.

MATERIALS AND METHOD

Plant extraction

Leaves of Myrtus communis (Myriaceae) were collected, washed thoroughly in tap water and dried at dark room temperature for one week. The dried leaves was ground using an electric blender, 25g of the ground material (Myriaceae) placed in a conical flask and 100 ml of methanol was added to the flask and put on a rotary shaker at 180 rpm for 24 h. The crude extracts were obtained by filtration through (Whatman No.1) filter paper. The filtrate was concentrated and dried at 60 ºC in oven. Extract was then prepared to 1, 2, 3, 4 and 5mg/ml by using sterilized distilled water [12].

Microorganisms

Thetest microorganisms,Escherichia coli,Klebsiella pneumoniae , Pseudomonas aeruginosa and Staphylococcus aureus were obtained from Central Health Laboratory in Baghdad. The organisms were inoculated onto Nutrient Broth (Hi-media) and incubated at 37 ºC for overnight.

Disc diffusion assay

The disc diffusion method of Iennette [13] was used with some modification to determine the rate of growth inhibition of bacteria by the examined plant extracts. The isolated suspensions broth were adjusted to an optical density of 0.5 McFarland, and then streaked with a sterilize cotton swap on plates containing Muller Hinton Agar (Hi-media).Sterilize Filter paper discs (6 mm diameter) which impregnated with a different concentrations of crude extract (1, 2, 3, 4 and 5mg/ml) were placed on the inoculated agar surfaces and incubated for 24hours. Antibiotic standard discsof erythromycin (E15), Ampicilin (AM10), Tetracyclin (TE30), Carbnicilin (PY100) and Gentamycin (CN10) supplied from (Difeco) were tested against the bacterial isolates and with same above conditions. Inhibition zone diameter mesurment after incubation period of 24hr.

RESULT AND DISCUSSION

The presented results show that Gram-positive bacteria Staphylococcus aureus were more resistance than Gram-negative bacteria E. coli, Klebsiella pneumonia and Pseudomonas aeruginosa Table(1).

Table(1): The mean of inhibition zone of the methanolic extract of Myriaceae against Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus .

Bacteria isolate	Inhibition zone (mm) at concentration (mg/ml)*				
	1	2	3	4	5
Klebsiella pneumonia	__	__	10	18	24
Escherichia coli	__	__	__	20	24
Pseudomonas aeruginosa	__	8	12	18	22

Staphylococcus aureus	__	__	6	10	16

* Myriaceae extract

The antibacterial activities of the extract of the plant varied in relation to the test organisms. The most active was 4 and 5 mg/ml concentration that inhibited completely the growth of all tested bacteria Figure (1). Abdullah et al. [14] reported that Gram-negative bacteria are generally more resistant compared to the Gram-positive ones. Also, Shan et al. [15] reported that Gram-positive bacteria (L. monocytogenes, Staphylococcus aureus and B. cereus) were generally more sensitive to the tested extracts than Gram-negative (Escherichia coli and Salmonella anatum). Pseudomonas aeruginosa was the most sensitive, while E. coli was the most resistant.

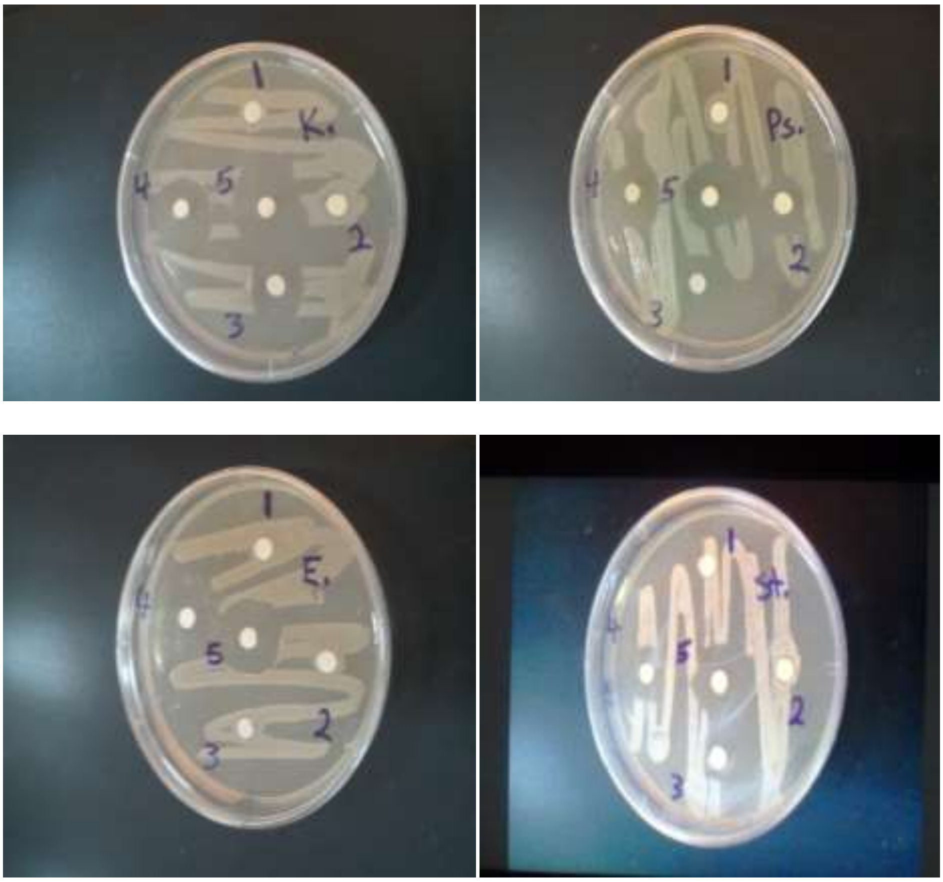


Figure (1): The antibacterial activity of methanolic extract of Myriaceae against Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus .

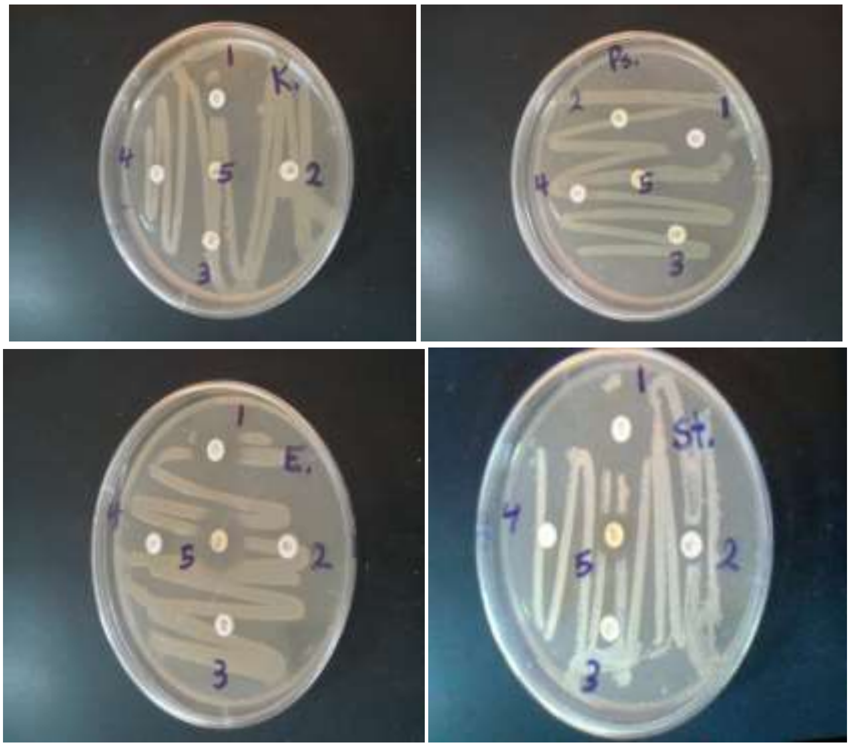
A possible explanation for these observations may lie in the significant differences in the outer layers of Gram-negative and Gram-positive bacteria. Gram-negative bacteria possess an outer membrane and a unique periplasmic space not found in Gram-positive bacteria [16]. The resistance of Gram-negative bacteria towards antibacterial substances is related to the hydrophilic surface of their outer membrane which is rich in lipopolysaccharide molecules, presenting a barrier to the penetration of numerous antibiotic molecules. It is also associated with the enzymes in the periplasmic space, which are capable of breaking down the molecules introduced from outside [17].Gram-positive bacteria do not possess this type of outer membrane and cell wall structure. Antibacterial substances can easily destroy the bacterial cell wall and cytoplasmic membrane and result in a leakage of the cytoplasm and its coagulation [18].

Antibacterial activity of methanolic extract of Myriaceae was compared with number of antibiotics that known for their ability included, Ampicilin (AM10), Tetracyline (TE30), Erythromycin (E15), Carbnicilin (PY100) and Gentamycin (CN10) using antibiogram test Table (2).

Table (2): The inhibition zone of standard discs against some pathogenic bacteria.

bacteria strain	Zone of inhibition(mm)				
	Ampicilin	Tetracylin	Carbnicilin	Gentamicin	Erythromycin
Klebsiella pneumonia	R	R	R	6	R
Escherichia coli	R	10	R	12	R
Pseudomonas aerogenosa	R	R	R	10	R
Staphylococcus aureus	6	4	R	20	R

Antimicrobial activity of latest concentration of of methanolic extract of Myriaceae was better than that to gentamicin against Klebsiella pneumoniae, Escherichia coli, Pseudomonas aerogenosa and Staphylococcus aureus. The resistance of pathogenic bacteria to Carbnicilin, Ampicilin and Erythromycin was noticed Figure (2). The frequent use of antibiotics stimulates the emergence of new strains of pathogenic bacteria, show resistance to these antibiotics and these findings in our study and in other studies.



(A)

(B)

Figure (2): The antimicrobial activity of some antibiotics (A) against Klebsiella pneumoniae, Escherichia coli, (B)Pseudomonas aeruginosa and Staphylococcus aureus .

In this study, the extract was significantly active against Gram-positive bacteria and Gram-negative bacteria. Some studies claim that the phenolic compounds present in spices and herbs might also play a major role in their antimicrobial effects [19]. There has been no large scale, systematic investigation of the relationship between bacterial inhibition and the total phenolic content of spices and herbs. Previous studies [20] showed that a highly positive linear relationship exists between antioxidant activity and total phenolic content in some spices and herbs.

According to the findings of this study, most of the extract concentrations (1, 2, 3, 4 and 5mg/ml) showed relatively high antibacterial activity against all the tested bacteria.

Finally we can conclude from this study that the active compound of leaf extract is a potential source of natural antibacterial agents.

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