

ANTIMICROBIAL ACTIVITY OF VARIOUS PLANTS EXTRACTS AGAINST SOME COMMON PATHOGENIC MICROORGANISM

Sinai Waleed

Tropical-Biological Research Unit, College of Science, University of Baghdad.

Abstract

The effects of plants aqueous extracts (Myrtle, Harmal, Henna, Thyme and Fenugreek) against some clinical species of microorganisms were studied. These isolates included: *Pseudomonas sp.*, *Klebsiella sp.*, *Escherichia coli*, *Proteus sp.*, *Staphylococcus aureus* and *Candida albicans*. The susceptibility of these isolates was tested against plants aqueous extracts by the agar well diffusion method. The results showed that the aqueous extract of Myrtle inhibited the growth of all tested microorganisms at concentration 5%, followed by Harmal when all tested bacteria were inhibited at concentration 10% except *C. albicans* inhibited at 20%. Compare with Henna when all bacteria were inhibited at concentration 20% except *C. albicans* was resistant, followed by Thyme which only two bacteria (*Klebsiella sp.* & *Staphylococcus aureus*) were inhibited at 20% while the remaining microorganism were resistant. On other hand all tested microorganisms were resistant to Fenugreek even at high concentration.

Introduction

Traditionally, plants are used as source of treatment of disease in different parts of the world (1). They are able to produce different compounds that be used to protect themselves against different types of pathogens (2).

Plants are rich in a wide variety of secondary metabolites such as: tannins, terpenoids, alkaloids and flavonoids which have been found in vitro to have antimicrobial properties (2, 3, 4).

The wide range of current antibiotics available for treatment of bacterial infections, there are still some challenges to be met in microbial chemotherapy. One of the problems is the development of resistance to chemotherapeutic agent due to abuse of these drugs (5).

This study was conducted in order to study the antimicrobial effect of aqueous extracts of Thyme (*Thymus vulgaris L.*), Myrtle (*Myrtus communis*), Harmal (*Peganum harmala L.*), Henna (*Lawsonia inermis L.*) and Fenugreek (*Trigonella foenum-graecum*) against six clinical species of microorganisms.

Materials and Methods

Tested Microorganisms

Isolation and identification

Samples were collected from urine and burn wound infections, from patients attending Al-Yarmouk Teaching Hospital during the period from 1/2/2004 to 1/5/2004. All samples

were cultured directly on blood agar, MacConkey agar and Sabouraud dextrose agar. The cultures were examined after overnight incubation at 25 °C - 37 °C.

Identification was based on gram stain, culture methods and biochemical tests (6,7,8).

These microorganisms are: *Pseudomonas sp.*, *Klebsiella sp.*, *Escherichia coli*, *Proteus sp.*, *Staphylococcus aureus* and *Candida albicans*.

- Identification of gram negative bacteria was based on the following tests: indole test, TSI test, citrate utilization, urease test, motility test and oxidase test.
- Identification of *Staphylococcus aureus* was based on the following tests: catalase test, hemolysis test, manitol salt agar, coagulase test.
- Identification of *Candida albicans* was based on the following tests: germ tube formation, chlamydospore production.

Plants collection

Five plants (Thyme, Myrtle, Henna, Harmal and Fenugreek) were collected from different localities in Iraq. They were air dried and packed in plastic containers until used as shown in Table (1).

Extracts preparation

About 50 gm of plant powder mixed with 250 ml of distilled water. After 24 hrs, the extracts were filtered through Whatman No.1 and concentrated to dryness and kept in

labeled dark bottle at 4°C until used. Various concentrations were made from aqueous extracts 5%, 10% and 20% in order to study the effect of these concentrations on different microorganisms.

Agar well diffusion method

The antimicrobial activity was determined by the well diffusion method (9). Wells were made in Mueller Hinton agar for bacteria and sabouraud dextrose agar for *C. albicans*. Plates were seeded with 0.1 ml of 10^8 CFU/ml of bacteria, 10^6 CFU/ml of *C. albicans*. (0.1) ml of plant extracts were added to the wells. Triplicates of each concentration for each microorganism species were prepared. The inoculated plates were incubated at 37°C for 24 hours for bacteria and 25°C for 24-48 hours for *C. albicans*. The diameter of the inhibition zones were measured for each plate. The standard Tetracycline disk (30 mcg) was used as a control (10).

Statistical analysis

Duncan's test was used in the analysis of results.

Results and Discussion

In this study the extracts of the following medicinal plant (Myrtle, Harmal, Henna, Thyme and Fenugreek), were tested for their in vitro antibacterial activity against six isolates of microorganisms (*Pseudomonas sp.*, *Klebseilla sp.*, *Proteus sp.*, *E. coli*, *Staphylococcus aureus* and *Candida albicans*) isolated from urinary tract and burn wound infections as shown in Table (2).

The results showed that the aqueous extract of Myrtle inhibits the growth of all tested microorganisms at concentration 5% and the best effect was on growth of *Proteus sp.* inhibition zone diameter 30 mm., followed by Harmal when all tested bacteria were inhibited at concentration 10% except *C. albicans* inhibited at 20%, the best effect was on growth of *Proteus sp.* and *Klebseilla sp.* inhibition zone diameter 30 mm. In regard to Henna, all bacteria were inhibited at concentration 20% except *C. albicans* was resistant, the best effect was on growth of *Klebseilla sp.* inhibition zone diameter 25 mm. Followed by Thyme which only two bacteria (*Klebsiella sp.* & *Staphylococcus aureus*) were inhibited at 20%, the best effect of inhibition

zone diameter on them was 10 mm. while the remaining microorganisms were resistant. On other hand all tested microorganisms were resistance to Fenugreek even at high concentration.

The zone of inhibition produced by aqueous extracts of Myrtle, Harmal and henna were higher than that produced by Tetracycline.

According to Duncan's test showed that plants extracts have significant difference ($p \leq 0.05$) between them.

Plant extracts have been used for many thousands of years, in food preservation, pharmaceutical, alternative medicine and natural therapies (10, 11, 12).

Myrtle is strongly antibacterial and antifungal herb. This effect may be due to its component which includes:

myrtucommulone-B, myrtucommulone-A, Saponins, phenols, steroids, gallic acid, ellagic acid, acetic acid, citric acid, carvacrol and tannin, They can be toxic to bacteria and yeasts (13, 14). Several studies (15, 16) have shown that Myrtle have strong and consistent inhibitory effects against various pathogens.

The antimicrobial activity of Harmal extract may be due to presence of harmaline, harmine and harmalol (17); these compounds had antibacterial and antiparasitic activities (18, 19). In addition, the Henna extract also showed good antimicrobial effects but high concentrations were required to kill these bacteria except *C. albicans* was resistant, this finding is agreement with Muhammad *et al.* (20) which found that water extract has no activity against the *C. albicans*.

Whereas Thyme and Fenugreek show less or no effect frequently on tested microorganisms, this may be the plant compounds are not dissolved in aqueous solutions (21).

The present study showed that Myrtle, Harmal and Henna extracts were capable of inhibiting the growth of microorganisms that are involved in causing urinary and burn wound infections.

Table (1)
Plants uses in the study, their names and uses part.

Common name	Arabic name	Scientific name	Family	Uses part
Myrtle	الياس	<i>Myrtus communis L.</i>	Myrtaceae	leaves
Thyme	الزعتر	<i>Thymus vulgaris L.</i>	Lamiaceae	leaves
Henna	الحناء	<i>Lawsonia inermis L.</i>	Lythraceae	leaves
Fenugreek	الحلبة	<i>Trigonella foenum-graecum</i>	Fabaceae	seeds
Harmal	الحرمل	<i>Peganum harmala</i>	Zygophyllaceae	seeds

Table (2)
Diameter of inhibition zones caused by five crude plant extracts at various concentrations on some microorganisms.

Plants	Conc. of plants extracts	Diameter of inhibition zone (mm) of microorganisms						Inhibition zone (Mean \pm S. E)*
		<i>Pseudomonas sp.</i>	<i>Proteus sp.</i>	<i>Klebsiella sp.</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>C. albicans</i>	
<i>Thyme</i>	5%	-	-	-	-	-	-	- ^a
	10%	-	-	-	-	-	-	- ^a
	20%	-	-	10	-	10	-	3.3 \pm 2.1 ^{ab}
<i>Henna</i>	5%	-	-	-	-	-	-	- ^a
	10%	-	-	-	-	-	-	- ^a
	20%	20	18	25	15	20	-	16.3 \pm 3.5 ^{cd}
<i>Myrtle</i>	5%	21	18	20	20	22	20	20.2 \pm 0.5 ^{de}
	10%	23	20	25	23	24	23	23.0 \pm 0.7 ^{ef}
	20%	27	30	27	25	26	27	27.0 \pm 0.7 ^f
<i>Harmal</i>	5%	-	20	10	-	10	-	6.7 \pm 3.3 ^b
	10%	19	25	15	14	15	-	14.7 \pm 3.4 ^c
	20%	22	30	30	26	21	10	23.2 \pm 3.1 ^{ef}
<i>Fenugreek</i>	5%	-	-	-	-	-	-	- ^a
	10%	-	-	-	-	-	-	- ^a
	20%	-	-	-	-	-	-	- ^a
Tetracycline	30 mcg	9	10	10	13	20	NT	12.4 \pm 2.0^c

(-) No inhibition zone, (NT) not tested, (*) Different letters: significant difference ($P \leq 0.05$) between means.

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الخلاصة

تم دراسة تأثير المستخلص المائي للنباتات التالية: الياس والحرمل والحنه والزعر والحبلة على تثبيط نمو بعض الاحياء المجهرية المعزولة من عينات سريرية مختلفة وهي:

Pseudomonas sp., *Klebseilla sp.*, *Escherichia coli*, *Proteus sp.*, *Staphylococcus aureus*, *Candida albicans*

أختبرت حساسية تلك العزلات تجاه المستخلصات النباتية باتباع طريقة الانتشار بالحفر. حيث اعطى مستخلص الياس فعالية تثبيطية قوية لنمو تلك العزلات عند التركيز 5% يتبعه مستخلص الحرمل حيث كان له فعالية تثبيطية لنمو الاحياء المجهرية عند التركيز 10% ماعدا *Candida albicans* ثبُتت عند التركيز 20%، في حين مستخلص الحنه كانت لها فعالية تثبيطية عند التركيز 20% للعزلات البكتيرية ماعدا *Candida albicans* بقيت مقاومة لمستخلص الحنه. اما الزعر فكانت له فعالية تثبيطية فقط لبكتريا *S. aureus* و *Klebseilla sp.* عند التركيز 20%، في حين لم يلاحظ اي فعالية تثبيطية للاحياء المجهرية تجاه مستخلص الحلبه المائي.