Efficiency of antibacterial agents extracted from Thymus vulgaris l. (lamiaceae)

I Al-Saimary, S Bakr, B Khudaier, Y Abass

Citation

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Abstract

Antibacterial activity of various concentrations of leaves, flowers and mixtures of Thymus vulgaris L. extracted with distilled water and 90% ethanol were carried out in this study.
Alcoholic extracts are more efficient on various pathogenic bacteria and mixed extracts have a highly antibacterial activity. In general, all extracts in various concentrations with few exceptions are more efficacious on Gram positive bacteria than on Gram negative bacteria. The biggest inhibition zone recorded for 1000 µg/ml of mixed alcoholic extracts was (35 mm) against Strept. faecalis.
The limits of minimal inhibitory concentrations (MICs) of all extracts are between (400-1300) µg/ml.
The efficiency of antibacterial activity of T. vulgaris extracts were compared with efficiency of standard antibiotics against the same bacterial types.

INTRODUCTION

All cultures from ancient times to the present day have used plants as a source of medicine. Today, as many as 80 % of the world's people depend on traditional medicine for their primary health care needs. The greater part of traditional therapy involves the use of plant extracts on their active principle. ([1])

The Iraqi flora are rich in plants unsubmitted to any previous study. The possibility of finding new antimicrobial agents is still widely ahead.

The studied plant is one of these Iraqi flora Thymus vulgaris L. (Lamiaceae or also known as labiatae), common name: Za'ater, Thyme.([2])

It is grown native in Iraq, in the area between Al-Mousl and Dhoook districts. The uses of Thymus vulgaris described in folk medicine: as an emmenagogue, sedative, antiseptic, antipyretic to control menstruation and cramps and in the treatment of dermatitis. ([3]) In vitro studies have shown that flavones and thyme extracts inhibit responses agonists of specific receptors such as acetyl cholin, histamin and L-norepinephrine as well as against whose actions do not require specific receptors such as barium chloride. ([4,5])

This investigation is the first study designed to study the in vitro effects of extracts of Thymus vulgaris L. (lamiaceae) against various pathogenic bacteria and compare it with the antibacterial activities of standard antibiotics.

MATERIALS & METHODS

The plant and Extraction:

Thymus vulgaris L. (labiatae) was collected from areas between Mousl and Dhook district and nearest Karkook City.

Studied plant parts: leaves, flowers and mixed (all plant).

1 gm of each dried plant parts was mixed with 100 ml of distilled water and 90 % Ethyl alcohol (Ethanol). The mixture was mixed by hot plate magnetic stirrer for 48 hours at 50-55 Oc. The mixture was put in centrifuge (5000 xg) for 30 minutes, then made various concentrations from crude extracts (50, 100, 250, 500, 750, 1000) mcg/ml (mcg = µg), in order to study the influence of these concentrations on different bacteria. ([6,7])

The antibacterial activity of plant extracts was compared with ([12]) standard commercial antibiotics (as a single pharmacological dose of concentration for each one). Penicillin, Ampicillin, Carbencillin, Chloramphenicol, Nitrofurantoin, Nalidixic acid, Cephalexin, Tetracycline, Kanamycin, Erythromycin, Gentamicin and Neomycin.

Antibacterial activity:

Various types of pathogenic bacteria isolated from clinical specimens and identified by roatine methods were used. These bacteria are: Escherichia coli, Enterobacter sp., Klebsiella sp., Proteus sp., Pseudomonas aeroginosa, Staphylococcus aureus, Staph. epidermidis, Streptococcus faecalis and Bacillus subtilis.

Blood agar (BA) and Nutrient agar (NA) (Difco) prepared for isolation of bacteria. The antibacterial activity was carried out by two techniques. ($_8$)

1- Agar diffusion technique: To determine the diameters of inhibition zone by using Mueller Hinton agar (MHA) (Oxoid).

2- Tube technique: To determine the minimal inhibitory concentrations (MICs) by using Brain Heart Infusion (BHI) (Difco).

0.1 ml (103 cell/ml) from each bacterial isolates were inoculated on to MHA and BHI.

RESULTS

In general, the present study showed that the alcoholic extracts are more effective on various pathogenic bacteria than aqueous extracts.

The result summarized in Table 1.

Figure 1

Table 1: Effects of various concentration of alcoholic and aqueous extracts of various plant parts of on pathogenic bacteria determine by diameters of Inhibition Zones (mm) and MICs (mcg/ml).

Bacterial types	Plant Parts	Diameters of Inhibition Zone (mm), MIC (mcg/ml)									
		Concentrations of alcoholic extracts (mcg/ml)					Concentrations of aqueous extracts (mcg/ml)				
		250	500	750	1000	MIC	250	500	750	1000	MIC
Escherichia coli	M	3**	7	11	16	500	NE	4	11	16	750
	L	0	6	10	15	600	NE	NE	8	13	750
	F	2	5	9	12	750	NE	NE	6	13	900
Klebsiella	M	6	13	18	23	500	NE	NE	6	16	600
	L	4	9	16	24	600	NE	NE	9	15	750
	F	5	10	14	20	500	NE	NE	4	13	750
Enterobacter	M	2	9	15	20	500	NE	4	10	18	600
	L	2	7	12	20	500	NE	5	12	16	600
	F	4	4	10	16	750	NE	NE	10	16	750
Proteus	M	NE	6	10	16	500	NE	3	7	12	900
	L	NE	NE	8	13	750	NE	NE	4	10	1000
	F	NE	NE	7	12	500	NE	NE	3	6	1100
Ps. aeruginosa	M	NE	NE	4	10	500	NE	NE	4	9	1000
	L	NE	2	8	10	750	NE	NE	2	9	1300
	F	NE	NE	6	8	1000	NT	NT	NT	NT	1250
B. subtilis	M	NE	NE	8	12	600	NE	NE	4	10	750
	L	NE	3	7	10	750	NT	NT	NT	NT	1000
	F	NE	2	7	11	1000	NE	NE	NE	8	1000
Staph. aureus	M	3	12	18	24	500	NE	NE	5	12	600
	L	5	14	20	26	600	NE	3	10	14	800
	F	6	10	15	22	500	NE	NE	4	10	900
Staph. epidermidis	M	6	13	20	28	500	NE	5	12	22	500
	L	4	12	21	26	500	NE	4	11	16	500
	F	8	14	21	28	500	NE	NE	9	16	500
Strept. faecalis	M	8	16	28	35	400	3	5	10	18	500
	L	12	20	26	32	500	NE	6	11	15	500
	F	9	16	22	30	500	NE	2	10	18	600

M: Mixture, L: Leaves, F: Flowers. NE: the concentration was not effected. NT: Not tested

** Highly significant differences between diameters of Inhibition Zones (P<0.01).

Table (1) illustrates efficacious of various concentrations of alcoholic and aqueous extracts from Thymus vulgaris on eight bacterial types. The highest antibacterial activity recorded for (1000 µg/ml) of mixed alcoholic extract (35 mm) for Strept. faecalis and the lowest antibacterial activity noticed for flower extracts (at the same concentration) (6 mm) for Proteus sp. and all other effects of both extracts on bacterial types are located between these records.

The limits of minimal inhibitory concentrations of all (mixed, leaves and flowers) extracts are between (400-1300 µg/ml).

Also we can see that all extracts in concentrations 50 and 100 µg/ml are not effected on all isolated bacteria, while some of concentrations (250 and 500) also are not effected on some bacterial types.

Depending on statistical analysis (ANOVA: Analysis of Variance and/or f-test carried by Havard computer program), we can showed that the mixed extracts in each alcoholic and aqueous extracts have a highly efficacious ($P<0.01$), followed by leaves and flowers ($P<0.05$).

We illustrated the antibacterial activity of twelve standard antibacterial measured by also two methods (I.Z. and MICs) and taken from previous study of the same authors. ([9])

The inhibition zone diameters of antibiotics were as follow: 23 mm for Proteus sp. for Penicillin, 12 mm 14 mm, 16 mm for Ps. aeruginosa for Ampicillin, Carbencillin and Chloramphenicol respectively, 22 mm for Nalidixic acid, 21 mm for Staph. aureus for Cephalexin, 18 mm for N. gonorrhoea for tetracycline, 17 mm for proteus for Kanamycin, 15 mm for Strept. faecalis for Erythromycin, 18 mm for E. coli for Gentamicin, and 20 mm for Klebsiella for Neomycin.

The minimal inhibitory concentrations (MICs) for all antibiotics ranged from 30-350 mcg/ml.

DISCUSSION

The result of the present study showed that Thymus valgaris has antibacterial activities against Gram positive and negative pathogenic bacteria. Mixed extracts have highly activities followed by leaves and flower extracts. Also the alcoholic extracts have a greater effects than aqueous extracts, this may be indicate that the main plant compounds are dissolved or extracted in alcohol extractors more than in aqueous solutions. ([10])

Our results revealed that all extracts in general are more effective on Gram positive bacteria than on Gram negative bacteria, these results may be due to the nature of bacterial cell membrane. The cell membrane of Gram positive bacteria contain mucopolysaccharides, proteins, and less amounts of phospholipids, while Gram negative bacteria have a huge amounts of phospholipids, and more pores in cell envelope. So, the permeability, entrance and reaction of the most antibiotics and/or antimicrobial agents through cell envelope (the outer and cytoplasmic membrane) are highly efficient for Gram positive bacteria depending on reaction with the protein layer (mucopolysaccharides or peptidoglycans). ([8],[11])

The antibacterial activity of Thmus vulgaris extracts may be due to presence of phenolic constituents (thymol and carvacrol), which make up a large percentage of the volatile oil. ([12],[13])

Our results supported the results of advanced studies that used Thymus spp. extracts as antimicrobial agents depend on presence of both thyme essential oil and thymol. Also, these studies suggested use of thyme as an antibiotic. Thymol is 25 times as effective as phenol, but less toxic. ([14], [15], [16], [17])

Other experimental evidence suggests that the in vitro activity of thyme preparations are due to the presence of polymethoxy flavones that have antibacterial activity. ([3])

CONCLUSION & RECOMMENDATION

We concluded that both alcoholic and aqueous extracts of Thymus vulgaris have a high antibacterial activity in comparison with standard antibiotics. And we recommended to identify the chemical compositions of T. vulgaris and in vivo testing of their extracts.

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Author Information

Ihsan Edan Al-Saimary
Dept. of Microbiology, Coll. of Medicine, Univ. of Basrah

Sundus S. Bakr
Dept. of Microbiology, Coll. of Medicine, Univ. of Basrah

Bassam Y. Khudaier
Dept. of Microbiology, Coll. of Veterinary Medicine, Univ. of Basrah

Yass K. Abass
College of Thi Qar Education, Univ. of Basrah