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Full Length Research Paper

Efficiency of antibacterial agents extracted from *Thymus vulgaris* L. (Lamiaceae), part 2

Ihsan Edan Al-Saimary[1]*, Sundus S. Bakr[1], Bassam Y. Khudaier[2] and Yass K. Abass[3]

[1]Department of Microbiology, College of Medicine. University of Basrah, Iraq.
[2]Department of Microbiology, College of Veterinary Medicine. University of Basrah, Iraq.
[3]College of Thi Qar Education. University of Basrah, Iraq.

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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 *Streptococcus faecalis*. The limits of minimal inhibitory concentrations (MICs) of all extracts are between (400 to 1300) µg/ml. The efficiency of antibacterial activity of *T. vulgaris* extracts were compared with efficiency of standard antibiotics against the same bacterial types.

Key words: *Thymus vulgaris*, antibacterial agents, plant extracts, antibiotics.

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 (WHO, IUCN, WWF, 1993). The Iraqi flora are rich in plants unsubmitted to any previous study, the possibility of finding new antimicrobial agents is still widely a head. So, the studied plant is one of these Iraqi flora *Thymus vulgaris* L. (Lamiaceae or also known as labiatae), common name: Za'ater, Thyme (WHO, 1999).

It is grown native in Iraq, in area between Al-Mousl and Dhoook Districts. The uses of *T. vulgaris* described in folk medicine: as an emmenagogue, sedative, antiseptic, antipyretic to control mensturation and cramps and in the treatment of dermatitis (Ghazanfar, 1994). *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 (Vanden and Lemli, 1983; Vanden, 1980). This investigation is the first study designed to study the *in vitro* effects of extracts of *T. vulgaris* L. (lamiaceae) against various pathogenic bacteria and compare it with the antibacterial activities of standard antibiotics.

MATERIALS AND METHODS

The plant and extraction

T. vulgaris L. (labiatae) is collected from areas between Mousl and Dhook District and nearest Karkook City.

Studied plant parts

*Corresponding author. E-mail: ihsanalsaimary@yahoo.com

Leaves, flowers and mixed (all plant). 1 g of each dried plant parts mixed with 100 ml of distilled water and 90% ethyl alcohol (Ethanol). The mixture is mixed by hot plate magnetic stirrer for 48 h at 50 to 55°C. The mixture was put in centrifuge (5000 x g) for 30 min, 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 (Al-Saimary, 1999; Al-Saimery et al., 2002).

The antibacterial activity of plant extracts was compared with (Janssen et al., 1987) 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, Staphylococcus 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 (Baron et al., 1994).

(i) Agar diffusion technique: To determine the diameters of inhibition zone by using Mueller Hinton agar (MHA) (Oxoid).
(ii) 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 results are summarized in Tables 1 and 2.

Table 1 illustrates efficacious of various concentrations of alcoholic and aqueous extracts from *T. vulgaris* on eight bacterial types. The highest antibacterial activity recorded for (1000 µg/ml) of mixed alcoholic extract (35 mm) for *Streptococcus 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 to 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. Depend 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 ($P<0.05$).

Table 2 illustrate the antibacterial activity of twelve standard antibacterial measured by also two methods (I.Z. and MICs) (the results in the Table 2 were taken from previous study of the same authors (Al-Saimary and Baker, 2002).

The inhibition zone diameters of antibiotics were as follow: 23 mm for Proteus sp. for Penicillin, 12, 14 and 16 mm for *P. aeruginosa* for Ampicillin, Carbencillin and Chloramphenicol respectively, 22 mm for Nalidixic acid, 21 mm for *S. aureus* for Cephalexin, 18 mm for *N. gonorrhoea* for tetracycline, 17 mm for proteus for Kanamycin, 15 mm for *S. 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 to 350 mcg/ml.

DISCUSSION

The result of the present study showed that *T. valgaris* have 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 (Al-Saimary and Baker, 2001). Our results revealed that all extracts in general are more effected 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) (Baron et al., 1994;Lennette, et al., 1985).

The antibacterial activity of *T. vulgaris* extracts may be due to presence of phenolic constituents (thymol and carvacrol), which make up a large percentage of the volatile oil (Janssen et al., 1987; Juven et al., 1994).Our results supported the results of advanced studies that used *Thymus* spp. extracts as antimicrobial agents depend on presence of both

Table 1. Effects of various concentration of alcoholic and aqueous extracts of various plant parts of *T. vulgaris* 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
E. 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
P. 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
S. 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
S. 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
S. 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 ($P<0.01$).

thyme essential oil and thymol, also these studies suggested use thyme as an antibiotic, thymol is 25 times as effective as phenol, but less toxic (Panizzi et al., 1993; Cosentino et al., 1999; Marino et al., 1999; Dorman and Deans, 2000). Other experimental evidence suggests that the *in vitro* activities of thyme preparations are due to the presence of polymethoxy flavones that have antibacterial activity (Ghazanfar, 1994).

CONCLUSION AND RECOMMENDATION

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

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TABLE 2 IS CITED IN THE WORK BUT IS NOT GIVEN PLEASE KINDLY PROVIDE.