ANTIBACTERIAL ACTIVITY *IN VITRO* OF *THYMUS CAPITATUS* FROM JORDAN

HAITHAM N QARALLEH, MUAYAD M ABBOUD*, KHALED M KHLEIFAT*, KHALED A TARAWNEH* AND OSAMA Y ALTHUNIBAT

Department of Biomedical Science, International Islamic University Malaysia, 25200 Kuantan Pahang Darul Makmur, Malaysia

**Department of Biology, Mutah University, Karak, Mutah, 61710, Jordan*

ABSTRACT

This study was carried out to evaluate the antibacterial activity of aqueous and organic extracts of *Thymus capitatus* L. (Lamiaceae) leaves and stems. Dried ground powder leaves and stems were extracted with water (aqueous extracts), ethanol, dichloromethane and hexane (Soxhlet extracts). The antibacterial activity of these extracts was evaluated against bacteria using disc diffusion method. The result obtained showed that the leaves had stronger antibacterial activity than the stems extracts. The ethanolic extract had the highest yield products and the high antibacterial activity than all other solvents. The results suggest that essential oil as non-polar organic compounds could be the main active compounds in this plant. Therefore the antibacterial activity of leaves ethanol extracts (LEE) was compared with essential oils leaves extracts (LEO) of *T. capitatus*. The LEO showed greater antibacterial activity than LEE. The LEO showed a broad spectrum of antibacterial activity and the *Pseudomonas aeruginosa* was the most sensitive bacteria.

Keywords: *Thymus capitatus*, antibacterial, soxhlet extraction, essential oil.

INTRODUCTION

Thyme (Thymus) is a genus containing about 350 species of aromatic perennial herbs and sub-shrubs to 40 cm tall, belong to the family Lamiaceae. This family is distributed throughout the arid, temperate and cold regions including Europe, North Africa and Asia. It is in leaf all year, flowering from July to September (Gruenwald *et al*., 2004). In Jordan, Thyme (local name Zahter) is one of many edible plants that grow in mountainous region (e.g. Al karak). It is often used to flavor meats, soups and stews. The edible parts of the plant are the young stem and leaves. It is one of many plants that are used as raw material without any preliminary preparation. Furthermore, thyme is used as salad ingredients and utilized for garnishes or seasonings. The condiment zahtar contains thyme as vital ingredient (Tukan *et al*., 1998).

Many reports have shown that *T. capitatus* possesses biological properties: antimicrobial activity (Alves *et al*., 2000; Al-Tarawneh, 2004; Bounatirou *et al*., 2007; Ebrahimi *et al*., 2008), antifungal activity (Grayer and Harborne, 1994; Kalemba and Kunicka, 2003; Ricci *et al*., 2005) and antioxidant activity (Ricci *et al*., 2005; Bounatirou *et al*., 2007; Al-mustafa and Al-thunibat, 2008).

Generally, the ability of *T. capitatus* to inhibit the growth of pathogenic bacteria has been numerous studies (Alves *et al*., 2000; Al-Tarawneh, 2004; Bounatirou *et al*., 2007). Thyme extracts have shown broad antibacterial activity by inhibiting the growth of both gram-positive and gram-negative bacteria (Al-Tarawneh, 2004; Bounatirou *et al*., 2007). Ethanol extracts of thyme, thyme essential oil, thymol and carvacrol were found to have strong inhibition activity against *S. aureus*, *Bacillus subtilis*, *Shigella sonne* and *E. coli* (Fan and Chen, 2001; Ebrahimi *et al*., 2008). Aqueous extracts of thyme significantly inhibited the growth of *Helicobacter pylori* (Tabak *et al*., 1996). Nimri and his co-workers (1999) have screened 15 of ethanol plant extracts for antibacterial activity. These plants are commonly used in traditional medicine of Jordan and other Middle East countries. *Thymus capitatus* had shown the least effective antimicrobial activity against *P. aeruginosa* and *E. coli*. Furthermore, Mahasneh and El-Oqlah (1999) indicated that methanol and hexane extracts of *T. capitatus* had displayed no antibacterial activity against tested gram-positive and negative bacteria or fungi.

Among the many studies to determine the antimicrobial activities of *T. capitatus*, few screening studies in Jordan have been mentioned the antimicrobial activities of *T. capitatus* (Mahasneh and El-Oqlah, 1999; Nimri *et al*., 1999; Al-Tarawneh, 2004). So far no specific study has been to evaluate the antibacterial activity of *T. capitatus* in Jordan. Therefore, the aim of this study was to investigate antibacterial activities of *T. capitatus* extracts obtained by different extraction methods. The efficacy of water, ethanol, dichloromethane and hexane extract from leaves and stems of *T. capitatus* against four bacteria were investigated and described.

**Corresponding author:* e-mail: haithym2006@yahoo.com

MATERIALS AND METHODS

Plant material

Collective samples of the aerial parts from *Thymus capitatus* growing wild in Aiy region within Al karak district in Jordan were collected during the August 2005. The taxonomic identification was determined by Mu'tah University, Department of biological sciences. A voucher specimen has been deposited at department of biological sciences, Mu'tah University, Jordan.

Collected plant materials were dried in the shade, and the plant leaves were separated from the stem, and grounded in a grinder to small particles.

Extraction

Aqueous extraction

A total of 15 g of dry powdered *T. capitatus* (leaves and stems) were infused in distilled water, and the mixture was heated for 15 min. The extract was then filtered using gauze and Whatman filter paper no.l, before it was sterilized by filtration through sterile syringe filter with 0.2 -0.45 µm pore. Finally the filtered extract was stored as aliquots until it was used.

Soxhlet extraction

The finely ground sample (15 g) of *Thymus capitatus* (leaves and stem) was successively extracted with ethanol, dichloromethane and hexane using the Soxhlet apparatus for 24 h each. The mixture was filtered and dried using a rotary evaporator. The final dried materials were stored in labeled sterile bottles and kept as aliquots until it was used.

Steam distillation Extraction

Air-dried of *T. capitatus* leaves were submitted for 3 h to steam distillation using a Clevenger apparatus to produce the essential oil in a yield of 5.6% (w/w). Oil was dried over anhydrous sodium sulphate and, after filtration, stored at 4°C until used.

Microorganisms

The test bacterial strains *Escherichia coli*, *Enterobacter aerogenes* and *Staphylococcus aureus* were obtained from the Dr. Khaled Khleifat (Department of Biology, Mu'tah University (Jordan)). Their morphological characteristics were re-verified and their biochemical identity was verified using the REMEL kit (RapID^{TM} ONE and RapID^{TM} NF plus systems) procedure by Dr. Khaled Khleifat (Khleifat, 2006 and Khleifat *et al.*, 2006). While *Pseudomonas aeruginosa* was obtained from Mr. Amjed Al-Tarawneh. Later on *P. aeruginosa* was isolated clinically. The isolated *P. aeruginosa* was characterized by using Api 20 NE testing system by Mr. Amjed Al-Tarawneh (Al-Tarawneh, 2004). The test organisms were sub-cultured at 37°C and maintained on nutrient agar media.

Disc Diffusion Method

The agar disc diffusion method was employed for the determination of antibacterial activities of the *T. capitatus* extracts (NCCLS, 1997). Briefly, inoculums containing 10^6 bacterial cells/ml was spread on Nutrients agar plats. Using sterile forceps, the sterile filter papers (6mm diameter) containing 5 µg of *T. capitatus* extracts were laid down on the surface of inoculated agar plate in addition to a positive control (Ciprofloxacin, 5 µg) and negative control (10% DMSO). The plates were incubated at 37°C for 24 h and the zone of inhibition was measured as millimeter diameter.

STATISTICAL ANALYSIS

Data were presented as means ± standard deviation (SD). A computer program (SPSS version 11) was used for statistical analysis. The one-way ANOVA and post hoc multiple comparison tests (Scheffe) were performed to examine the differences among the groups. A P value of <0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

In order to test optimum condition of *Thymus capitatus* extraction for antibacterial activity, different solvents with increasing polarity such as water, ethanol, dichloro-methane and hexane were used. Water was used in aqueous extraction while other solvents were used for Soxhlet organic extraction. Extracts Yield of leaves and stems *T. capitatus* are shown in (table 1). In general, leaves yield crude extracts were higher than stems crude extracts. The ethanolic leaves extract furnished the highest yield (w/w) (0.163g) followed by dichloro-methane (0.125g) and then hexane (0.036g).

The results of antimicrobial activity test of *T. capitatus* extracts by different solvents are shown in table 2. The different extracts of this plant varies in their antibacterial activity against the tested bacteria. The aqueous extraction of the *T. capitatus* gave less antibacterial activities than all other organic solvents extraction. The most active organic extract was the ethanolic extract. An ethanolic extraction from the leaves of *T. capitatus* produced an inhibition zone of almost 19.5 mm against *P. aeruginosa*. The aqueous extract displayed no antibacterial activity, while the extraction with the dichloromethane and hexane gave zone of inhibition equal to 15 and 13 mm, respectively. The stem extract had low and sometimes negligible activity as compared to the leaf extract.

Any part of the plant may contain active antimicrobial compounds but the present study suggested that leaves of *T. capitatus* possess stronger antimicrobial activities than stem. Many reports show that plants leaves possess high antimicrobial activity than other parts (Ndukwe *et al.*, 2007; Nwaogu *et al.*, 2008). In sixty species of plants

Table 1: Yields of Extracts for the *T. capitatus* leaves and stems by using various solvents

Extraction method	Solvents	Leaves		Stems	
		Crude products (g)[a]	Yield (w/w)[b]	Crude products (g)	Yield (w/w)
Aqueous	Water	ND	ND	ND	ND
Soxhlet	Ethanol	2.45	0.163	1.9	0.126
	Dichloromethane	1.87	0.125	0.41	0.027
	Hexane	0.54	0.036	0.12	0.008

[a] Weight of the crude result from 15g of grounded plants leaves and stems after 24 h by Soxhlet extraction.
[b] Yield = weight of crude /15g.
ND; not determined.

Table 2: Antibacterial activity of the various extracts of leaves and stems of *T. capitatus* using agar disc diffusion method

Bacterial strains	Water		Ethanol		Dichloromethane		Hexane	
	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems
E. coli	7	0	12	10	7.75	6.5	8.75	6
E. aerogenes	7	0	12.5	9.25	8.5	0	7.75	0
P. aeruginosa	0	0	19.5	15	15	9	13	8.75
S. aureus	7	0	15	8.5	9	6	15	9

Mean diameter of zone of inhibition in mm including the diameter of the disc 6 mm.

being tested, Alves *et al* (2000) noted that plants leaves have the main antimicrobial activities.

The extraction of active ingredient compounds from plant material depends on the type of solvent used in the extraction procedure (Parekh *et al*., 2005; Majhenic *et al*., 2007). In our study, it was observed that plant extractions with organic solvents provide stronger antibacterial activity than extraction with water. This study confirms the results from previous studies, which reported that water is not a suitable solvent for extraction of antibacterial compounds from medicinal plants compared to other solvent, such as methanol, or ethanol (Karaman *et al*., 2003; Moniharapon & Hashinaga, 2004; Parekh *et al*., 2005; Majhenic *et al*., 2007). Conclusively, this study indicates that most of the active compounds in this plant are concentrated in leaves, insoluble in water and are predicted to be non-polar, hydrophobic organic compounds. This implies that essential oil as non-polar organic compounds could be the main active compounds in this plant. These results agree with that of the previous study (Ali-Shtayeh *et al*., 1997). Ali-Shtayeh *et al*. (1997) reported that the ethanolic extract from *Micromeria nervosa* was the most active extracts compared with ethyl acetate and freeze dried water extracts against all tested bacteria. Based on this result they suggest that the activity of *Micromeria nervosa* is as a result of the essential oil, and so they successfully isolate carvacrol and thymol as active antimicrobial compounds.

Therefore, our results of antibacterial activity of leaves ethanol extracts (LEE) are compared with antibacterial activity of *T. capitatus* essential oils leaves (LEO) extracted by steam distillation (based on the unpublished result of Qaralleh *et al*., 2008).

The antibacterial activity by disc diffusion method of LEE extracts and LEO extracts were compared together and compared with Ciprofloxacin activity against four bacterial strains (table 3). The results reveal that the LEO extracts significantly showed higher inhibition effect than LEE extracts on the growth of *E. coli* and *P. aeruginosa* but not against *E. aerogenes* and *S. aureus*. Ciprofloxacin significantly showed higher antibacterial effects (21.5 - 26.4 mm) than all extracts except LEO extracts against *P. aeruginosa* (24±1.1 mm) and *S. aureus* (18±2.2 mm). The control culture that contained 10% DMSO did not show any antibacterial activity.

Table 3: Antibacterial activity of the leaves Soxhlet extracts (LEE) and leaves essential oils extracts (LEO) of *T. capitatus* using agar disc diffusion method compared to reference antibiotic

Bacterial strain	Negative control	Positive control	LEE extracts	LEO extracts
E. coli	0	26.4±1.5	12±1.8*	16±1.5*[#]
E. aerogenes	0	24.8±2.2	12.5±1.9*	16±1.6*
P. aeruginosa	0	25.1±1.6	19.5±1.4*	24±1.1[#]
S. aureus	0	21.5±1.8	15±1.8*	18±2.2

Negative control, 10% DMSO; Positive control, Ciprofluxacin
Values represent mean ± standard deviation of triplicates.
*Significant deference in comparing with positive control at ($p < 0.05$).
[#]Significant deference in comparing with LEE extraction at ($p < 0.05$).

A large number of studies have been performed concerning the antimicrobial activity of *T. capitatus* (Kandil *et al*., 1994; Nimri *et al*., 1999; Cosentino *et al*., 1999; Bouzouita *et al*., 2003; Al-Tarawneh, 2004; Bounatirou *et al*., 2007). The evaluation of antimicrobial activity of these oils is difficult because of their volatility and their water insolubility (Janssen *et al*., 1987; Celiktas *et al*., 2007). In this study the *T. capitatus* leaves extracts effectively inhibited the Gram positive bacterium *S. aureus* and the Gram negative bacterium *P. aeruginosa* when LEE and LEO extracts were used. The sensitivity of bacteria to an ethanol extract of *T. capitatus* agrees with earlier observations (Nimri *et al*., 1999; Al-Tarwneeh 2004) and agrees with other reports related to plants containing essential oil (Ali-Shtayeh *et al*., 1997; Kunle *et al*., 2003; Sahin *et al*., 2004). The essential oils are more active against gram-positive than gram-negative bacteria (Delaquis *et al*., 2002). Some studies shown that no significant difference between gram positive and gram negative susceptibility after 24 h, however after 48 h gram negative was more susceptible than gram positive (Ouattara *et al*., 1997). These differences in susceptibility between bacteria related to the outer membrane of gram-negative bacteria which bestows the bacterial surface with strong hydrophilicity and acts as a strong permeability barrier (Ultee *et al*., 1999).

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