



Received on 20 June, 2013; received in revised form, 10 September, 2013; accepted, 10 October, 2013; published 01 November, 2013

## ANTIBACTERIAL AND CYTOTOXIC ACTIVITIES OF *THYMUS VULGARIS* LEAVES GROWN IN OMAN

Aisha Haroon Al-Balushi, Maryam Aljabri, Md. Sohail Akhtar\*, Sadri Said, Afaf Weli, Qasim Al-Riyami and Ahlam A. AlAbri

School of Pharmacy, College of Pharmacy and Nursing, University of Nizwa, Nizwa, Sultanate of Oman

### Keywords:

Thyme, *Thymus vulgaris*, Oman, Antimicrobial activity, cytotoxic activity, Brine shrimp test

### Correspondence to Author:

**Md. Sohail Akhtar**

33, Pc 616, Birkat Al-Mouz,  
Nizwauniversity, School of  
Pharmacy, Nizwa, Sultanate of  
Oman

E-mail: sohail@unizwa.edu.om

**ABSTRACT:** Thyme (*Thymus vulgaris* L.) is a pleasant smelling perennial shrub, which grows in several regions in the world. The plant is reported to have high economic and medicinal values. The objectives of this research was to determine antimicrobial activity of petroleum ether, chloroform and hydroalcoholic extracts obtained from leaves of *Thymus vulgaris* collected from Oman. The other objective was to determine cytotoxic activity of the three extracts. Antimicrobial activity was measured using disc diffusion method against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. Brine shrimp test was used to estimate cytotoxic activity. In antibacterial assay, *Thymus vulgaris* leaves extracts showed very strong results, inhibition zones ranged from 7 – 20 mm. So this plant can be used as a good source of potential antimicrobial agent. Petroleum ether showed the highest antibacterial activity. Furthermore, Petroleum ether and Chloroform extracts have almost killed all the shrimp larvae at higher concentration of 1000 mcg/ml. LC<sub>50</sub> values for the two extracts were 85.2 and 95.8 mcg/ml, respectively. Polar fractions like hydro alcoholic extract have displayed very low cytotoxic activities.

**INTRODUCTION:** Thyme (*Thymus vulgaris* L.) is a pleasant smelling perennial shrub, which grows in several regions in the world<sup>1</sup>. The plant is indigenous to the Mediterranean region and neighboring countries, northern Africa, and parts of Asia<sup>2</sup>. The thyme plants are used as antibacterial, antioxidant, natural food preservative. Its use in cosmetic industry has increased the economic importance of this medicinal crop worldwide. Agricultural practices have an influence on growth and yield of thyme plants<sup>3</sup>.

The non-medicinal use of thyme is worthy of attention, because thyme is used in the food and aroma industries; it is widely used as culinary ingredient and it serves as a preservative for foods especially because of its antioxidant effect<sup>4</sup>. Many pharmacological *in vitro* experiments carried out during the last decade revealed well defined pharmacological activities of both, the thyme essential oil and the plant extracts. Thymol and carvacrol constituted the main phenolic compound of Thyme oil.

The major nonphenolic compounds were linalool and p-cymene. Thyme oil with high thymol content strongly inhibited the bacterial growth. Also, thymol has the higher activity against fungi, followed by carvacrol and geraniol, but linalool, terpineol and thujone exhibited the least effect<sup>5</sup>. The cytotoxic activity of ethanolic extracts of



*Thymus vulgaris*, *Thujaorientalis* and *Achilleamillefolium* was investigated on various growing tumor cell lines. MTT colorimetric assay was used for measuring the inhibition of cell proliferation. All of the three extracts showed a relatively dose-dependent inhibition of proliferation of human breast cancer and leukemia cell lines<sup>6</sup>. One of the studies conducted in Morocco, the main chemical constituents isolated were Camphor (38.54%), Camphene (17.19%), Alpha-pinene (9.35%), Borneol (4.92%), and Beta-pinene (4.23%)<sup>7</sup>.

In one of the phytochemical investigation, the major constituents of the oil isolated were thymol (57.7%), p-cymene (18.7%) and carvacrol (2.8%). Other components were present in amounts less than 2%<sup>8</sup>. Cytotoxicity of *Thymus vulgaris* essential oil towards human oral cavity squamous cell carcinoma was seen<sup>9</sup>. The water extracts of thyme has detoxifying and antioxidant effects. Therefore, it is recommended to use them to avoid alcohol toxicity<sup>10</sup>.

Antimicrobial activities of Thyme extract from Oman have been reported<sup>11</sup>. Several *Thymus* species are available worldwide and it is also available in Sultanate of Oman. This plant is known as Zatar in Arabic by the Omani population. The present study was conducted with the objectives;

1. To estimate the cytotoxicity potential and;
2. To evaluate anti-bacterial activity of organic extracts from *Thymus vulgaris* leaves.

## MATERIALS AND METHODS

### Materials:

- All solvents were of analytical grade.
- Gram positive bacteria (*Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) were obtained from microbiology department, College of Arts and Sciences, Nizwa University.
- Filter paper discs of diameter 6mm were obtained from Whatmann Company, catalogue number: 8174900.

- Nutrient agar and Plastic Petri dishes were purchased from SharlauChemie Company.
- Brine shrimp eggs (ARTEMIA CYSTS) were purchased from GOAQUA, Taiwan.
- Sea salt was obtained from Al-Qurum, Muscat. All solvent were evaporated at low pressure using Yomato Rotary evaporator model RE810.
- Incubator using Genelac incubator Model no. Incubator: Gen Lab Model: MINO/75F.

**Sample collection:** *Thymus vulgaris* leaves were collected from Jabalakhdar green mountain of Oman in February 2012. After collection, the leaves were dried in shade for two weeks and weighed, it was then ground using kitchen blender to get powder.

**Preparation of organic extracts:** *Thymus vulgaris* leaves powder (150g) was soaked in ethanol. The solvent was then decanted out and filtered under vacuum using Buchner apparatus to give clear solution. The ethanol was evaporated at low pressure using rotary evaporator to obtain crude extract. This crude extract was re-dissolved in ethanol: distilled water 1:1 mixture and re-extracted successively with Petroleum ether and chloroform. All solvents were then removed using rotary evaporator to give corresponding crude extracts.

**Antimicrobial test:** Antibacterial activity was tested by the disc diffusion method<sup>12</sup>. The assay employed strains of gram positive bacteria, *S. aureus*, and gram negative bacteria *E. coli*, *Klebsiellapneumoniae* and *P. aeruginosa*. Extracts were diluted in four concentrations, 1000, 500, 250 and 125 µg/ml. Amoxicillin was used as a positive control drug.

**Cytotoxicity test:** Brine shrimp (*Artemia salina* Leach) larvae were used as indicator organisms for cytotoxicity assay. The Brine Shrimp Test (BST) was conducted as described by McLaughlin and his coworkers<sup>13</sup>. The BST used four concentrations, 125, 250, 500, and 1000 mcg/ml for each extract. LC<sub>50</sub> values and 95% confidence intervals of each extract were generated by Finney probit analysis of shrimp percent mortality data using a computer program<sup>14</sup>.

**RESULTS AND DISCUSSION:**

**Antibacterial activity:** Table 1 shows results of antibacterial activities of different extracts of *Thymus vulgaris*.

**TABLE 1: ANTIBACTERIAL ACTIVITY OF EXTRACTS AGAINST *E. COLI*, *S. AUREUS*, *P. AERUGINOSA* AND *K. PNEUMONIAE***

Name of extract	Concentrations ( $\mu\text{g/ml}$ )	Inhibition Zone (mm)			
		<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>
Hydro alcoholic extract	1000	13	8	13	15
	500	10	8	12	11
	250	9	7	9	8
	125	7	0	7	7
Chloroform	1000	14	9	9	11
	500	10	8	0	9
	250	9	0	0	7
	125	8	0	0	0
Petroleum ether	1000	13	20	10	15
	500	10	17	9	13
	250	9	11	8	10
	125	7	8	7	8
Control (Dimethylsulfoxide)		0	0	0	0
Amoxicillin	30	25	24	27	22

All the three extracts of *Thymus vulgaris* have shown antibacterial activity against all four micro-organism *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* at different concentrations as compare to the standard drug Amoxicillin. Especially, hydro alcoholic and Petroleum ether extracts showed good inhibitions against all micro-organisms.

Chloroform extract of *Thymus vulgaris* showed moderate inhibition against all the micro-organisms. In one of the previous study of Samiayah et al<sup>15</sup> reported that Antibacterial activity in the leaf extracts of *Thymus vulgaris* L. against pathogenic bacteria like *Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Proteus vulgaris* was evaluated by *in vitro* agar disc diffusion method. Results showed that all organic extracts of the leaves were found to possess strong antibacterial activity especially ethanolic extract followed by acetone.

So the previous studies on *Thymus vulgaris* supported this present study which is conducted in Oman. Medicinal potential of Thyme and its exploitation can certainly support achieving indigenous medical treatment in Oman. These results confirm the potential use of thyme essential

oils in food industry for the preservation of foodstuffs against bacteria and for increasing the shelf life of foodstuffs.

**Cytotoxicity activity:** The results for brine shrimp lethality test of different extracts of *T. vulgaris* are presented in Table 2.

**TABLE 2: MEAN MORTALITY OF BRINE SHRIMP LARVAE WHEN EXPOSED TO PETROLEUM ETHER CHLOROFORM, HYDRO-ALCOHOLIC EXTRACTS FROM *THYMUS VULGARIS* LEAVES.** (n = 10 larvae per treatment)

Concentration mcg/ml	Mortality%		
	Petroleum ether	Chloroform	Hydro alcoholic
125	53	40	26
250	60	73	16
500	76	86	26
1000	100	100	53

It is evident from the table that non polar extracts are more active against the brine shrimp larvae. Petroleum ether and Chloroform extracts have almost killed all the shrimp larvae at higher concentration of 1000 mcg/ml; (mean percent mortality is 100% for Petroleum ether and Chloroform). Polar fractions like hydro alcoholic extract have displayed very low cytotoxic activities.

The LC<sub>50</sub> values for the extracts deduced from the probit analysis results are shown in **Table 3**. As can be seen from the table that Petroleum ether and Chloroform showed very good activity against the test organism, Hydro alcoholic extract showed very less activity.

**TABLE 3: PROBIT ANALYSES OF MORTALITY (LC<sub>50</sub>) OF PETROLEUM ETHER, CHLOROFORM AND HYDRO ALCOHOLIC EXTRACTS OF *THYMUS VULGARIS* LEAVES AGAINST BRINE SHRIMP LARVAE (n = 10)**

Extract	LC <sub>50</sub> (95% CI) (ppm)
Petroleum ether	85.181(19.703 – 190.199)
Chloroform	95.768 (33.398 – 183.485)
Hydro alcohol	3071.510

LC<sub>50</sub> value of Petroleum ether and Chloroform is very less i.e. 85.18 and 95.76µg/ml; it means that these two extracts are highly active and showing very good cytotoxic activity. Hydro alcoholic extract is not showing any activity, its LC<sub>50</sub> value is very high.

Our results are similar to the study reported by Sertel Sand his coworkers about Cytotoxicity of *Thymus vulgaris* essential oil towards human oral cavity squamous cell carcinoma were seen. Thyme essential oil inhibits human HNSCC cell growth<sup>16</sup>. One of the radical scavenging activities reported by El-nekeety AA and his coworkers where non polar extracts exhibited significant activity.

This report also supported the present study which is conducted in Oman. These findings are useful for further research to identify, isolate and characterize the specific compound which is responsible for the higher cytotoxic activity.

**CONCLUSION:** Antibacterial activity of *Thymus vulgaris* leaves extracts conducted and very good results were obtained. So this plant can be used as a good source of potential antimicrobial agent. In addition, cytotoxic assay of *Thymus vulgaris* extracts of Petroleum ether and Chloroform showed very good cytotoxic activity against brine shrimp larvae.

Hydro-alcoholic extract showed very weak activity. The brine shrimp lethality test (BST) has been found to be a quick, versatile method for evaluating general bioactivity of plant extracts.

**ACKNOWLEDGMENT:** Authors are thankful to University of Nizwa, Sultanate of Oman, for providing the infrastructure and necessary research facilities to carry out the research work.

## REFERENCES:

- Hendawy SF, Ezz el-din A, Aziz EE and Omer EA: Productivity and oil quality of *Thymus vulgaris* under organic fertilization condition, Ozean Journal of Applied Sciences, 2010; 3(2): 203.
- El-Nekeety AA, Mohamed SR, Hathout AS, Hassan NS, Aly SE, Abdel-Wahhab MA: Antioxidant properties of *Thymus vulgaris* oil against aflatoxin-induce oxidative stress in male rats, Toxicol, 2011;57(7-8): 984-991.
- Ezz AL-Dein A: Plant growth strategies of *Thymus vulgaris* L. in response to population density, Industrial Crops and Products: 2009; 30: 389-394.
- Agrigore A, Paraschiv I, Colceru-mihul S, Bubueanu C, Draghici E, Ichim M: Chemical composition and antioxidant activity of *Thymus vulgaris* volatile oil obtained by two different methods, Romanian Biotechnological Letters; 2010;15(4) 2010.
- Mensure Ozgüven, Sezen Tansi: Drug Yield and Essential Oil of *Thymus vulgaris* L. as influenced by Ecological and Ontogenetical Variation, 1998; 537-542.
- Amirghofran Z and Karimi MH: Cytotoxic activity of *Thymus vulgaris*, *Achillea millefolium* and *Thuja orientalis* on different growing cell lines, MJIRI 2001, 15(3): 149-154.
- Imelouane B, Hamhamdi H, Wathelet J.P, Ankit M, Khedid K and El bachiri A: Chemical Composition and Antimicrobial Activity of Essential Oil of Thyme *Thymus vulgaris* from Eastern Morocco, Int. J. Agric. Biology, 2009; 11(2): 205-208.
- Rota M.C, Herrera A, Martinez R.M, Sotomayor J.A, Jordan M.J: Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils, *Food control*, 2008; 19: 681-687.
- Sertel S, Eichhorn T, Plinkert PK, Efferth T: Cytotoxicity of *Thymus vulgaris* essential oil towards human oral cavity squamous cell carcinoma. *Anticancer Research*, 2011; 31(1):81-7.
- Ali AS, Elsaid FJ: Effects of water extracts of thyme (*Thymus vulgaris*) and ginger (*Zingiber officinale*) on alcohol abuse, *Food and chemical toxicology*, 2009; 47: 1945-1949.
- Nzeako BC, Al-Kharousi, ZSN and Al-Mahrooqui, Z: Antimicrobial Activities of Clove and Thyme Extracts. *Sultan Qaboos Univ Med journal*, 2006; 6(1): 33-39.
- Singh B, Sahu PM, Sharma MK: Anti-inflammatory and Antimicrobial activities of

- triterpenoids from *Strobilanthes callosus* Nees, *Phytomedicine*, 2002; 9, 355-359.
13. McLaughlin JL, Rogers LL, Anderson JE: The use of biological assay to evaluate botanicals, *Drug Info. Journal*, 1998; 32, 513-524.
  14. (USEPA) U.S. Environmental Protection Agency: EPA Probit analysis program. Calculating LC/EC values Version 1.5. 1994. ([http:// www.epa.gov/nerleerd/stat2.htm](http://www.epa.gov/nerleerd/stat2.htm)) downloaded April 2012.
  15. Chitra S, SEBAREZE L: Antibacterial Activity of *Thymus Vulgaris* L. (Thyme) From Leaf Extracts – A Medicinal Plant, *International journal of Ecology Development*, 2011; 20(11): ISSN 0973-7308 (Online).
  16. Sertel S, Eichhorn T, Plinkert PK, Efferth T: Cytotoxicity of *Thymus vulgaris* essential oil towards human oral cavity squamous cell carcinoma, *Anticancer Res.* 2011; 31(1):81-87.

**How to cite this article:**

Al- Balushi AH, Aljabri M, Akhtar MS, Said S, Weli A, Al-Riyami Q and AlAbri AA: Antibacterial and cytotoxic activities of *Thymus vulgaris* leaves grown in Oman. *Int J Pharm Sci Res* 2013; 4(11): 4253-57. doi: 10.13040/IJPSR. 0975-8232.4(11).4253-57

All © 2013 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **ANDROID OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)