

## SCREENING OF ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL OF *THYMUS VULGARIS* SOME PATHOGENS

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### ABSTRACT

The inhibitory effects of *Thymus vulgaris* essential oils were screened against Coliform spp, *Pseudomonas* spp, *Saccharomyces cerevisiae* (EC1118), *Zygosaccharomyces bailii* (DSM 70492) and *Lactobacillus plantarum* (DSM2601) by agar well diffusion method. Results obtained in this study revealed that the essential oils of *Thymus vulgaris* possess a weak antibacterial effect against the tested microorganisms with small inhibition zones ranged between 0 and 4 mm. This activity was dose independent. *Z. baillii* was more susceptible to *T. vulgaris* essential oils, while Coliform spp was more resistant comparing to other tested microorganisms.

**Keywords:** *Thymus vulgaris*, Essential oils, Antibacterial activity, Bacteria, Yeast, Agar well diffusion method.

**Contribution/ Originality:** This study contributes to the existing literature and the scientific justification to the use of this plant in herbal medicine for the treatment of diseases of microbial origin and also introduces it as a potential candidate for drug development for the treatment of infectious diseases caused by these pathogens.

### 1. INTRODUCTION

Plants of the genus *Thymus* are among the most popular in the world for their aromatic and medicinal properties [1]. The genus of *Thymus* comprises 300–400 species, some of which are used in folk medicine. The main medicinal *Thymus* is *Thymus vulgaris* (common thyme) which is used for dry coughs, bronchitis, laryngitis, indigestion and gastritis [2] thanks to several properties such as antiseptic, antispasmodic, antitussive antimicrobial, antifungal, antioxidative, and antiviral [3, 4]. The main compounds of *Thymus vulgaris* are the natural terpenoid thymol, its phenol isomer carvacrol and flavonoids, which have antimicrobial effects [5, 6].

The aim of the present work was to investigate the antimicrobial effects of the liquid extract of *Thymus vulgaris* against Coliform spp, *Pseudomonasspp*, *S. cerevisiae*, *Z. bailii* and *L. plantarum*.

## 2. MATERIAL AND METHODS

### 2.1. Essential oils

The essential oils (EO's) used in this study were thyme (*Thymus vulgaris*). They were obtained from Farmalabor (Canosa di Puglia, Italy) as liquid extract.

### 2.2. Test Microorganisms

The microorganisms used for this experiment were Coliform spp and *Pseudomonasspp* belonging to the Culture Collection of the Laboratory of Applied Microbiology (University of Foggia, Italy), *Saccharomyces cerevisiae* EC1118 (Lallemand Inc.), *Zygosaccharomycesbailii* DSM 70492 and *Lactobacillus plantarum* DSM2601 were obtained from German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, DSMZ, Germany).

### 2.3. Antimicrobial Assay

The antimicrobial activity was assayed by a modification of the agar diffusion method (Kirby-Bauer) on Nutrient agar (except *L. plantarum* MRS). The overnight culture of the microorganisms cultures were inoculated on Nutrient agar plates using sterilized cotton swabs. In each of these plates, two wells were cut out using a sterilized cork borer. The *T. vulgaris* essential oils were used against test pathogen, 100 µL of extracts of different concentration (10, 50 and 100 ppm) were loaded into each well. The control was cultured without essential oil. Plates of bacteria were incubated at 37°C, while those of yeast at 25°C for 24 hours. After incubation, all plates were examined for the presence of zone of inhibition around the wells.

## 3. RESULTS AND DISCUSSION

The effects of the *Thymus vulgaris* extract on the organisms were summarized in Table 1. The antimicrobial activity of *T. vulgaris* essential oil shows positive result against all strains. It gives varied levels of antimicrobial activity against the tested organism strains.

The zones of inhibition produced against the tested organisms strains ranged between 00 and 04 mm. The largest mean zones of growth inhibition (3 and 4 mm) were produced by 10 ppm and 50 ppm of *T. vulgaris* oil against *Z. baillii*, followed by 10 ppm of the same extract against Coliform spp (2.5 mm diameter). While some concentrations did not show any antimicrobial activity against coliform spp (50 and 100 ppm), *Pseudomonasspp* (50 ppm) and *S. cereviceae* (100 ppm). Among the organisms, *Z. baillii* was the most sensitive strain (1.5-4 mm), while Coliform spp was the most resistant (0-2.5 mm), *Pseudomonasspp* (0-2 mm), *S. cereviceae* (0-2 mm) and *L. plantarum* (0.45-2 mm) have moderate antimicrobial activity to *T. vulgaris* oil.

Table-1. Effect of *T. vulgaris* essential oil on test organisms.

	Coliform spp	Pseudomonas spp	S. cereviceae	Z. baillii	L. plantarum
10ppm	2.5	1.5	2	4	0.45
50	NI	NI	2	3	2
100	NI	2	NI	1.5	2

NI: no inhibition

Several studies from different country in the world have demonstrated the antimicrobial activity the essential oils of *T. vulgaris* against many pathogens [7-18]. The antimicrobial activity of an essential oil is attributed mainly to its major compounds; however the synergistic or antagonistic effect of a minor component of the complex mixture has to be considered [19]. Several past studies identified thymol, carvacrol and p-cymene as the main components in *Thymus* essential oil [20-25]. The weak antibacterial activity of *Thymus vulgaris* essential oil founded in our study could be due to its lack of phenolic contents (thymol and carvacrol). Our results are in good agreement with the findings of Millezi, et al. [26] who reported production of a weak zone of inhibition in his study of the antimicrobial activities of plant essential oils *T. vulgaris*, *C. citratus* and *L. nobilis* against five important foodborne pathogens (*S.aureus*, *E.coli*, *L. monocytogenes*, *S. Enteritidis*, and *P. aeruginosa*).

In conclusion, the findings of the present study indicate that *T. vulgaris* essential oil has an antimicrobial activity against microorganisms that are pathogenic to humans. Given the results described above, particularly the possible mechanisms of action, which might induce side-effects in humans, this antimicrobial agent require further investigation. The results presented should stimulate studies on toxicity, improved formulations and the determination of optimal concentrations for clinical applications, as well as comparative studies alongside currently used drugs of the therapeutic efficacy of essential oils to control infections.

## REFERENCES

- [1] A. C. Figueiredo, J. G. Barroso, L. G. Pedro, L. Salgueiro, M. G. Miguel, and M. L. Faleiro, "Portuguese thymra and thymus species volatiles: Chemical composition and biological activities," *Current Pharmaceutical Design*, vol. 14, pp. 3120-3140, 2008.
- [2] H. Hajimehdipoor, M. Shekarchi, M. Khanavi, N. Adib, and M. Amri, "A validated high performance liquid chromatography method for the analysis of thymol and carvacrol in thymus vulgaris L. volatile oil," *Pharmacognosy Magazine*, vol. 6, pp. 154-158, 2010.
- [3] A. Ocaña and G. Reglero, "Effects of thyme extract oils (From *Thymus Vulgaris*, *Thymus Zygis*, and *Thymus Hyemalis*) on cytokine production and gene expression of oxLDL-stimulated THP-1-macrophages," *Journal of Obesity*, vol. 2012, p. 11, 2012.

- [4] M. A. Dubinnyi, D. I. Osmakov, S. G. Koshelev, S. A. Kozlov, Y. A. Andreev, and N. A. Zakaryan, "Lignan from thyme possesses inhibitory effect on ASIC3 channel current," *The Journal of Biological Chemistry*, vol. 287, pp. 32993-3000, 2012.
- [5] F. C. Fachini-Queiroz, R. Kummer, C. F. Estevão-Silva, M. D. D. B. Carvalho, J. M. Cunha, and R. Grespan, "Effects of thymol and carvacrol, constituents of thymus vulgaris L. essential oil, on the inflammatory response," *Evidence-Based Complementary and Alternative Medicine*, vol. 2012, p. 10, 2012.
- [6] S. Shabnum and M. G. Wagay, "Essential oil composition of thymus vulgaris L. and their uses," *Journal of Research & Development*, vol. 11, pp. 83 – 94, 2011.
- [7] B. A. Abdul Sattar, A. M. Hassan, and A. S. Hassan, "In vitro antimicrobial activity of thymus vulgaris, origanum vulgare and rosmarinus officinalis against dental caries pathogens," *Ibn Al-Haitham Journal for Pure and Applied Science*, vol. 25, pp. 33-39, 2012.
- [8] M. A. A. Al.Maqtari, S. M. Alghalibi, and E. H. Alhamzy, "Chemical composition and antimicrobial activity of essential oil of thymus vulgaris from Yemen," *Turkish Journal of Biochemistry*, vol. 36, pp. 342–349, 2011.
- [9] E. A. Al-Timimi and M. AL-Casey, "Effect of thymus vulgaris extract on streptococci and mutans streptococci, in comparison to chlorhexidine gluconate (In Vivo Study)," *Journal of Baghdad College of Dentistry*, vol. 24, pp. 116–121, 2012.
- [10] Imelouane, H. Amhamdi, J. P. Wathélet, M. Ankit, K. Khedid, and A. El Bachiri, "Chemical composition and antimicrobial activity of essential oil of thyme (Thymus Vulgaris) from Eastern Morocco," *International Journal of Agriculture and Biology*, vol. 11, pp. 205–208, 2009.
- [11] A. S. Klaus, D. V. Beatovic, M. P. Niksic, S. C. Jelacic, V. K. Nedovic, and T. S. Petrovic, "Influence of ethereal oils extracted from lamiaceae family plants on some pathogen microorganisms," *Proc. Nat. Sci, Matica Srpska Novi Sad*, vol. 115, pp. 65-74, 2008.
- [12] K. Kon and M. Rai, "Antibacterial activity of thymus vulgaris essential oil alone and in combination with other essential oils," *Nusantara Bioscience*, vol. 4, pp. 50–56, 2012.
- [13] M. Marino, C. Bersani, and G. Comi, "Antimicrobial activity of the essential oils of thymus vulgaris L. measured using a bioimpedometric method," *Journal of Food Protection*, vol. 62, pp. 1017-1023, 1999.
- [14] H. Miladi, R. Ben Slama, D. Mili, S. Zouari, A. Bakhrouf, and E. Ammar, "Essential oil of thymus vulgaris L. and rosmarinus officinalis L.: Gas chromatography-mass spectrometry analysis, cytotoxicity and antioxidant properties and antibacterial activities against foodborne pathogens," *Natural Science*, vol. 5, pp. 729-739, 2013.
- [15] K. S. L. Mota, F. O. Pereira, W. A. Oliveira, I. O. Lima, and E. O. Lima, "Antifungal activity of thymus vulgaris L. Essential oil and its constituent phytochemicals against rhizopus oryzae: Interaction with ergosterol," *Molecules*, vol. 17, pp. 14418-14433, 2012.
- [16] M. Sadiki, M. Balouiri, H. Barkai, H. Maataoui, S. I. Koraichi, and S. Elabed, "Synergistic antibacterial effect of myrtus communis and thymus vulgaris essential oils fractional inhibitory

- concentration index," *International Journal of Pharmacy and Pharmaceutical Sciences*, vol. 6, pp. 121-124, 2014.
- [17] D. F. Santurio, P. P. C. Moraes, P. R. Franchin, and S. H. Alves, "Atividade antimicrobiana dos óleos essenciais de orégano, tomilho e canela frente a sorovares de salmonella enterica de origem avícola," *Ciência Rural, Santa Maria*, vol. 37, pp. 803-808, 2007.
- [18] M. D. Soković, J. Vukojević, P. D. Marin, D. D. Brkić, V. Vajs, and J. L. D. Leo Van Griensven, "Chemical composition of essential oils of thymus and mentha species and their antifungal activities," *Molecules*, vol. 14, pp. 238-249, 2009.
- [19] F. M. Santos, J. E. B. P. Pinto, S. K. V. Bertolucci, A. A. Alvarenga, M. N. Alves, M. C. T. Duarte, and A. Sartoratto, "Chemical composition and antimicrobial activity of the essential oil from the leaves and flowers of aloysia gratissima," *A Revista Brasileira de Plantas Medicinai*s, vol. 15, pp. 583-588, 2013.
- [20] S. A. Burt and R. D. Reinders, "Antibacterial activity of selected plant essential oils against escherichia coli O157:H7," *Letters in Applied Microbiology*, vol. 36, pp. 162-167, 2003.
- [21] A. M. Janssen, J. J. C. Scheffer, and S. A. Baerheim, "Antibacterial activity of essential oils: A 1976-1986 literature review. Aspects of the test methods," *Planta Medica*, vol. 53, pp. 395-398, 1987.
- [22] B. L. Juven, J. Kanner, F. Schved, and H. Weisslowicz, "Factors that interact with the antibacterial action of thyme essential oil and its active constituents," *Journal of Applied Bacteriology*, vol. 76, pp. 626-631, 1994.
- [23] J. Kaloustian, L. Abou, C. Mikail, M. J. Amiot Carlin, and H. Portugal, "Southern French thyme oils chromatographic study of chemotypes," *Journal of the Science of Food and Agriculture*, vol. 85, pp. 2437-2444, 2005.
- [24] M. Özcan and J. C. Chalchat, "Aroma profile of thymus vulgaris L. growing wild in Turkey," *Bulgarian Journal of Plant Physiology*, vol. 30, pp. 68-73, 2004.
- [25] A. Sartoratto, A. L. M. Machado, C. Delarmelina, G. M. Figueira, M. C. T. Duarte, and V. L. G. Rehder, "Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil," *Brazilian Journal of Microbiology*, vol. 35, pp. 275-280, 2004.
- [26] A. F. Millezi, D. S. Caixeta, D. F. Rossoni, M. G. Cardoso, and R. H. Piccoli, "In vitro antimicrobial properties of plant essential oils thymus vulgaris, cymbopogon citratus and laurus nobilis against five important foodborne pathogens," *Food Science and Technology*, vol. 32, pp. 167-172, 2012.

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