# PREPARATION, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF CLINOPTILOLITE/THYMOL COMPOSITES

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## ABSTRACT

Composites based on natural zeolite – clinoptilolite and thymol were prepared using two different approaches: 1) wet impregnation (WI) and supercritical solvent impregnation (SSI). The SSI was more superior since it yielded the composite with higher amount of thymol and with stronger thymol-clinoptilolite interactions than WI. The composites exhibited antibacterial activity towards Gram-negative bacteria *Escherichia coli* and Gram-positive bacteria *Staphylococcus aureus* suggesting its applicability for disinfectant purposes.

Keywords: natural zeolite, thymol, antibacterial activity, supercritical solvent impregnation.

## INTRODUCTION

Essential oils derived from plants belonging to the *Lamiaceae*family (such as *Thymus vulgaris, Lippiagracilis* and *Origanumvulgare*) exhibit antiseptic properties. Thymol (2-isopropyl-5-methylphenol) is a major component of these oils and has been considered as a safe herbal drug without harmful health effect [1]. Nowadays, different materials have been studied as thymol carriers such as collagen, cotton, cellulose, polypropylene, starch, alginates, etc. In this work natural zeolite - clinoptilolite was tested for thymol loading and antibacterial performance of obtained composites towards Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus*.

## EXPERIMENTAL

*Preparation of the composites*

The zeolitic tuff (Z) from Vranjska Banja deposit (Serbia) with about 70 wt. % of natural zeolite – clinoptilolite and with particle size of 63-125 μm was used in experiments. The Z was transformed firstly into a $NH_4$-form ($NH_4$-Z) by its treatment with a solution of ammonia acetate (1 mol $dm^{-3}$) during 24 h at room temperature and then into H-form (H-Z) by two steps: 1) calcination of $NH_4$-Z in air at 550 ºC for 3 h and 2) treatment of the calcined product with 0.6 mol $dm^{-3}$ of HCl at 70 ºC for 1 h. Finally, the product was washed with distilled water until the negative reaction to $Cl^-$, dried overnight at 60 ºC to a constant mass.

Thymol loading was performed by two procedures: 1) WI using an ethanol solution of thymol (9 wt. %) and 2) SSI with $CO_2$ in a high-pressure view chamber (Eurotechnica GmbH), using a static method (at t= 35 ºC and p= 300 MPa for 18 h). At the end of the experiment, the $CO_2$ was released at the rate of 1.5 MPa $min^{-1}$. The obtained samples were denoted as ZT1 and ZT2, respectively.

*Antibacterial activity test*

Modified method based on disk diffusion was used in this work as a qualitative assessment of antibacterial activity toward Gram-negative *Escherichia coli* DSM 498 and Gram positive *Staphylococcus aureus* TL (culture collection-FTM, University of Belgrade, Serbia). The suspensions of bacteria, containing about $10^8$CFU $cm^{-3}$ of bacteria, were placed onto the Nutrient agar. The suspensions containing 5, 10 and 20 mg of H-Z, ZT1 or ZT2 per $cm^3$ in sterile solution of 0.085 % NaCl were examined. On the Petri dishes inoculated with

bacteria cells, the $10^{-2}$ $cm^3$ of the prepared suspensions were dropped. The Petri dishes were placed in the incubator for 24 h at 37 ºC and the zone of inhibition was determined.

*Desorption of thymol from ZT1 and ZT2*

ZT1 or ZT2 was suspended into a water solution of ethanol (10 wt. %), and left in a water bath (Memmert WNB 22) at 25 ºC for 15 min to 24 h. The solid/liquid ratio was 1:100. The thymol concentration released from the composites was determined photometrically by measuring the absorption intensity at $\lambda_{max}$= 274 nm using an UV-VIS spectrophotometer Cary 100 Scan (Varian).

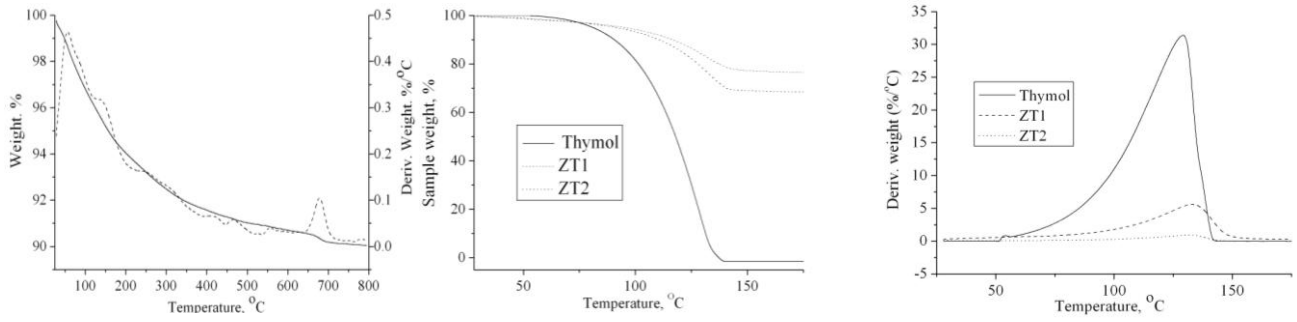
*Characterization*

Thymol amount in ZT samples were determined by thermogravimetric analysis (TGA) using a SDT Q-600 simultaneous DSC-TGA instrument (TA Instruments). Powder X-ray diffraction analysis (PXRD) was performed to check composite crystallinity. PXRD patterns were recorded using an Ital Structure APD2000 diffractometer. Specific surface area of the zeolite samples before thymol loading was calculated according to the Brunauer, Emmett, Teller (BET) method using the $N_2$ adsorption–desorption experiments (Micromeritics ASAP 2020). Interactions of thymol with clinoptilolite lattice were studied byFourier Transform Infrared (FTIR) Spectroscopy. The FTIR spectra were recorded in the range 4000–450 $cm^{-1}$ with a resolution of 4 $cm^{-1}$ at room temperature, using Nicolet iS10 (Thermo Scientific) spectrometer.

## RESULTS AND DISCUSSION

The PXRD analysis of H-Z, ZT1 and ZT2 confirmed that the clinoptilolite lattice was not significantly affected by the conversion of Z into H-Z and by thymol loading onto H-Z (data not shown). The conversion of the Z into H-Z significantly increased the specific surface area from 42 $m^2 g^{-1}$ (Z) to 230 $m^2 g^{-1}$ (H-Z) suggesting that the clinoptilolite pore system was partially opened by the modification procedure.

Figure 1 shows the results of TGA. The TG curves of H-Z, ZT1 and ZT2 display different weight loss (wt.%): H-Z - 9.7, ZT1 - 29.3 and ZT2 - 34.9.




a) b) c)

Figure 1. (a) TG/DTG curves of H-Z; (b) TG curves of thymol, ZT1 and ZT2; (c) DTG curves of thymol, ZT1 and ZT2.

In order to compare TG profiles of the studied samples with the TG profile of pure thymol, TGA of thymol was also performed and the coresponding curve is given in Figure 1. It is evident that the thermal decomposition of thymol proceeds in a single step up to 150 °C with the DTG maximum centered at 129 °C. Both ZT1 and ZT2 exhibit similar thermal behaviour. The DTG maximum corresponding to this thermal event is at 134 °C for both samples. On the other hand, the TG curve of H-Z displays a different profile and continuous weight loss up to 700 °C which can be attributed to water loss. All of these lead to conclusion

that both impregnation method resulted in thymol loading onto H-Z and that the loading amount depends on the impregnation method. A higher thymol loading is obtained by SSI (~25 wt %) than by WI (~20 wt %).

Figure 2 shows FTIR spectra of thymol, H-Z, ZT1 and ZT2. The thymol spectrum displays characteristic stretching vibrations at ($cm^{-1}$): 3169 and 3035 due to phenolic =C-H, and 2929 due to phenolic –OH; 1622, 1585 and 1458 due to C=C of benzene ring [2]. The spectrum of H-Z displays characteristic pattern below 1100 $cm^{-1}$ corresponding to the vibrations of $SiO_4$ and $AlO_4$ tetrahedra [3]. The thymol characteristic bands appear in the spectra of ZT1 and ZT2.

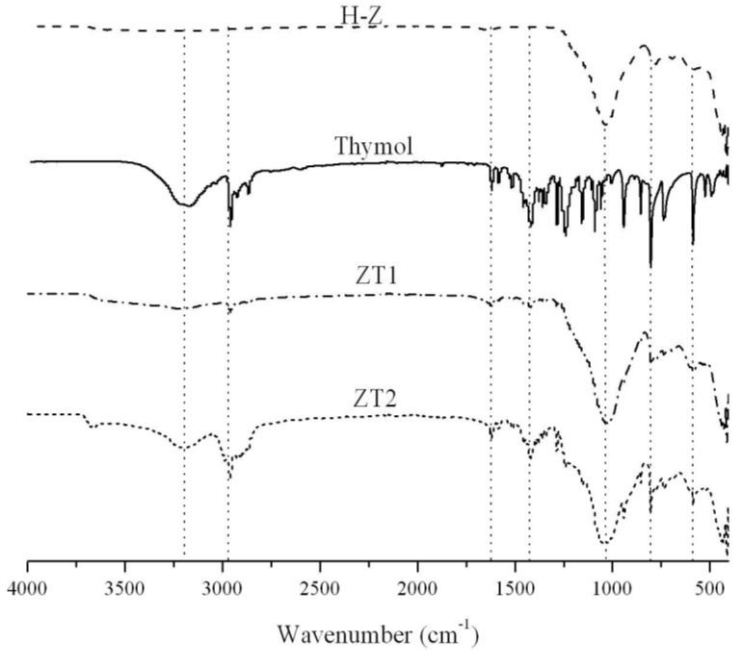



Figure 2. FTIR spectra of H-Z, thymol, ZT1 and ZT2 sample.

*Antibacterial activity*

Antibacterial activity of H-Z, ZT1 and ZT2 was determined toward *E. coli* and *S. aureus* by a disc diffusion method (Figure 3). It is evident that the H-Z does not exhibit the antibacterial performance whereas the strains are sensitive toward both ZT1 and ZT2. *E. coli* is more sensitive than *S. aureus*. Both ZT1 and ZT2 exhibit activity in the concentration range 10-20 mg $cm^{-3}$ toward both strains. According to the inhibition zone, it could be concluded that the ZT2 is more active than ZT1.

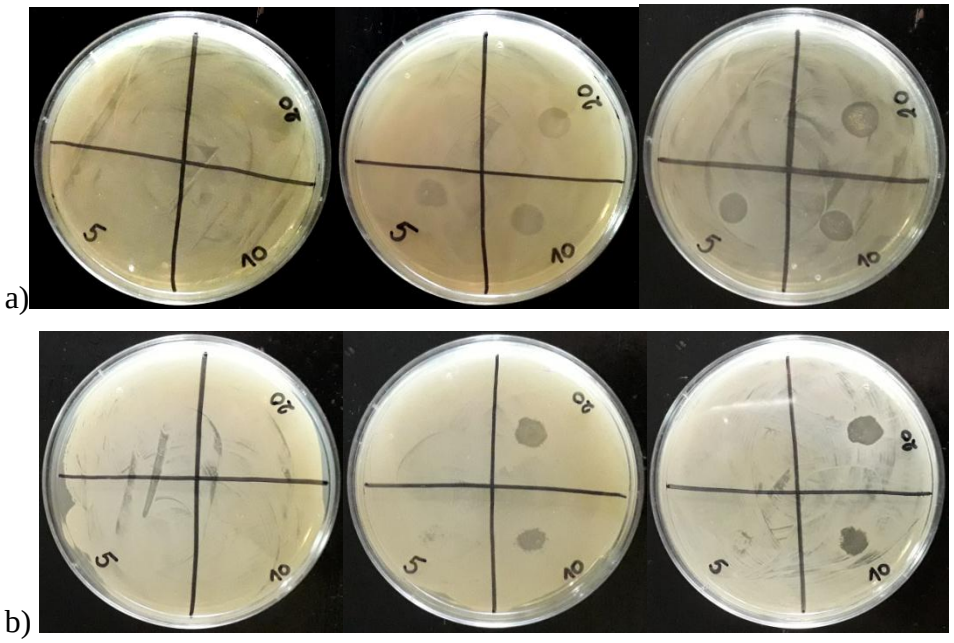



Figure 3. Antibacterial activity of H-Z, ZT1 and ZT2, respectively, toward *E. coli* (a) and *S. aureus* (b). Initial number of bacteria ($t_0$): *E. coli* = $2.9 \cdot 10^8$; *S. aureus* = $8.6 \cdot 10^8$ CFU $cm^{-3}$.

*Desorption of the thymol from composites*

Thymol release is more pronounced from the ZT1 than from ZT2 (data not shown). After 24 h a complete release occured from ZT1 and partial from ZT2 (50 %). This indicates the stronger interactions of thymol and clinoptilolite lattice in the composite obtained by SSI than in composite obtained by WI.

## CONCLUSION

Loading amount of thymol on clinoptilolite depends on the impregnation method. The supercritical solvent impregnation is more superior to wet impregnation. Thymol loaded by wet impregnation more readily desorbs from the composite, suggesting stronger thymol-clinoptilolite interactions in the composite obtained by supercritical solvent impregnation. The prepared composites are active against Gram-negative *E. coli* and Gram-positive *S. aureus* suggesting the disinfectant applicability.

## ACKNOWLEDGEMENT

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