STUDY ON ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF DENTOSEPT

The study has been performed in the Chair and Department of Pharmaceutical Bacteriology of the Karol Marcinkowski Medical University in Poznań, under the supervision of Prof. Dr. hab. Zygmunt Muszyński and Dr. Ilona Mirska.

Batch number and composition of the product tested: batch no. 010607

active substances declared by the manufacturer:

100 g Extractum fluidum (0.65 :1) ex: Metricariae flos – 13.0 g; Quercus cortex – 13.0 g; Salviae folium – 13.0 g; Arnicae herba – 6.5 g; Calami rhizoma – 6.5 g; Menthae piperitae herba – 6.5 g; Thymi herba – 6.5 g.

Excipients: ethanol 60 - 70% (V/V)

Product concentration tested: 15%

Scope and methodology of testing:

In the studies, the antimicrobial activity of 15% Dentosept solution has been established in laboratory conditions. The studies were performed with the use of the quantitative suspension method described in the European Standardisation Committee (CEN) standards (CEN) (1,2).

The purpose of the study was to determine the number of bacterial cells and fungal spores which would survive the specific contact time following their introduction into the sample.

To this end, the reduction factor (RF) was determined:

$$RF = \frac{No}{N_t}$$

N – number of viable bacterial cells and fungal spores entered into the sample

N_0 – baseline number of cells $\left(N_0 = \frac{N}{10} \right)$

N_t - number of cells after antiseptic agent use

Micro-organisms:

For the studies 10 of micro-organisms originating from the American Type Culture Collection (ATCC) and from the collection of the Chair and Department of Pharmaceutical Bacteriology of the Medical University (BF) were used:

- *Moraxella catarrhalis* ATCC 25238
- *Haemophilus influenzae* ATCC 49247
- *Streptococcus pyogenes* ATCC 10389
- *Streptococcus pyogenes* BF
- *Streptococcus anginosus* BF
- *Streptococcus pneumoniae* ATCC 49247
- *Streptococcus pneumoniae* BF
- *Staphylococcus aureus* ATCC 6538
- *Pseudomonas aeruginosa* ATCC 15442
- *Candida albicans* ATCC 10231

Study procedure:

The study was performed with the use of the quantitative suspension method, at 20° C, in accordance with the diagram on *Fig. 1.* For the purposes of the study, a working 18.75% solution of Dentosept in sterile water was prepared; its concentration was 1.25 times the intended concentration for use (15%).

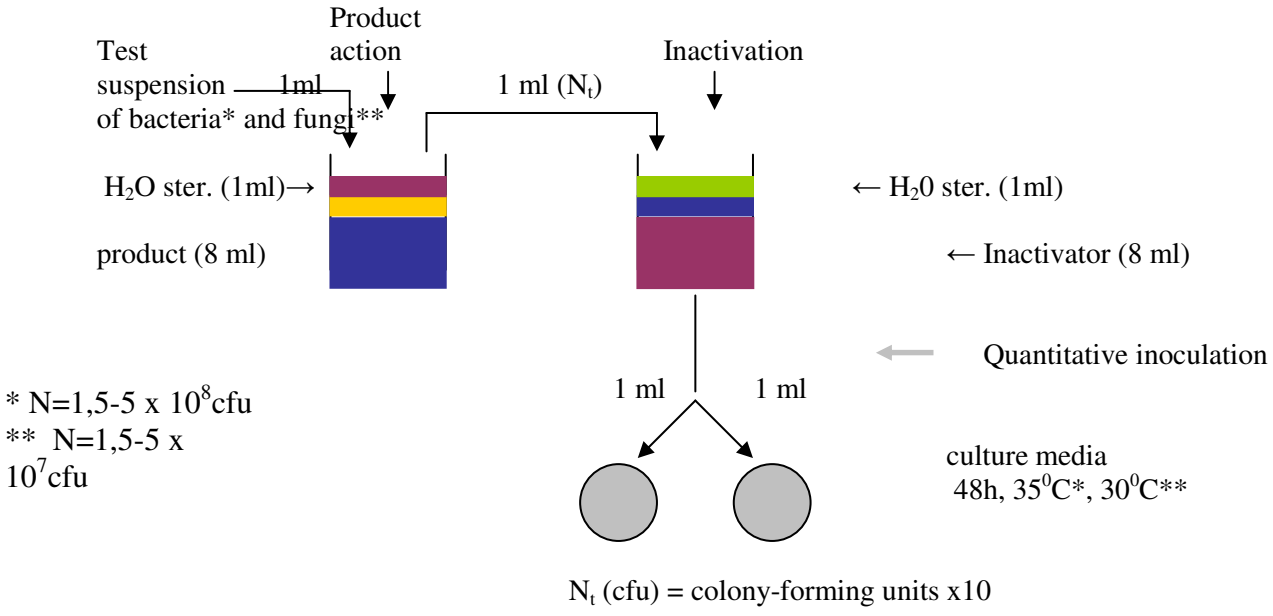

Figure 1. The basic diagram of antimicrobial activity testing, consistent with EN 1040 and EN 1275 standards.

Validation tests:

Verification of methodology and validation of the method were performed for undiluted Dentosept. The studies were performed at the same time and under the same conditions (test bacterial suspension,

inactivator, diluent) as in the study of activity of 15% Dentosept solution, and the results are presented in Table 2.

Verification of the methodology

Only such results of tests which complied with the requirements listed below were taken into account for evaluation of bactericidal and fungicidal activity of the product:

- the number of cells in the baseline suspension (N) should be contained within the range of 1.5 – 5 x 10^8 cfu/ml (bacteria) and 1.5 - 5x10^7 cfu/ml (fungi)
- the number of cells in the test suspension (Nv) should be contained within the range of 6 x 10^2 – 3 x 10^3 cfu/ml (bacteria) and 6 x 10^2 – 1.5 x 10^3 cfu/ml (fungi)
- the numbers of cells (Nx) should be equal or larger than 0.05 Nv
- the numbers of cells (Ny) should be equal or larger than 0.05 Nv

Results

Dentosept is an herbal medicinal product intended for mouth rinsing and gargling. This activity is most often performed over 1 to 5 minutes. The product is used in the form of 15% aqueous solution unless the doctor prescribes otherwise. Therefore, in the study antimicrobial activity of this drug was assessed on the basis of its ability to reduce the number of bacteria and fungal spores over 1 and 5 minutes. The antimicrobial activity of the product was tested in accordance with the methodology described in PN-EN 1040 and PN-EN 1275 standards. Seven microbial strains representing the species which often cause pharyngitis and upper respiratory tract inflammation: *S. pyogenes, S. pneumoniae, M. catarrhalis, H. Influenzae* and three strains of *S. aureus, P. aeruginosa* and *C .albicans* were used for testing. The study included validation tests for each bacterial and fungal strain used for testing (Table 2).

The results of testing are presented in Table 1.

Bacteria representing the aetiological factors of pharyngitis and upper respiratory tract inflammation proved to be the most susceptible to the Dentosept action.

15% aqueous solution of Dentosept reduced the number of cells of one of the two tested *Streptococus pneumoniae* strains (S. pneumoniae BF) and of the *Moraxella catarrhalis* ATCC 25238 strain by over 10^5 already after one minute, and after 5 minutes also of two other strains: *Streptococcus pyogenes* ATCC 10389 and *Streptococcus anginosus BF* isolated from clinical materials. The drug displayed ten times weaker activity against the remaining bacteria (*Streptococcus pyogenes BF*, *Haemophilus influenzae ATCC* 49247). Nevertheless, it should be emphasised that the reduction factors RF calculated for both these strains were contained within the range of >10^4 to <10^5, which means that the drug in the concentration for use destroyed at least 99.99% of the bacterial cells tested. The reduction factors obtained for *S. aureus* ATCC 6538 and *P. aeruginosa* ATCC 15442 strains were below 10^4, and the reduction factors obtained for the spores of *C. albicans* ATCC 10232 were below 10^3.

Conclusions

1. **Dentosept in 15% aqueous solution displays potent antibacterial activity causing more than 10^4 (> 99.99%) reduction in the number of bacterial cells of some of the species classified as aetiological factors of pharyngitis and upper respiratory tract inflammation such as *Streptococcus pyogenes, Streptococcus anginosus, Streptococuus pneumoniae, Haemophilus influenzae* and *Moraxella catarrhalis*.**

2. **Dentosept in 15% aqueous solution displays weak antibacterial action against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* strains, causing less than 10^4 (< 99.99%) reduction in the number of their cells.**