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# EXAMINATION OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF SELECTED NON-ANTIBIOTIC PRODUCTS

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A variety of compounds which are involved in the management of diseases of non-infectious aetiology have shown some antimicrobial activity *in vitro*, against bacteria and other microorganisms (1-3). Such compounds are called "non-antibiotics". By the end of the nineteenth century the dyes were known to possess antimicrobial activity. Paul Ehrlich used methylene blue – one of phenothiazines compounds as an antimicrobial agent (4). So far, a lot of attention has been focused on phenothiazines, thioxanthenes and other agents with affinities to cellular transport systems or show other inhibition mechanism (5-6).

The search of "non-antibiotics" among drugs distributed at Polish pharmaceutical market have been performed in Drug Institute in Warsaw for 7 years. So far, about 700 drugs which were randomly chosen from different groups of pharmaceutical products were examined. During this study (7-10), it was indicated that some of preparations inhibited growth at least one of the examined strains. The drugs with the following active substances showed significant antimicrobial activity: amlodipine, acepromazine, butorphanole, cisapride, cisplatine, clomipramine, diltiazem, emadastine, fluvastatine, ketamine, levocabastine, matipranalol, methotrexate, nicergoline, perazine, perphenazine, proxymetacaine, sertraline, tegaserole, tetrahydrozoline, ticlopidine and tropicamide. The surveillance study was performed on standard microbial strains.

The aim of this study was the continuation of searching and characterizing the antimicrobial activity expressed by selected non-antibiotic drugs, analyzed during state control performed in National Medicines Institute in Warsaw.

## MATERIALS AND METHODS

### Materials

The following microorganisms: *Escherichia coli* – ATCC 8739*, Pseudomonas aeruginosa* – ATCC 15442, *Staphylococcus aureus* ATCC – 6538P, *Candida albicans* – ATCC 10231, were used in the study.

The following pharmaceutical products available at the Polish market were randomly chosen for the analysis: Ac. E-aminocapronicum 200 mg/mL syrup (E-aminocapronic acid), Accupro 10 mg tabl. (quinapril), Alcaine eyes drops 0.5% (proximetacaine HCl), Alitol caps. (*Allii sativae extr.*), Allupol 100 mg tabl. (allopurinol), Aprazomerck 0,5 g tabl. (aprazolam), Arcoxia 60 mg, 90 mg, 120 mg tabl. (etoricoxib), Argol rheuma oint. (*etheric oils from: Melisse sp, Cinnamomi cassiae, Caryophylli sp, Myristica sp, Thymi sp, Citri sp, Coriandri sp, Menthae pip., Rosmarini sp, Cajeputi sp*), Artelac eyes drops (hypromelose), Arteoptic eyes drops 2% (carteololi HCl), Bazetham 0,4 mg caps. (tamsulosine), Beto 200 ZK 190 mg tabl. (metoprolol succinate), Betoptic S eyes drops 0.25% (betaxolol), Bisacodyl 5 mg tabl. (bisacodyle), Bobotic 66,66 mg/mL drops (simeticotine), Boldovera (0,015 g; 0,01 g)/0,2 g tabl. (*Aloe extr. sicc., Fumariae extr. sicc.*), Captopril 50 mg tabl. (captopril), Ceratio 10 mg tabl. (cetirizine), Cet alergin drops 1% (cetirizine), Cholestil 200 mg tabl. (hymecromone), Citabax 40 mg tabl. (citalopram), Clopidix 75 mg tabl. (clopigrel), Cosopt 20 mg + 5 mg/mL eyes drops (dorzolamide, tymol), Cyclo 3 Fort 150 mg caps. (*Rusci aculeati extr. sicc.*), Daivobet (50 µg + 0,5 mg)/g ointment (calcipotriole, bethametha-

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Table 1. Antimicrobial activity of selected non-antibiotic drugs.

| Active substance (*Drug*) | Minimal inhibitory concentration (MIC) in mg/mL of active substance in drug | | | |
|---|---|---|---|---|
| | Strain | | | |
| | ***S. aureus*** | ***E. coli*** | ***P. aeruginosa*** | ***C. albicans*** |
| Analgeticum, Antypyreticum, Antiphlogisticum* • naproxen (Nalgesin 550 mg tabl) | -** | - | - | 35 |
| Antiandrogenicum • flutamide (*Flutamid 250 mg tabl*) | 63 | - | - | - |
| Antiatheromaticum • simvastatine (*Simvacor 10 mg tabl.*) | - | - | - | 5 |
| Anticoagulantia • clopigrel (*Clopidix 75 mg tabl.)* | 24 | - | - | - |
| Antidepressivum • citalopram (*Citabax 40 mg tabl.*) | 13 | 7 | - | - |
| Antidiabeticum • acarbose (*Glucobay 100 mg tabl.*) | - | 7 | - | - |
| Hepatoprotectivum • sylimarine (*Sylicaps 100 mg tabl.*) | 67 | - | - | - |
| Laxantia • docusate sodium (*Doculax 50 mg caps.*) | 3 | - | - | - |
| Spasmolytica • solifenacin succinate (*Vesicare 10 mg tabl.*) | 7 | - | - | - |

(*) – the names of pharmaceutical groups according to Podlewski et al. (16)
(**) – lack of microbial growth inhibition

sone), Danazol 200 mg tabl. (danazole), Debelizyna paste (*Dolichos biflori sem. extr.*), Debridat 7,87 mg/g granulate (trimebutine), Delatar ointment 2 g/100 g (pitch coal), Dexak 25 mg tabl. (dexketoprofen), Doculax 50 mg caps. (sodium docusate), Duphalac 667 mg/mL syrup (lactulose), Ecomer 50 tabl. (alkylglycerols), Eryptaminum tartaricum 1 mg tabl. (eryptamine tartrate), Fenoterol 5 mg tabl. (fenotenoli hydrobromidum), Flutamid 250 mg tabl. (flutamide), Geronpol 40 mg tabl. (*extr. Panax ginseng* + vitamins), Ginkofar Vita tabl. (*extr. Ginco biloba*), Gliatilin 400 mg caps. (choline alfoscerate), Glucobay 100 mg tabl. (acarbose), Gromeloksin 1 mg, 5 mg tabl. (meloxicam), Hascovir 200 mg, 400 mg tabl. (aciclovir), Helicid 10 mg caps. (omeprazole), Hepacom 100 mg tabl. (timonacic), Iberogest solut. (*extr. of: Iberis amara, Angelicae rad., Matricariae flos, Carvi fruct., Silybi mariani fruct.; Melissae folii, Menthae piperitae folii, Chelidonii herbae, Liquiritiae radicis*), Iwermektyna 0.12 g/6.42 g paste for horses (ivermectine), Ketrel 100 mg, 200 mg, tabl. (quetiapine), Kortaddon 100 mg caps. (flupirtine maleate), Lactulosum 7.5 g/15 mL syrup (lactulose), Lovastin 20 mg tabl. (lovastatine), Mefacid 250 mg tabl. (mefenamic acid), Mentoval 65% drops (methyl valerate), Metazidine 20 mg tabl. (trimetazidine HCl), Metohexal 200 ZK 190 mg tabl. (metoprolol succinate), Miansec 30 mg tabl. (mianserine), Miozepam 50 mg tabl. (tetrazepam), Mitrip 2.5 mg, 5 mg tabl. (ramipril), Mononit 80 Retard 80 mg tabl. (isosorbide mononitrate), Myopam 25 mg tabl. (tetrazepam), Nalgesin 550 mg tabl (naproxen), Neatin 16 mg tabl. (betahistine HCl), Pabi-Acenocoumarol 4 mg tabl. (acenocumarole), Phlebodia 600 mg tabl. (diosmine), Polcortolon 4 mg tabl. (triamcinolone), Posorutin 50 mg/ml eyes drops (troxerutinum), Prestarium 4 mg tabl. (perinoloprile erbumine), Prograf 0,5 mg caps. (tacrolimus), Promethazine 5 mg/mL syrup (promethazine), Pulsaren 5 mg, 20 mg tabl. (quinapril), Salbutamol 4 mg tabl. (salbutamol), Scopolan 10 tabl. (Hyoscine butylbromide), Sifor 1000 mg tabl. (metformini HCl), Simvacor 10 mg tabl. (simvastatine), Siofor 850 mg tabl. (metformin), Sorel liquid 50 ěg/mL (calcipotriole), Starazolin eyes drops 0.5% (tetryzoline HCl), Sylicaps 100 mg tabl. (sylimarine), Talcid 500 mg tabl. (hydrotalcit), Tamsudil 0,4 mg caps, (tamsulosine), Tanyz 0,4 mg caps, (tamsulosine), Tegretol CR 200 mg tabl. (carbamazepine), Tenox 5 mg tabl. (amlodipine), Tertensif SR 1.5 g tabl. (indapamide),

Trental 400 mg tabl. (pentoxifylline), Troxeratio 300 mg tabl. (troxerutin), Tyzine nose drops 0.1% (tetryzoline HCl), Ulgastran 1 g tabl. (sucralfate), Vesicare 5 mg, 10 mg tabl. (solifenacin succinate), Vitagin 60 mg caps. (*Ginseng radix extr. sicc.)*, Xentic 20 mg tabl. (zolpidem tartrate) and Zovirax 200 mg tabl. (acyclovir).

**Initial screening of antimicrobial activity**

The sterile blotting-paper disks were soaked with 10% (v/v or w/v) solutions of tested drugs in 0.08 M phosphate buffer, pH 7, and placed onto Mueller-Hinton 2 Agar (BioMerieux). Plates were inoculated with standardized cells suspension 0.5 unit (Mc Farland scale) of tested strains. The inhibition of bacterial growth was seen as a halo around the disk containing the tested compound. Size of inhibition zone was correlated with the antimicrobial activity of the drug.

**Minimal inhibitory concentration (MIC) determination**

Appropriate dilution of the drug in 0.08 M phosphate buffer, pH 7.0 was mixed with 19 mL of a Mueller-Hinton 2 Agar, cooled to 45°C. The suspension of particular strain of density 0.5 unit (Mc Farland scale) – 2 µL was applied on agar surface. The lowest concentration of tested drug, which totally inhibited growth of examined strain was evaluated as MIC value.

## RESULTS AND DISCUSSION

It was shown that the drugs listed below inhibited growth of at least one of the examined strains: Citabax 40 mg tabl. (citalopram), Clopidix 75 mg tabl. (clopigrel), Doculax 50 mg caps. (sodium docusate), Glucobay 100 mg tabl. (acarbose), Nalgesin 550 mg tabl (naproxen), Simvacor 10 mg tabl. (simvastatine), Sylicaps 100 mg tabl. (sylimarine) and Vesicare 5 mg, 10 mg tabl. (solifenacin succinate) (Table 1).

*Staphylococcus aureus* was susceptible to about 80% of the drugs listed in Table 1. Docusate sodium inhibited growth of *S. aureus* in concentrations 3 mg/mL. Other chemical compounds showed activity against this microorganism in concentrations between 5 and 70 mg/mL. Acarbose and citalopram in concentration 7 mg/mL showed the strongest activity against *E. coli*. In addition, citalopram inhibited growth of. *S. aureus* in concentration 13 mg/mL. *C. albicans* showed the strongest susceptibility to simvastatine (MIC – 5 mg/mL). Interestingly, natural product – sylimarine, extracts from *Silybum marianum* inhibited *S. aureus* in concentration 67 mg/mL. *P. aeruginosa* was resistant to all of the examined active substances.

Kristiansen et al. (1, 5, 11) confirmed that non-antibiotic compounds enhance the *in vitro* activity of certain antibiotics against specific bacteria. For instance, omeprazole and nizatidine enhance the inhibition effect of metronidazole on *Helicobacter pylori* growth (12) or phenothiazines could be helper compounds in the treatment of multidrug-resistant Gram-negative rods (13). So, our further investigations will focus on this type of activity. Moreover, the antimicrobial activity of such non-antibiotic drugs emphasizes a necessity of the neutralization of their activity during the microbial purity tests of pharmaceutical products (14, 15). In eyes drops the content of benzalkonium chloride varies from 0.05 to 0.1 mg/mL. After 10 times dilution of eyes drops they still inhibited *S. aureus* growth, therefore, sterility test should be carried on in 100 times dilution of the drug. In case of syrups Duphalac and Lactulosum, preservatives are carbohydrates, in Promethazine, sodium benzoate (0.1-0.2%) is preservative compound. These products after 10 times dilution did not inhibit growth of any tested strains therefore test of microbial purity does not required any neutralization of these products.

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